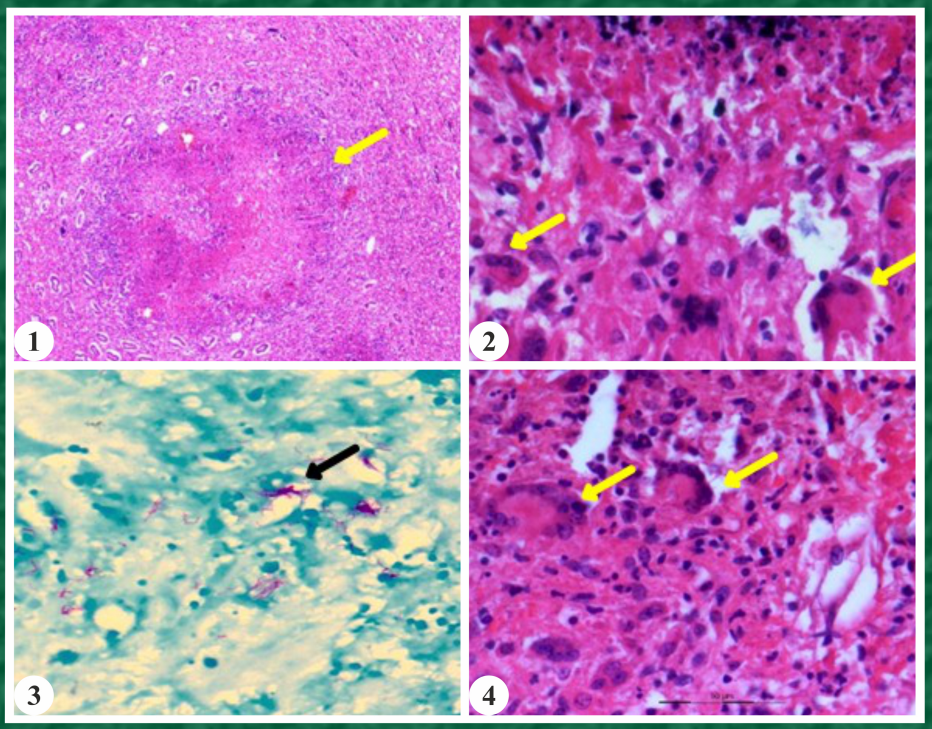
Vol.: 50(1)
March, 2026
ISSN: 0250-4758
Online ISSN: 0973-970X

IJVP-2026

INDIAN JOURNAL OF VETERINARY PATHOLOGY



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Vol. 50 (1)
March, 2026
ISSN: 0250-4758

INDIAN JOURNAL OF VETERINARY PATHOLOGY

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INDIAN JOURNAL OF VETERINARY PATHOLOGY

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INDIAN JOURNAL OF VETERINARY PATHOLOGY

INDIAN ASSOCIATION OF VETERINARY PATHOLOGISTS
(Estd. 1974)

Cover Page Photo (Clockwise) : Tuberculosis: Photomicrographs showing microscopic lesions in Mycobacterium orygis associated generalized tuberculosis in cattle - Kidney sections showing caseous granuloma and intense cellular reaction (Left top), Meninges showing granulomas with multinucleated giant cells (Right top), Cerebellum showing acid-fast bacilli and caseous material (Left bottom) and Mammary gland showing caseous granuloma with giant cell formation (Right bottom).

Indian J. Vet. Pathol., 50(1) : 1-14, 2026: DOI: https://doi.org/10.56093/f5pdx955

REVIEW ARTICLE

Synergizing academia, industry and clinical practice: Role of veterinary pathology

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Received: 06.1.26; Accepted: 21.1.26

Clarion call: "Building India a product-driven nation to become 'Viksit Bharat' (Developed India) by 2047 (100th anniversary of independence) and achieve a $5 trillion economy" -Shri Narendra Modi, Hon'ble Prime Minister of India.

How to cite this article: Balachandran, C. Synergizing academia, industry and clinical practice: Role of veterinary pathology. Indian J. Vet. Pathol., 50(1) : 1-14.

INTRODUCTION

Industry-academia collaboration refers to partnerships between educational institutions (universities, colleges) and businesses or industries. These collaborations aim to leverage the strengths of both sectors, fostering knowledge transfer, innovation and practical application in research. This involves a mutually beneficial relationship where industries gain access to research, expertise and a pipeline of skilled graduates, while academia benefits from real-world insights, funding opportunities and the ability to translate theoretical knowledge into practical applications. Industry-academia partnerships result in a 'win-win' situation for both since each other's deficiencies are bridged through these linkages. The 'science' developed by the academic institution is converted into 'technology' by the industry which makes them available to the ultimate stakeholders. During this process, the industry has to make a 'profit' while the academia is 'incentivized' and they obtain satisfaction as their endeavours reach the end users. More recently, science and technology development needs to be complemented by the commercialization for the ultimate benefit. This entire process is called "productization". The vision of many world-class universities / research organizations have changed over the time from a mere "teaching-research" model to "societal contribution through innovation" approach. The 'driver' and the 'enabler' for such an approach is the industries readiness to adopt strategic innovations. Such a favourable ecosystem is created by increased two-way collaborations through industry-academia linkages. Industry should, not only highlight the issues that need to be addressed but also uptake the technologies available, scale up and market them to make them available to the stake holders[1].

Current focus on demand driven agriculture

Agriculture is to produce food, feed, fodder, and fiber for the country. There is an urgent need for commercial market driven techno savvy production with emphasis on value addition, quality, efficiency and export. Let our research be user/farmer friendly and marketable. Practicing 'Lab to Land' in the right spirit to achieve 'Farm to Fork'. In the light of these considerations, collaboration/ partnership between universities and industries and other related organizations is critical for education and training, research, innovation and technology transfer and the promotion of start-ups and spin-offs (entrepreneurship). The trend of modernization of industries has set in, and the bases of competition have also changed. Agriculture and allied sectors can't be an exception to these pressures. With increased opportunities and competitive scenario in this open economy, agriculture sector has the power and potential of ensuring livelihood security to our people, providing employment opportunities to millions of people, and fostering agripreneurship. With proper planning, India can certainly rise as the proud food basket of the world[2]. The synergy between academic institutions and industry has emerged as one of the most significant components of India's research and innovation journey. Over the decades, these partnerships have demonstrated the ability to translate research outcomes into tangible societal benefits, spanning from advancements in healthcare and rural technologies to sustainable agriculture and digital solutions[3].

In India, addressing collaboration challenges between industry and academia-such as the monetization of shared intellectual property, high commercialization costs of academic research and differing project management approaches-can help leapfrog the nation towards greater technological leadership. This is where the government can play a catalytic

role in strengthening industry-academia linkages to further bolster our innovation ecosystem. Long-term investment in both fundamental research and advanced technological pursuits, aligned with national priorities, is essential for creating a robust innovation ecosystem. These thoughts were echoed at the 26th meeting of the PM-STIAC (Prime Minister's Science, Technology, and Innovation Advisory Council), held on 18th October 2024, which brought together experts from academia, industry, think tanks and government who aligned on identifying certain mission-critical priority areas where such partnerships can drive significant multiplier effects in the country's growth[3].

BASIC NEEDS[4-6]

Focus on veterinary education

Veterinary education provides human resource required for augmenting public good. In India, where up to 80% population is rural, and animal husbandry is the livelihood. Veterinary profession provides the desired professional coverage. Agriculture and livestock are turning towards a broader concept of "bio-economy". Bio-economy refers to all economic activities derived from scientific and research activities focused on biotechnology. State Agricultural/Veterinary Universities need to be well integrated with traditional universities that emphasize basic sciences. Veterinary education approach should be multifaceted to ensure the betterment of both humans and animals as individuals and populations with problem solving interdepartmental expertise teaching. It should also focus on animal welfare.

Scope of veterinary education

i. Livestock sector provides employment opportunities, livelihood and socio-economic security to small holding livestock farmers.
ii. By 2050, the population in India would increase by 34%.
iii. To fulfill the dietary recommended levels of the livestock products by ICMR for a population of 1.7 billion people, the livestock sector should produce 186.2 million tons of milk, 18.7 million tons of meat and 306 billion eggs per annum which is an increase of 1.5, 3.7 and 4.7 folds of milk, meat and eggs respectively.
iv. The additional challenge is to fulfill the feed requirements for the increased livestock population from the same resource base of land and water
v. India need to produce more food in the next 40 years than has been produced in the last 8,000 years
vi. Agriculture and livestock is turning towards a broader concept of bio-economy
vii. The evolution of the biotechnology industry and its application to agriculture, health, chemical, or energy industries is a classic example of bio-economy. Thus, veterinary education can contribute towards fulfilling the above needs.

Veterinary Research Contribution

Animal health

i. New generation vaccines: chronic infections bovine TB, JD, brucellosis in goats, Marker (e.g. Current indigenous LSD DIVA vaccine developed), Recombinant and multicomponent vaccines
ii. Point of care diagnostics
iii. Antimicrobial resistance
iv. Food safety: Food borne pathogens
v. Biomedical instrumentation
vi. Lab on chip
vii. Novel delivery systems
viii. Traceability of foods
ix. Stem cell and regenerative medicine
x. Emerging diseases
xi. Zoonotic diseases
xii. Poultry disease diagnosis: Production integrated diagnostics; Conventional poultry diagnostic investigation is done as a response to increased mortality, impaired production and losses at processing. Although occurrence of a single disease is significant, the problem is mostly multifactorial in poultry farming.

Priorities for Veterinary Research

Veterinary Research should implement the concepts of "One Medicine" and interdisciplinary and translational research in the broader biomedical research agenda. i. Widen our knowledge on detection and control of infectious diseases ii. Develop databases and other resources that are freely accessible to the scientific community iii. Improve research on the areas of human-animal interfaces iv. Optimize effective, sustainable and socially responsible food-animal production v. Implement the concepts of "One Health" along with interdisciplinary and translational research.

There would be a dramatic increase in the demand for livestock products, driven largely by human population growth, income growth and urbanization. However, the changing scenarios and the need to adapt to climate change and to mitigate greenhouse emissions will add to the costs of production. Livestock production would become industrialized. The "vicious circle" involving the challenges of teaching, researching, publishing and competing for competitive grants, coupled with pursuing career aims and ambitions, can seem daunting. However, it can also be deeply satisfying when the fruits of the experimental laboratory research are translated into

improved healthcare delivery to our animal patients. To do this, the basic motive to do research has to change in terms of addressing a pressing problem and not to start with another publication. We need to be up to the challenge and above technologies is our tool box!![1].

We all know the disease triad. Disease occurs due to the interaction of host, pathogen and the environment. Similarly, the technology response to the prevention, therapy or control of disease has to encompass nanotechnology, immunotechnology or biotechnology for rapid and effective results. The application of these technologies, either alone or in combination, for protective and therapeutic interventions in translational veterinary medicine is required for the animal sciences sector, to achieve the goal of doubling the farmers income and to accelerate growth of this sector[7].

Animal Welfare Requirements

In future, public would insist consuming animal food that is certified as produced with due animal welfare considerations. The welfare of animal on-farm, during transport, at markets and at slaughter has to be considered[4-6].

Vetpreneurship Development – the Need of the Hour

Livestock business plays a crucial in role in the economy of farmers. It is a major source of subsidiary income for farmers during off-season. But their income through this source is mainly affected due to a plethora of animal diseases. The demands for increased production of livestock and need to manage these diseases have become imperative. This could only be achieved by adopting novel technologies and sustainable practices. At the same time, many innovators in animal sciences need handholding in developing their ideas into commercializing products or services. Veterinary Start-up links innovators, mentors, investors, farmers and end users in a fashion that accelerates the adoption and exploitation of novel technologies for the betterment of human life. Start-up India builds a strong eco-system for nurturing innovation and start-ups that will drive sustainable economic growth and generate large scale employment opportunities. Start-ups like other companies have number of risk factors that hinders their growth in achieving their goals. They need to develop a business model that de-risks the known risk factors or at least mitigate the challenges[6].

Skill development: The livestock farmers are effectively contributing to the GDP (Gross Domestic Product) of the country. Still they need to be imparted about certain skills to make the livestock business more profitable and sustainable. The gap between the raising technologies and adoption and utilization of those technologies shall be gradually reduced to attain maximum benefit from the novel technologies.

Entrepreneurship development: Government of India has taken definitive steps to encourage entrepreneurs in the country. Micro, Small and Medium Enterprises (MSME) Development Act was enacted in 2006 to promote entrepreneurship and to provide incentives, schemes or subsidies for the development of entrepreneurship in India. Market players in veterinary businesses are actively engaged considering the market size. Business opportunities in veterinary sector cannot be ignored anymore. They are also required for delivering veterinary clinical services and marketing novel veterinary products. Vetpreneurship help the innovators to translate the technologies for field exploitation while enjoying the monetary benefits out of it. Vetpreneurs can also involve in setting up of modern dairy farms and animal rearing centres[6].

Encouraging entrepreneurship in animal husbandry sector will ultimately lead to high quality food, increased income for farmers and employment generation. This could be possible by providing access to information about technical advancement and market. Complex issues in making livestock entrepreneurship, a successful one, shall be addressed to realize the potential of this animal husbandry industry. Start-up culture in veterinary sector is gaining momentum as time passes thanks to the demand for increased productivity and emerging diseases. Services of livestock enterprises are much needed at this stage to enhance the livelihood of farmers. Skill development will contribute to effective adoption of novel technologies. Successful entrepreneurs in animal husbandry will greatly benefit all the stakeholders that mainly include farmers and innovators and will lead to the overall economic development[6].

The Academic's Dimension

The advantages perceived by the universities or research institutions by the Industry-Academia partnerships ranges from obtaining extra-mural research funding, opportunities to work on cutting edge research, share biological resources, understand market requirements, assess technology feasibility, facilitate cGMP production and obtain regulatory approvals and so on. University research is usually 'curiosity-driven' and its market potentials are not immediately apparent. This strategy is the strength of these institutions and majority of their research are proof of concept studies. The outcomes of these studies most often contribute a new addition to the existing knowledge pool and most often are in its early stages and do not have immediate 'real-world' applications. A lot of additional work is essential to fine tune this technology and make it presentable, feasible and market-ready. Although these are less appealing from the industry's perspective, only universities can invest on basic 'blue-sky' research that companies cannot realistically do[1].

Academia is more flexible e.g. working hours and autonomy in deciding where and how you work; disagreeing with the boss or the university's 'corporate line' is considered par for the course. Academia provides more room for intellectual autonomy (Reason choosing to pursue academic careers). As universities become more geared to industry-driven research and commercialisation opportunities, the tide may be changing. The only people in academia are those who can't make it to industry. Teaching: Students often don't respect if your focus is other than them. Industrialist do a guest lecture. Teaching in academia sets you up well for private industry training opportunities. Publications: Refereed, peer reviewed publications ('high quality' journals, if academia is the goal or industry publications, if industry is your goal) are the only ones that have considerable weight on an academic curriculum vitae; Ph.D., as a basis for publications. *Be flexible and visible*[8].

From academia side: Apathy towards applied research and comfort zone of pure teaching restrictive internal policies, procedures and politics, leisurely-paced approach of academia v/s time-bound strategy of industry, lack of inner urge in academic fraternity due to absence of appropriate incentives/recognitions and absence of exclusive "University/Academia-Industry Interaction Cell"[2].

The Industry's Dimension

The advantages perceived by the industry by such partnerships are direct access to the knowledge pool and potential opportunities to utilize the wide range of expertise available in the university. Credibility and branding of the university are added advantages. In addition, the industries need not spend their resources on exploratory research instead can selectively obtain the leads that have real world applications from its academic partners[1,6]. Industry pays more. This is not convincing, and depends on the employment market, skills shortages/surpluses etc., and the knowledge market relative to your discipline and specialisation. Salaried versus specialist contract/consultancy work. How well you can sell yourself and your skills? Industry is more practical i.e. Industry is oriented to achieving practical outcomes in the short-term while academic work tends to have a long-term view. Industry-based professionals do not have the same level of insight into complex problems as academics[8]. Insensitivity to the resource potential of academia is unhealthy obsession with expensive 'so-called' consultants focus on short-term results, mostly quantifiable heavy dependence on easily available foreign 'know-how'. Earlier bitter experience with academia fear of losing competitive edge. Anxiety to keep secrecy of IPR and ensuring profit at least for some time[2].

The Cultural Divide – 'critical acclaim' vs 'block busters'

There exists a large difference between the working cultures of the academia and industry. Such cultural divide is largely driven by the way research is focused in these sectors. The academics more often want their research to be published in peer reviewed journals since the number and impact of their scientific publication evaluate their merit. On the contrary, industry research aims to develop an intellectual property that is marketable. Such innovations are not published but are protected by patents etc. Since the academia's research questions are not based on market requirements, the academics have the comfort of not working in a time frame. In addition, the academics are not incentivized enough for engaging in marketable technology development which in-turn leads to disinterest in the part of the academics. But the industry works on a strict time line and driven by result oriented work plans. Such difference in work cultures largely impact the way these two entities interact. The industry wants to tap into a marketable idea at the earliest with minimal investments and aims at large profitability from such ideas. This is obvious by the way the industry adapts the foreign 'know-hows' instead of relying on 'in-house' research and development e.g. the universities, particularly those that are government funded have a lot of internal constraints in the way of handling funds, obtaining permissions, utilization of external consultants, outsourcing research components etc. These restrictions heavily delay the output in terms of the industry's time frame[1].

Bridging Disconnect: The Government's Initiatives

In recent years, Government of India has initiated many activities to bridge this large disconnect between the industry and academia by providing suitable funds e.g. DBT, Biotechnology Industry Research Assistance Council (BIRAC), a not-for-profit Public Sector Enterprise, The Biotechnology Ignition Grant (BIG), Start-up's or an incubator who have an exciting idea which may be in the nascent and planning stage and there is an unmet need for mentorship and initial funding. The BIG would help to support and nurture these high-risk early starters and their concepts. The DBT-Small Business Innovation Research Initiative (SBIRI) scheme of the DBT is facilitating innovation, risk taking by small and medium companies and bringing together the private industry, public institutions. The Biotechnology Industry Partnership Programme (BIPP) promotes partnership with industries for support on a cost sharing basis for path-breaking research in frontier futuristic technology areas having major economic potential and making the Indian industry globally competitive. BIRAC has launched Promoting Academic Research Conversion to Enterprise (PACE) scheme[1]. This list is not exhaustive. There are additional schemes viz., to contribute to the vision, Government has established National Missions

i. ANRF-Anusandhan National Research Foundation

ii. The Manthan digital platform

iii. The National Quantum Mission

iv. The Deep Ocean Mission v. The National Mission on Interdisciplinary Cyber-Physical Systems

vi. India AI mission promotes synergistic efforts between academic researchers and industry experts.

vii. The National One Health Mission is a testament to how these partnerships can address complex challenges at the intersection of human, animal, and environmental health, fostering comprehensive and sustainable solutions[3]. About 13 government-facilitated initiatives to promote industry-academia partnerships are

i. Uchhatar Avishkar Yojana (UAY) – 2015

ii. IMPRINT-2015

iii. DBT-BIRAC Amrit Team Grants-2024

iv. Biotechnology Industry Partnership (BIIP)-2008.

v. IIGP 2.0-2017

vi. Industry Relevant R&D Scheme

vii. TIDE 2.0-2019

viii. SERB-Industry Connect-2021

ix. RUSA-2013

x. National Biopharma Vision-2017

xi. Skill Vigyan Programme-2022

xii. Biofoundries and Biomanufacturing Hubs-2024

xiii. DRDO Industry Academia Centres of Excellence (DIA-CoEs)-2022 and so on[9]. There are two elements in an industry-academia partnership. Industries today include multinationals, Indian industries, industry associations, MSMEs etc. Academia includes both private and public institutes and 300 Incubators as well as their thousands of incubated startups. These stakeholders are large in number with distinct specialisations[10].

Industry-Academia Collaborations

Academia-industry partnering relationship is not like technology donator-acceptor; but of interactive and collaborative nature. For a "Win-Win" partnership, the prerequisites are: Paradigm shift in the attitude and approach of both the entities for obtaining mutually beneficial outcomes[2]. Networks are the key: Start with your supervisors. Don't be afraid to contact other academics to discuss opportunities. Ask around at the relevant research institute[8].

Evolution of Industry-Academia Partnerships in India

1941: Dr. Shanti Swarup Bhatnagar established the Industrial Research Utilization Committee and in 1942 this evolved into the Council of Scientific and Industrial Research (CSIR), a crucial bridge between scientific research and industrial application in India, India's first institutional attempt to forge meaningful connections between research, development (R&D) and industry needs. Then established, 1950-60: Indian Institutes of Technology; 1971: DST; 1986: DBT; 1991: Economic liberalization marked another pivotal shift. These industry-academia partnerships in India have taken various operational forms viz., 1. Industry driven projects 2. Projects of mutual interest 3. Partnerships of scientific infrastructure with various expansions like i. Private industry and public academia ii. Public industry (PSUs) and public academia iii. Private industry and private academia iv. PSU and Private academia. Incubators and Technology Transfer, a Core Component of Collaboration. IIT Bombay supported 245 startups and Biomedical Engineering and Technology Innovation Centre (BETIC) and IIT Kharagpur's Agri Business Incubation Foundation addressing agricultural challenges. Beyond incubators, this collaborative ecosystem's strength lies in its diverse support mechanisms, as evidenced by IIT Madras Research Park, which houses over 200 R&D companies and facilitated more than 1300 patent filings. The vibrant ecosystem steadily dissolving traditional boundaries in this space[11].

IIT-Madras

In 1998, IITM-Research Park (IITMRP) was established for creating industry-academia-startup ecosystem, so far incubated 375 deep tech companies worth Rs. 50,000/ crores. Once the Industry-Academia-Startup Ecosystem works, it grows from strength to strength, attracting more industries, international organizations and governments and also youngsters dreaming big could do the impossible. The Venture Capital finds the ecosystem increasingly attractive and is willing to take up big bet[12].

Deep Tech

The future of innovation lies in "deep tech". Deep tech refers to technologies rooted in substantial scientific research and engineering breakthroughs. Breakthroughs in transformative fields such as quantum technologies, artificial intelligence and advanced biotechnology are set to revolutionize almost all sectors, drive sustainable solutions, and create unparalleled societal impacts. Unlike incremental innovations, deep tech solves complex challenges and disrupts existing markets by introducing ground breaking solutions e.g. development of CRISPR-Cas9 genome editing technology. This groundbreaking innovation allows precise editing of DNA, paving the way for curing genetic disorders, improving crop yields, and tackling diseases like cancer and revolutionizing fields like medicine, agriculture, and bioengineering.

Other e.g. AI-driven diagnostic tools-Google's AI to detect diabetic retinopathy. In deep tech, no single stakeholder can achieve breakthroughs in isolation but with strong academia-industry government partnerships and needed infrastructure. One of the core characteristics of such partnerships is smooth bidirectional flow of knowledge, and technology transfer. Universities and research institutions often serve as incubators of breakthrough innovations. For India, moving towards a product oriented economy does not imply abandoning the existing strengths in IT and digital services but complementing[13].

Triple Helix Model

The triple helix model represents a transformative approach to innovation, where academic institutions, industry partners, and governmental bodies converge to drive technological advancement and economic development. This collaborative framework has emerged as a critical approach for addressing complex challenges across diverse sectors, enabling knowledge transfer, research commercialization, and sustainable innovation. This is a proven framework for fostering research and innovation. This dynamic partnership leverages the complementary strengths of each stakeholder to address pressing societal and sectoral challenges. This covers life sciences, agriculture, defense and sustainable energy. Life sciences: Invention of library of organic small molecules with violet-blue absorption and bright green emission in the solution state, greatly helped bioimaging in fluorescence microscopy compared to conventional dyes in biological research with great market potential in the biotech, pharmaceutical and clinical research. Agriculture: Chemical pesticide challenges: Pesticide-resistant pests, environmental pollution, and soil degradation leading to development and deployment of a pheromone-based bio-insecticide system (ATGC Biotech, a pheromone chemical synthesis company with Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR) and University of Agricultural Sciences (UAS), Bengaluru resulted in a nano-matrix pheromone dispenser. This dispenser broadcasts synthetic pheromones to disrupt mating cycles of pests, offering an eco-friendly and effective alternative to traditional pesticides and is validated in field in Karnataka[14].

National Funding

The key imperative is for Indian industry to increase its expenditure on R&D. In FY2023, the total expenditure on R&D by Indian industry was USD 7.4 billion. This was less than a fifth of what Alphabet as a single firm spent on R&D. If India has to see high economic growth of 8 to 10 percent over the next two decades to cross the USD 20 trillion mark, Indian industry would need to lead the way through its investments in R&D. A multi-policy approach would be needed that goes beyond funding models and incentives for R&D and innovation. One that prioritizes building the talent pipeline, addresses regulatory bottlenecks, considers trade policies on a case by case basis (not only protectionism), focuses on supply chains, invests in skilling the workforce on AI complementary skills, and guides firms on good management practices to integrate into global value chains. We must collectively and continuously engage in this effort to help India fulfil her R&D and innovation ambitions[15].

Public-private partnership being seen as a synonym to industry-academia partnerships. Large grants often come from the government and the scientific facilities get established. Once you create the facilities, this is where industries come in. They give the relevant problem statements, and then use these facilities created through government funding to do work which will be of interest to the industry. In India, even the government funding is just 0.6-0.65% of GDP which is less than what other BRICS (Brazil, Russia, India, China, South Africa) nations support in their countries[16].

The Barriers to Academia-Industry Collaborations

These can be categorized into two main types: transactional and orientation-related. Transactional barriers primarily involve intellectual property rights and conflict resolution mechanisms among stakeholders. Orientation-related challenges stem from fundamental differences in how academia and industry operate. Academic research typically follows PhD student timelines, which may not align with industry's need for immediate results. Additionally, academia thrives on publishing and sharing knowledge, while industry often requires confidentiality. There should be flexibility and overcome the limitations e.g. Fraunhofer operate on a unique model where professors divide their time between university and applied research, enabling efficient translation of academic work into industry applications and was highly successful, generating 600 patents annually and €3 billion in revenue, with 70% from industry-driven, demand-focused research. The more crucial opportunity lies in supporting MSMEs (Micro, Small, and Medium Enterprises), forming India's economic backbone but lack the resources and confidence to approach major institutions. Government intervention is essential for facilitating these connections, as demonstrated by initiatives like RuTAG-Rural Technology Action Group[16].

Overcoming the Impediments

Breaking mental barriers on the part of industry and academia. Industry should build confidence in capabilities of universities. Public policy (may influence the propensity to collaborate through a direct role) in providing funds for R&D projects to universities. Necessary infrastructure for technology transfer offices, and business incubation centers. Action steps for long lasting, symbiotic relationship between the academia,

industry and other related organizations:

1. Encouragement of efficient work flow, Creation of "Chairs" in the name of renowned academicians and industrialists, Provision of incentives establishing center of excellence in collaboration, Reformation of students' internships / training / projects / master seminars / RAWE / Student-ready, etc.
2. Setting up interface structures R&D incentives and grants for collaborative projects.
3. Performance-based funding of universities and reward systems for researchers.
4. Education, training, skill enhancement guest lectures, expert talks, training, discourses etc. by industry veterans.
5. Establishing in-house training centers for sharpening skill sets of industry personnel and other related organizations by offering customized skill development initiatives. Effective revenue sharing mechanisms for the concerned ones involved in training[2].

Key Aspects of Industry-Academia Collaboration

Knowledge Transfer: Sharing of research findings, expertise, and best practices between academia and industry.

Skill Development: Providing students and researchers with opportunities to gain practical experience and industry-relevant skills.

Innovation and Research: Driving innovation through collaborative research projects, leading to new technologies, products, and services.

Resource Sharing: Pooling resources (funding, equipment, expertise) to support research and development activities.

Commercialization of Research: Facilitating the translation of academic research into commercially viable products and services.

Talent Pipeline: Creating a pathway for students to transition into the workforce, addressing the skills gap in various industries.

Examples of Industry-Academia Collaboration

Industry-driven projects: Industries partner with universities to solve specific problems or develop new technologies e.g. a pharmaceutical company might collaborate with a university research lab to develop a new drug. Joint research projects: Both industry and academia collaborate on research projects of mutual interest, sharing knowledge and resources.

Partnerships for infrastructure: Collaborations focused on building and maintaining research infrastructure e.g. research labs, specialized equipment. Internships and placements: Providing students with opportunities to gain practical experience in industry settings. Consulting and advisory roles: Industry experts providing guidance and advice to academic institutions on curriculum development and research priorities.

Technology transfer: Licensing or commercializing academic research outputs to industry. Various permutation and combinations of collaboration can be had between and among the academia (Government or private), industries, government, government agencies etc.

Industry-Academia Collaborations in Veterinary/Science/Pathology

This involves partnerships between universities and research institutions with companies in the animal health industry. These collaborations aim to leverage expertise from both sectors to advance research, education, and ultimately, animal health. Such collaborations can take various forms as detailed above paragraphs.

Key Aspects of Industry-Academia Collaboration in Veterinary Pathology

Research: Joint research projects are a cornerstone of these collaborations, allowing researchers to pool resources and expertise to tackle complex problems in veterinary pathology e.g. a pharmaceutical company might collaborate with a university to study the efficacy of a new drug in treating a specific animal disease, or a diagnostics company might partner with a university to develop new diagnostic tools.

Education: Industry-academia partnerships also play a crucial role in training the next generation of veterinary pathologists. These collaborations can provide students with valuable hands-on experience in industry settings, access to specialized equipment and technologies, and mentorship opportunities with experts in the field.

Knowledge transfer: These collaborations facilitate the transfer of knowledge and technologies from academia to industry, and vice versa. This can lead to the development of new products, services, and improved practices in animal health.

Funding: Industry funding can provide a significant boost to research and training programs in veterinary pathology. This can be in the form of grants, sponsored research agreements, or donations.

Types of Partnering Relationship between Academia and Industry

i. *High intensity of partnering:* Inter-organizational research partnership-collaborative R&D projects; Research services-Commissioned by industries like contract research, consulting, quality control, testing, certification and prototype development. Shared

infrastructure- Use of university labs and equipment by companies, business incubators and technology parks located within universities.

ii. *Medium intensity of partnering:* Academic entrepreneurship-Commercialization of technologies developed by academic inventors own (spin-off companies), student start-ups through incubation centres supported by industries. Human resource training and transfer-At universities, internship / training/ visits for students in industries and research staff to industry and appointing adjunct faculty from industry.

iii. *Low intensity of partnering:* Commercialization of intellectual property-Transfer of university generated IP/Patents to industries via licensing. Scientific publications- Use of codified scientific knowledge within industry. Informal interactions-Formation of social relationships e.g. conferences, meetings, social networks, farmer exhibitions[2].

The TANUVAS Experience – Linkages vs Convergence[1]

Translational Research Platform for Veterinary Biologicals (TRPVB), a vision of the DBT, GoI, was formed in partnership with one of the premier Animal Science Universities of the country, TANUVAS with an ultimate aim of converting science into commercializing technologies in animal sciences by creating an inclusive ecosystem involving academia, industry, biosafety personnel and regulatory agencies etc., to have all the requirements needed to traverse the path of idea to market under a single roof. This is a 'convergent' strategy Vs a 'linkage' interconnect. This would enable conversion of leads / vaccine candidates / kits in to prototypes for industry and thereby foster 'productization' in the field of veterinary vaccines and diagnostics. TRPVB was functioning at TANUVAS since 2012. In its 14th year of existence, TRPVB has fostered collaborations with various industries and has commercialized and produced >30 products and also commercialized most of them and offered biotechnology services including unique cell culture facilities. A sizeable number of these technologies have reached the market and are presently used by various stakeholders. TRPVB has obtained various BIRAC, CRS and SBIRI grants for joint product development with the industries. It holds the Schedule-M Current Good Manufacturing Practices certificate for its manufacturing unit with required state-of-the-art infrastructure facilities for formulating and manufacturing external veterinary products such as ointments, creams, gel, spray, shampoos. TRPVB supplies many of these products to the Department of Animal Husbandry, Government of Tamil Nadu for use by the veterinarians. Veterinary Incubation Foundation @ TANUVAS, to nurture budding entrepreneurs, has been established as a not for profit company with funding support from the Tamil Nadu Manufacturing Business Incubation Infrastructure Development Project of the Entrepreneurship Development & Innovation Institute of Tamil Nadu (EDII -TN)[1].

Benefits of Industry-Academia Collaboration

Industry-academia interaction is one of the key determinants of a nation's competitiveness. The most prosperous and technologically advanced nations, which have achieved innovation-led growth, are those where industry-academia interaction is the strongest. Globally, we see this cycle: Money is invested into research, generating new knowledge, and then the industry innovates to convert that knowledge into economic value, taxes, and further investment in research. Academia can generate ideas, but to make an impact on society, the industry must play a significant role. Secondly, academia strives for excellence, while industry focuses on relevance, economic gain and societal impact. Competition drives this. When we talk about the economics of knowledge, the generation, production, consumption, and conversion of knowledge are all equally crucial.

Two track systems: i. Public research-CSIR, ICAR, ICMR ii. Higher education institutions and universities. Sometimes these two tracks align and collaborate effectively, but at other times, they operate in silos, limiting the collective impact of academic R&D. Think of opportunities, challenges and ways to collaborate between them and interact with industry. Overall, the framework is semi-quantitative, combining both quantitative and qualitative elements, making it rigorous yet practical. This framework helps build trust, which is crucial in India. Talent and technology are abundant, but trust is the biggest challenge. By incorporating frameworks like this into our policies, monitoring systems, and evaluations, we can start creating trust, which ultimately fosters innovation and progress[17]. For academia: Access to funding, real-world problems for research, practical training for students, and opportunities for commercialization of research. For industry: Access to cutting-edge research, a pipeline of skilled graduates, innovative solutions to problems, and potential for new product development.

For society: Economic growth, technological advancements, and solutions to societal challenges.

Benefits of Industry-Academia Collaboration in Veterinary Science/Pathologists

Veterinary pathologists actively engaged in research are well trained. Industry-academia collaboration involves partnerships between veterinary institutions and commercial entities, enhancing veterinary education with curriculum development incorporating industry-relevant knowledge and practical skills. Graduates are better prepared for their careers, gain practical experience and knowledge in real-world applications.

Industry partners offer internships, mentorship and job opportunities and providing students with practical experience and career guidance. Joint research projects and knowledge exchange foster innovation and facilitate the transfer of new technologies from the academic setting to the commercial market leading to development of new/ improved products, technologies and practices in animal health field covering areas such as disease diagnosis, treatment and prevention. Thus, industry-academia partnerships bridge the gap between theoretical knowledge and practical application with economic growth and job opportunity. Industries benefit from accessing specialized knowledge, research findings and training programs from academic institutions to address specific challenges and improve their operations. American College of Veterinary Pathologists (ACVP) promotes collaboration between veterinary pathologists and industry which outline the scope of work, funding and IPRs.

Challenges

It's important to manage potential conflicts of interest that may arise from industry-academia partnerships. Intellectual property: Establishing clear guidelines for intellectual property rights is crucial for successful collaborations. Communication and coordination: Effective communication and coordination between academic and industry partners are essential for successful collaborations.

Industry-Academia Collaboration Successes

India: Since its launch in August 2022, the Manthan platform has successfully delivered over 350 projects and sourced over INR 8277 Cr for research and innovation, addressing 780 problem statements and supporting 285 R&D projects, 138 early stage innovations, and 66 market-ready challenges, awarding 2354 scholarships, benefitting 794 startups and 246 academic institutions. Notable collaborations: InDx COVID-19 and Dengue Diagnostics-Rockefeller Foundation; ChemAmaze edtech-Pune STI cluster by BASF Germany; by Rockefeller Foundation; Vaccine Immunology Studies and Analysis of Immune Protection by Hindustan Unilever; and Clinical Research Platform for Rare Infectious Diseases by HUDCO with National Centre for Biological Sciences. Manthan gathered global recognition wherein foreign universities like Cornell and Ohio expressed their interest[10].

Technologies developed/commercialized in India: e.g. Animal health technologies/products developed. ICAR-IVRI: DIVA LSD vaccine, PPR vaccine, negative marker, thermostable FMD vaccines, canine distemper vaccine technology and alternative model for QC testing of vaccines; TANUVAS: Pentavalent blue tongue vaccine, RD vaccine, multiplex PCR diagnosis of MD, LL and ALV and *Babesia spp*., *T. evansi* and *E. canis* in dogs, quick heal, ABT choice, Bovine TB Alert kit, Nano ND vaccine, CPV VLP/2B vaccine, LSD virus strain, antibody detection ELISA kit for *Babesia gibsoni;* SVVU, Tirupathi: Blue tongue and foot rot vaccines, molecular diagnosis for malignant catarrhal fever, FAT for ante-mortem diagnosis for bovine sarcocystosis and screening of meat.

By other nations: The Silicon Valley, USA epitomizes how strategic partnerships between universities and industry can not only meet market demands but can also position a country as a technology leader. Research conducted by Germany's Fraunhofer Institutes indicates that their targeted research collaboration model between industry and academia yielding 18 times return on investments through improved regional economies, enhanced workforce capabilities, and higher firm and government revenues[3].

ICAR initiatives on Industry-Academia collaborations[18-21]

The Indian Council of Agricultural Research (ICAR) - Indian Veterinary Research Institute (IVRI) organized series of industry-academia interface meets to foster collaboration and discuss research and product development opportunities. In the ICAR-IVRI "Industry-Academia 1st Interface Meet, the major decisions made were, MoU between institutions and industries to formalize partnerships, facilitate knowledge exchange, and collaborate on specific projects; Establishing dedicated Industry Liaison Cells or Corporate Relations Offices within veterinary colleges; Joint research projects: Academia and industry can collaborate on research projects that address specific veterinary challenges, such as disease outbreaks, drug development, or animal welfare i.e., is crucial for advancing the field, improving animal health, and ensuring a skilled and knowledgeable veterinary workforce. The 2nd ICAR-IVRI Industry-Academia Interface meet insisted use of IVRI animal health products such as negative marker, thermostable FMD vaccines, canine distemper vaccine technology and alternative model for QC testing of vaccines by the industry. As vaccination not only protect animal populations, but also safeguard public and environmental health[22]. Recently, Agrinnovate India Ltd, an ICAR commercial wing, facilitated successful transfer of three Foot and Mouth Disease (FMD) vaccines developed by ICAR-National Institute of FMD, Bhubaneswar to the industry (January, 2026).

In the ICAR-IVRI organized 3rd Industry-Academia Interface meet academia urged industry participation in CSR-funded initiatives aligned with ICAR guidelines, emphasizing on the mutual benefits of supporting teaching and research infrastructure development, and extension and social welfare activities, dealt on significance of the events for all stakeholders and the contemporary challenges confronting the livestock sector and the delegates to collaborate effectively for

the collective betterment of livestock farmers across India. ICAR-NMRI National Workshop and Industry-Academia Interface meet **d**iscussed on key findings from ICAR-NMRI's collaboration with Sealed Air Packaging, Mumbai, ICAR's crucial role in ensuring food security and safety and importance of standards and best practices for maintaining global competitiveness are stressed and consensus in harmonizing Indian meat and poultry standards with internationally recognized benchmarks to strengthen the industry and enhance its global competitiveness.

Role of Veterinary Pathologists in Industry-Academia Set UP

In the field of veterinary pathology, this is further driven by the fact that traditional funding sources cannot keep pace with the innovation needed in digital pathology and artificial intelligence. The role of pathologists during different phases of an Academia Industry Projects[23] and the top three goals are "feasibility, verification and validation".

Man Power and Training Needs

Veterinary pathologists traditionally have been actively engaged in research as principal investigators and as collaborators. Pathologists frequently obtain advanced training in research; however, it appears that in the last 10 years there has been a reversal of a previous trend toward increasing numbers of pathologists obtaining PhD degrees. This has arisen despite an established shortage of veterinarians engaged in research[24]. Employability in the veterinary context is a set of personal and professional capabilities that enable a veterinarian to gain employment, contribute meaningfully to the profession, and develop a career pathway that achieves satisfaction and success. The congruence of stakeholder responses was notable, regardless of age and geographical location, with minor differences noted in academics and para-veterinary staff responses, and gender. The most important capabilities were honesty, ethical behaviour, communicating effectively and collaboratively with clients, knowing when to ask for help, and the willingness to learn. The categories of communication and teamwork ranked the highest[25].

The man power shortage in veterinary profession including pathology have been highlighted for about two decades[24,26-28]. The various factors attributed are globalization, urbanization, changes in agriculture, pandemics of zoonotic diseases, changing demographics, an altered economic landscape, new and continually advancing information technologies, the pending retirement of a broad swath of veterinary medical professors, and other outside pressures are all affecting veterinary medical education. The recommended core competencies for all veterinary medical students to achieve by graduation include: i. multispecies knowledge plus clinical competence in one or more species or disciplines; ii. "One Health" competency related to the intersection of animal, human, and environmental health and iii. The development of professional competencies which include communication, collaboration, management, lifelong learning related to scholarship and research, diversity and multicultural awareness, and the ability to adapt to changing environments[28].

Benefits of Research Training for the Veterinary Pathologists

Widest spectrum of job opportunities and potential for crafting responsibilities to satisfy personal career interests: The 2008 ACVP Demographic Survey indicated that more than one-third of employers considered research training an important requirement, whereas only 24% considered such training unimportant. There is a high demand for veterinary pathologists with research training to evaluate safety and efficacy of new tests and treatments in animal models, including bringing these advancements into human veterinary and human medical practice in the form of clinical trials. Most veterinary pathologists have acquired both pathology (anatomical and clinical) and experimental research training in their backgrounds which greatly influenced the accomplishments and stature of the discipline. Research training provides a unique opportunity for developing critical thinking and problem-solving skills, learning laboratory methods and experimental design in the context of hypothesis driven experimentation, enhancing knowledge of disease and normal biology, evaluating safety and efficacy of new pharmaceuticals, designing new biomedical devices, diagnostic tests, and instruments, contributing to drug discovery and developing and evaluating new teaching strategies and enables veterinary pathologists to be more competitive for most professional positions, enriches their work experiences regardless of the ultimate career pathway and will continue to enhance our ability to interact with the larger biomedical community. To maintain our role in advancing biology and medicine and remain relevant in the world of global health with animals as potential sources of zoonotic disease or novel infectious agents and the reliance on information from animal studies for major medical advances, comprehensively trained veterinary pathologists will continue to be critical to the design and interpretation of research studies. Some pathologists will focus their careers on research as principal investigators, but many pathologists will continue to seek a career that includes both research and diagnostic pathology. Trainees are encouraged to consider the personal and professional value of research training as being equal to or greater than the necessary investment both for their own careers and for veterinary medicine and global health. Mentors and training institutions should engage in continual reflection on mechanisms for optimization

of educational programs[24].

Coalition of ACVP and Society of Toxicologic Pathology established resulted in 13 new training positions for veterinary pathologists with the financial support of the biopharmaceutical industry and dissolved[29,30]. The Royal College of Pathologists involved in board certification with the support of Veterinary Pathology Specialty Advisory Committee.

Biomedical Devices/Research

For veterinary pathologists to continue to make critical contributions to biomedical research and collaborative efforts such as the One Health Initiative, it will be important for the discipline to increase the number of pathologists who have the knowledge and skills to function as primary investigators as well as collaborative researchers[24]. Because the focus is on the development of the fundamental technology in medical devices, little effort is placed on product performance, reliability, manufacturability, robustness, or the cost of an eventual commercial device. Often, these devices are little more than an alpha prototype developed to verify a hypothesis. Thus, a large gap exists between the discovery of a new medical technology and a commercial device available for public use. These include low-cost products for the developing world and custom medical devices for people with disabilities e.g. Eyeglasses for vision correction that use water-filled lenses that are inflated to change optical properties and a prosthetic arm for a limbless child[31].

Toxicological Studies

Different toxicological experiments are briefly dealt

i. *Discovery toxicology:* Evaluating the safety of potential drug candidates throughout the drug development process. Early screening and lead optimization: To identify compounds with unacceptable toxicity profiles, allowing researchers to focus on more promising candidates. Studies may involve *in vitro* assays (using cell lines) and *in vivo* animal studies to assess genotoxicity (potential to damage DNA), cytotoxicity (cell toxicity), and other potential adverse effects; to identify early warning signs, like target organ toxicity, specific toxicities, and maximum tolerated doses (MTD), to eliminate unsuitable compounds early

ii. *Preclinical toxicology / Exploratory toxicology / Non-GLP toxicology:* Studies on selected candidate drug-toxicity profile, dose-response relationship, target organs, and potential mechanisms of toxicity and on its behaviour in different systems-Absorption, Distribution, Metabolism, and Excretion (ADME), vital for designing safer clinical trials and identifying parameters to monitor for potential side effects

iii. *Clinical trial support:* Toxicology studies provide crucial information for designing and conducting clinical trials for safe starting doses for human trials and determine appropriate monitoring parameters risk assessment and to decide on continuing or discontinuing studies

iv. *Post-Market Surveillance:* Toxicology plays a role even after a drug is approved and marketed, with ongoing monitoring for adverse drug reactions (ADRs). This helps identify rare or long-term toxicities that may not have been apparent during preclinical or clinical testing

v. *Safety Pharmacology:* Studies that assess the effects of a drug on vital organ systems e.g. cardiovascular, respiratory, central nervous systems.

vi. *Genetic toxicology:* Studies that evaluate the potential of a drug to cause mutations or damage DNA

vii. *Organ toxicity:* Studies that identify which organs are most susceptible to the drug's toxic effects. ADME on drugs

viii. *Reproductive and developmental toxicity:* Studies that assess the potential of a drug to affect fertility, pregnancy, or fetal development. Carcinogenicity: Studies that assess the potential of a drug to cause cancer

ix. *GLP toxicology / regulatory toxicology:* Crucial role in drug discovery and development by ensuring the safety of potential drug candidates before they are used in humans by rigorous testing to assess the adverse effects of drugs in animals

x. *Safety assessment:* The primary goal of regulatory toxicology is to assess the safety of drug candidates through various studies, including *in vitro*, *in vivo*, and in silico methods

xi. *Non-clinical studies:* Before clinical trials: Genotoxicity, general toxicity, and safety pharmacology, are conducted to support the safety of the drug candidate.

xii. *Specific toxicity studies:* Regulatory toxicology - Assess different types of toxicity, including carcinogenicity, reproductive toxicity, and drug-drug interactions. Regulatory guidelines for animal experimentation and drug development for human use in pharmaceutical industry-Safety. Regulatory agencies are many: Fulfil according to the conditions of the country e.g. USA: FDA- Food and Drug Administration; *The Federal Food, Drug, and Cosmetic Act*: Basic food and drug law, most extensive law of its kind in the world. *Code of Federal Regulations* **(CFR) for Investigational New Drugs (INDs), New Drug Applications (NDAs), Abbreviated New Drug Applications (ANDAs) and Biologics License**

Applications (BLAs); The International Council for Harmonization (ICH-1990): Technical Requirements for regulatory body - increasingly global face of drug development. OECD (Organization for Economic Co-operation and Development) Guidelines for the Testing of Chemicals (OECD TG)-Internationally accepted specifications for the testing of chemicals. The European Medical Agency's Committee; India: Central Drugs Standard Control Organization (CDSCO); Good Clinical Practice-Guidelines for biomedical studies[32].

Lessons from an academician's experience in industry[33]

A Medical Pharmacologist after 30 years of academic service entered industry discusses her experiences. Leadership means how am I doing than others? Humility is facing the failures. By resilience (learning from failures) achieved success and developed 20 new products. Selecting a company by you or the company? Career advancement in industry is by active contribution and experience but may seem initially less intellectually stimulating than an independent faculty position. Academic faculty is primarily evaluated by their individual contributions while in industry it is part of success of a team. If the project the team fails, the performance evaluation and compensation will be affected. Academia is salaried class as per rank. In industry workers have a base salary, a bonus and incentives. A misperception is that industry science is inferior to that in academia. But actually the opposite is true. All the works are rigorously monitored, reviewed and evaluated internally and externally by regulatory authorities. Industry teams are under constant pressure to deliver and adhere to timelines. Hence, proceed with caution in choosing a career in the pharmaceutical industry, understand the job opportunity and discuss with members of the company[33].

The Way Forward

1. Successful industry-academia collaboration needs to cater to the interests and needs of both the partners with mutual benefits and also respect each other's role and contributions.
2. The academics should be incentivized to promote their research appetite, greatly promoting industry oriented research and directly linked to their career development.
3. Universities should encourage academics to create 'spin offs' and should allow them to act as 'Directors' for such spin off companies.
4. The industry scientists can be incentivized by means of monetary benefits, can also be given adjunct/ honorary faculty positions at universities.
5. University administrative regulations governing the industry collaborative research to be made flexible to accommodate to promote and nurture such collaborations.
6. Industries can set up specific funding for university collaborative research, by making policy decisions that gives tax rebate (or exemptions) for such funds.
7. Partnerships with industries should be long-term and multifaceted with increased faculty/ scientist exchange between the partners for training.
8. Universities/ industries can provide mutual consultancy services with their technical / manufacturing/ regulatory expertise.
9. Create adequate awareness on Regulation, Translational Research, Biosafety, GMPs etc., for the students to feel comfortable with entrepreneurship[1].

The clinical practice undertaken by the practicing veterinarians is also a form of entrepreneurship and is an expertise-driven business model. VETPRENEURSHIP is hopefully going to be the buzzword for the veterinary research institutions in the near future, ensuring that the research outcomes of these institutions are taken to the users and thereby the existence of these institutions are not questioned[1]. Higher education system is a powerful tool for social, political and economic change, which will have greater pressures because of demands for providing training ground for skilled manpower to meet the needs of industry or for self-employment. With very promising initiatives like "Skill India", "Start-up India", India has started taking big steps and all stakeholders to build the "New India" need to be brought together to achieve grand success in this direction[2].

Best-practice recommendations for program infrastructure, mentorship, time management, and a team approach to research and research training are advocated to facilitate the development of successful programs and to encourage a continued emphasis on integrated training for pathologists as both clinical diagnosticians and experimentalists[24]. Such training programmes are offered by organizations like, ACVP, ECVP and Royal College of Pathologists, Japanese College of Veterinary Pathologists and in India by Indian College of Veterinary Pathologists (ICVP), the only board certifying body in veterinary science in India giving diplomat to the successful candidates after examination since 2008. As per the Global Innovation Index USA stands 2nd, China 6th and India 66th. Hence, India has a long way ahead and make us to be prepared for achieving the heights.

Indian livestock sector growth

Projected 2025-2030 livestock sector contribution to CAGR for value addition is around 9.5%; animal health market: 13.9%; cattle management software: 14.8%; poultry sector: over 8%; animal feed market: 6.8%;

livestock population: overall 4% with specific growth in sheep and goat at an 8%; poultry population 12% (BAHS, 2025). Being first in world milk production, Indian dairy industry contributes around 4.5% to the national GDP accounting for over one-fourth of the agricultural GDP and growing at the rate of 10% per annum. Livestock sector GVA is expected to increase by an additional Rs. 2.5 trillion by 2030[34-37]. Indian edible meat market will reach over $16 billion by 2030 (MoFPI). These developments will lead to industry-academia collaboration with disease challenges.

Government of India Initiatives

Animal Husbandry and Dairying Dept:

i. Rashtriya Gokul Mission

ii. National Programme for Dairy Development

iii. National Livestock Mission

iv. Animal Husbandry Infrastructure Development Fund;

v. Livestock Health and Disease Control Programme v. Expanding the network of Colleges for Veterinary Education- 39 in 2014 to 79 in 2024

vi. Livestock Census & Integrated Sample Survey Scheme

vii. Kisan Credit Cards for Dairy Farmers of Milk Cooperatives and Milk Producer Companies and through other fora. These schemes are supporting and encouraging quality veterinary education, animal husbandry services, cooperatives, farmer producer organizations, livestock farming to increase the animal production, quality feed, entrepreneurship, employment, women empowerment, quality livestock products, efficient use of animal resources, disease protection etc., for socioeconomic development of people and food and nutritional security, and safety (Livestock Production Statistics of India, 2024).

CONCLUSIVE REMARKS

Collaborations among industry, academic and government organizations would result in better outcomes than work done independently in isolation. Collaborations can benefit industry by enabling them to hire a subject matter specialist knowledgeable on a given topic. Where, academics have the most flexibility and can pursue projects of interest, apply for suitable funding and are obliged to teach, serve the university and conduct research. Projects that could be completed in months in industry can take years to complete in an academic environment. Eliminating the normal commercial requirements for performance, quality, reliability and profitability enables these projects to be completed by students and faculty with little or no industrial experience. While industry is the most focused group and must identify opportunities that meet a public need for a specific product or service, generate sufficient profit to sustain the business and maintain their fiduciary responsibility to shareholders and ethical responsibilities to customers and regulatory agencies. Investment are assessed based on the balance of these considerations. The government is a large comprehensive entity with multiple responsibilities and minimal overlap[31].

Such strong growth is driven by increasing demand for animal-based products (milk, eggs, and meat), government initiatives and the adoption of modern technologies (like sex-sorted semen, IVF, genomics) in feeding and health management (BAHS, 2025) which will be hampered by the occurrence of diseases, wherein Veterinary Pathologists have a crucial role to play in imparting quality education, inculcate research aptitude, involving in diagnosis and control of animal and poultry diseases. Besides, the scenario of entry of organized sectors and the GoI initiatives to achieve various goals including FMD/PPR freedom by 2027, improving milk yield by 8% which will uplift the national economy and provide livelihood to the people and by supporting industry-academia-Government collaborations[34].

Let the HEIs in Agriculture and allied sciences take the lead and serve as the 'torch bearers' by fostering such 'education revolution' through integrated approach for building, strengthening and sustaining partnership between academia, industry and other related organizations[2]

"Sabka Saath, Sabka Vikas, Sabka Vishwas, Sabka Prayas (Everyone's support, everyone's development, everyone's faith, everyone's effort) through industry-academia partnerships that will continue to drive the nation towards realizing its highest aspirations in science, technology, and economic prosperity". Vision of Shri Narendra Modi, Hon'ble Prime Minister of India

ACKNOWLEDGEMENT

I sincerely thank Dr. G. Dhinakar Raj, Retired Professor and Head, Dept. Animal Biotechnology, Madras Veterinary College, Chennai, for the partly help rendered.

Conflict of interest: There is no conflict of interest.

Financial assistance: There is no financial support.

AI: The article is not AI based preparation.

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Indian J. Vet. Pathol., 50(1) : 15-23, 2026: DOI: https://doi.org/10.56093/446a2f69

RESEARCH ARTICLE

Clinico-pathological studies on Theileriosis in cattle

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Received:30.8.25; Accepted: 19.11.25

ABSTRACT

The present study was conducted to study the clinical profile, hemato-biochemical, post mortem and histopathological changes along with molecular detection of theileriosis in cattle. Out of 290 suspected cases, 40 (13.79%) cattle were found to be positive for theileriosis. Age-wise prevalence was higher in animals above 12 months (65%) of age. Breed-wise and sex-wise prevalence was highest (45%) in Holstein Friesian females. The important clinical signs observed in theileriosis affected cattle were fever, pale, papery white or icteric mucous membranes, swollen lymph nodes and tick infestation. Hemato-biochemical changes were marked anaemia, thrombocytopenia, elevated levels of serum hepatic and renal biochemical parameters in affected animals. Blood smear examination of affected cattle revealed the presence of pleomorphic intra-erythrocytic piroplasm stages of *Theileria annulata* and *Theileria orientalis* including mixed infection (*T. annulata* + *T. orientalis*) in few cases. These cases were also confirmed by PCR. The characteristic post-mortem lesions were pale, icteric or papery white mucous membranes, swollen oedematous or haemorrhagic lymph nodes, splenomegaly, punched out ulcers in abomasum and hepatomegaly with icteric discoloration. Histopathology revealed multiple haemorrhagic ulcerations and necrosis of mucosal epithelium of abomasum, lymphoid depletion in lymph nodes and hepatocellular degeneration. Overall percent positivity of theileriosis was 13.79 % among cattle population in and around of Udgir city of Maharashtra involving either alone one pathogen or mixed infections of *Theileria annulata* and *Theileria orientalis*.

Key Words: Cattle, molecular detection, pathological lesions, Theileriosis, *Theileria annulata, Theileria orientalis*

INTRODUCTION

Tick-borne haemoprotozoan diseases poses a significant risk to the health and management of domesticated cattle in tropical and subtropical climates[1]. Due to tropical environment, India's climate is particularly conducive to the survival and growth of tick vectors, which facilitates the transmission of haemoparasitic diseases such as theileriosis, babesiosis and anaplasmosis[2].

Haemoprotozoan diseases, particularly babesiosis, tropical theileriosis, trypanosomiasis and anaplasmosis are considered significant barriers to the health and productivity of cattle[3]. Among all tick-borne diseases (TBD), theileriosis is one of the most economically detrimental illnesses affecting livestock globally. It is estimated that around 250 million cattle across the globe are susceptible to theileriosis[4].

Several tools are available for the diagnosis of theileriosis in cattle, which includes blood or lymph node biopsy smear examination, post-mortem findings, polymerase chain reaction (PCR) and serological evaluation. Although traditional diagnostic procedures lack sensitivity and specificity, they are still widely used and valuable aids for clinical and epidemiological investigations[5]. Among these methods, the PCR assay was found to be more sensitive and accurate than traditional microscopic examination because it enables the accurate detection and differentiation of various *Theileria* species, even in carrier animals with very low parasitemia that might be missed by microscopy[6].

Considering the economic importance of the disease and the need to develop effective therapeutic and control strategies, it is essential to investigate the clinico-pathological alterations and employ molecular techniques for species level detection of etiology of theileriosis in cattle. Therefore, the present study was undertaken to evaluate the clinical profile, haemato-biochemical and pathological alterations, along with the molecular diagnosis of theileriosis in cattle.

How to cite this article : Bhange, R.R., Chavhan, S.G., Awandkar, S.P., Kondre, B.M., Jadhav, R.K. and Kulkarni, R.C. 2026. Clinico-pathological studies on Theileriosis in cattle. Indian J. Vet. Pathol., 50(1) : 15-23.

MATERIALS AND METHODS

Selection of Animals

The present study was conducted at the Department of Veterinary Pathology, College of Veterinary and Animal Sciences (COVAS), Udgir, Maharashtra on two hundred and ninety (n=290) cases of cattle with a

history of fever, pale or icteric mucous membranes, swollen lymph nodes and tick infestation. These cases were admitted to Teaching Veterinary Clinical Complex, COVAS, Udgir and reported from areas surrounding to Udgir during period May 2024 to March 2025. For obtaining control data of hematological, biochemical and clinical parameters, the blood samples were collected from healthy cattle (n=17) housed at the Livestock Farm Complex of the college.

Ethical Approval for Animal Studies

The present study was approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Udgir and Committee for the Control and Supervision of Experiments on Animals (CCSEA) (Permission Letter No. VCU/IAEC/CPCSEA/XI/24). The blood samples from suspected cattle were collected with the consent of the farm/animal owners in the clinical case admission form during admission of case to college clinic. All the procedures were performed as per the ethical guidelines provided by CCSEA/IAEC and Maharashtra Animal and Fishery Sciences University, Nagpur.

Blood Smear Preparation, Staining and Screening of Theileriosis

Thin blood smears were prepared from a blood collected from suspected cattle by puncturing jugular vein with 18-gauge needle. These smears were air dried, fixed with methanol and subjected to Giemsa staining as per routine protocol[7]. Positive samples were selected or diagnosed on the basis of the presence of intra-erythrocytic rod or bayonet for *Theileria orientalis*[7]and signet ring, pyriform and dot shaped for *Theileria annulata*[8].

Haematological Estimations

The blood samples for analysing various haematological and biochemical parameters were collected under the aseptic condition from the jugular vein in K_2EDTA vacutainers (K_2EDTA concentration: 3.6 mg, 2 ml capacity) and clot activator vacutainers (Clot activator, silicone coated, 4 ml capacity) respectively (Becton, Dickinson and Company, USA). The haematological parameters such as total erythrocyte count (TEC), haemoglobin concentration (Hb), packed cell volume (PCV), MCV, MCH, MCHC, platelet count, total leucocyte count (TLC), absolute granulocyte, lymphocyte and monocyte count were estimated by using fully automated haematology analyser (Make: Nihon Kohden Corporation, Japan, Model: MEK-6550K- 4 Part Vet Haematology Analyser).

Biochemical Estimations

Serum samples separated and used to estimate various biochemical parameters such as alkaline phosphatase (ALP), aspartate transaminase (AST/SGOT), alanine transaminase (ALT/SGPT), total bilirubin, direct bilirubin, indirect bilirubin, total protein, albumin, blood urea nitrogen (BUN), creatinine, calcium (Ca), phosphorus (P), magnesium (Mg), creatine kinase-MB (CK-MB), creatine kinase-NAC (CK-NAC) and Adenosine Deaminase (ADA) on semi-automated biochemical analyser (Make: Erba Mannheim GmbH, Germany, Model: Chem-7) by using standard commercial biochemical kits (Make: Erba Mannheim GmbH, Germany).

Pathological Examination

A detailed post-mortem examination of seven (n=7) cattle suspected to have died due to theileriosis was conducted, and the gross lesions observed in various organs and body systems were recorded. During the necropsy, the tissue samples measuring about 0.5 cm thickness were collected in 10% neutral buffered formalin. The formalin fixed tissue samples were processed and stained using haematoxylin and eosin staining technique for histopathology as per standard routine protocol[9].

DNA Extraction, Primer Designing and PCR Cycling Conditions

The blood samples of theileriosis suspected cattle for PCR detection were collected under aseptic condition from the jugular vein in commercial K_2EDTA vacutainers (Becton, Dickinson and Company, USA) and stored at -20°C. The genomic DNA was extracted from 250 µl of whole blood by using Phenol-Chloroform-Isoamyl alcohol method[10]. The extracted DNA samples were stored at -20°C for further analysis. The species-specific primer sets for detection of *T. annulata* {Merozoite-Piroplasm Surface Antigen gene (MPSA gene)/ *annulata* Merozoite Surface Antigen 1 gene (Tams1 gene)} and *T. orientalis* {Major Piroplasm Surface Protein gene (MPSP gene)} were designed by using National Center for Biotechnology Information (NCBI) Primer Designing Tool (NCBI Primer-BLAST). All the oligonucleotide primers were custom synthesized (Eurofins Genomics India Pvt. Ltd., Bengaluru, India). The PCR reaction mixture of 25-µl was prepared containing 12.5-µl master mix (2X) (GoTaq® Green Master Mix, Promega, USA), 1-µl of template DNA, 1-µl (10 pmol) of each primer and nuclease free water (9.50 µl). PCR reaction was performed with 25-µl reaction volume using Mastercycler Nexus Gradient 96-Well Thermal Cycler (Eppendorf, Hamburg, Germany). The primers sets and PCR cycling conditions used for detection of *T. annulata* and *T. orientalis* are shown in table 1,2 and 3.

The PCR products were subjected to agarose gel electrophoresis (2%) with 0.5 µg of ethidium bromide/ml of agarose and visualized by using BIO-PRINT CX4 EDGE Gel Documentation System (Vilber Lourmat, France).

Table 1. Primer Sets used for PCR detection of *T. annulata* and *T. orientalis.*

Sr.	Organism	Gene	Primer Sequence	Amplicon Size	Reference
1.	*T. annulata* (Forward)	MPSA (Tams1) Gene	CCTTTGATACTCGCGACCCT	674 bp	Designed
2.	*T. annulata* (Reverse)	MPSA (Tams1) Gene	GACGATGAGTACTGAGGCGA		
3.	*T. orientalis* (Forward)	MPSP Gene	TCCTCATCGTCTCTGCAACT	826 bp	Designed
4.	*T. orientalis* (Reverse)	MPSP Gene	TGTGAGACTCAATGCGCCTA		

Table 2. PCR Cycling conditions for amplification of MPSA(Tams1) gene of *T. annulata.*

Sr. No.	Condition	Temperature	Time	No. of cycles
1	Initial Denaturation	95 °C	5 min	--
2	Denaturation	95 °C	30 sec	
3	Annealing	57.2 °C	30 sec	30
4	Extension	72 °C	30 sec	
5	Final Extension	72 °C	10 min	--
6	Hold	4 °C	∞	--

Table 3. PCR Cycling conditions for amplification of MPSP gene of *T. orientalis.*

Sr. No.	Condition	Temperature	Time	No. of cycles
1	Initial denaturation	95 °C	5 min	--
2	Denaturation	94 °C	45 sec	
3	Annealing	55 °C	30 sec	35
4	Extension	72 °C	1 min	
5	Final extension	72 °C	5 min	--
6	Hold	4 °C	∞	--

Statistical Analysis

The data generated from different parameters was subjected to independent samples t-test (at the level of P≤0.01 or P≤0.05) by using IBM SPSS software (version 20) for windows.

RESULTS

Out of two hundred and ninety (n=290) suspected cases, forty (n=40) cattle were found positive for theileriosis by blood smear examination indicating an overall 13.79% prevalence. Among forty (n=40) cases, twenty-eight (n=28, 70%) cases infected with *annulata*, eight (n=8, 20%) with *orientalis* and four (n=4, 10%) had mixed infection (*annulata* + *orientalis*).

Blood smear examination of cattle infected with *orientalis* revealed intra-erythrocytic rod- or bayonet-shaped piroplasms (Fig. 1). In contrast, cattle positive for *annulata* showed numerous intra-erythrocytic piroplasms, predominantly signet ring, pyriform, and dot-shaped forms. Moreover, in cases of mixed infection, intra-erythrocytic piroplasms characteristic of both *orientalis* and *annulata* were observed (Fig. 2).

Out of forty blood samples (n=40) positive for theileriosis by smear examination, randomly seven representative samples (n=7) were subjected for molecular diagnosis at species level by PCR. PCR detected two samples (n=2) as positive for *annulata*, one as *orientalis*, and four samples (n=4) for mixed infection with *annulata* and *orientalis* (Fig. 3, 4).

The age wise prevalence of theileriosis in cattle was higher in animals aged above 12 months (n=26, 65%) followed by age group of 3-12 months (n=8, 20%) and lowest prevalence was recorded in age group below 3 months (n=6, 15%). The sex-wise prevalence of theileriosis was higher in females (n=24, 60%) as compared to males (n=16, 40%).

Breed-wise prevalence of theileriosis was found highest in Holstein Friesian breed (n=18, 45%) followed by Deoni (n=10, 25%), Red Kandhari (n=5, 12.5%), non-descript (n=3, 7.5%), Gir (n=2, 5%), Khillar and Jersy (n=1, 2.5%) in present study.

The theileriosis affected cattle showed important

clinical signs *viz;* fever, mucous membrane as pale (n=21, 52.5%), icteric (n=9, 22.5%), congested (n=8, 20%) or papery white (n=2, 5%), enlargement of superficial lymph node (n=25, 62.50%) and presence of tick infestation. However, in a few cases, clinical signs such as diarrhoea (n=7,12.50%), melena (n=2,5%), haemoglobinuria (n=2, 5%) and brisket oedema (n=4,10%) were also noticed.

Theileriosis affected cattle showed highly significant increase (P≤0.01) in body temperature (103.44±0.12 *vs* 100.90±0.15°F), heart rate (82.32±3.97 *vs* 45.76±0.38 beats/min), respiration rate (42.57±4.88 *vs* 25.76±0.34 breaths/min) and pulse rate (79.83±3.35 *vs* 46.47±0.44 pulse/min) as compared to healthy control animals.

The haematological analysis of blood samples of theileriosis affected cattle showed highly significant decrease (P ≤ 0.01) in total erythrocyte count, hemoglobin concentration, packed cell volume and MCHC, while the significant increase (P ≤ 0.05) in total leucocyte count was recorded in affected cattle as compared to control animals. Platelet counts of theileriosis affected cattle revealed highly significant decrease (P ≤ 0.01) as compared to healthy control animals. Moreover, the non-significant increased values of MCV, MCH and absolute counts of neutrophils, lymphocytes, monocytes and eosinophils were observed in affected cattle as compared to healthy control animals (Table 4).

Serum biochemical level of ALP, AST, total bilirubin, direct bilirubin, indirect bilirubin, BUN, creatinine, CK-MB, CK-NAC and adenosine deaminase (ADA) were significantly elevated in theileriosis affected cattle while the serum levels of total protein, albumin, calcium and phosphorus showed significant decrease in affected animals. Biochemical parameters such as ALT showed non-significant increase while magnesium showed non-

Table 4. Haematological changes in theileriosis affected in cattle (Mean ± SE).

Sr.No.	Parameter	Infected (n=40)	Healthy Control (n=17)	't' value
1.	TEC (10^{12}/L)	4.92 ± 0.31	7.19 ± 0.24	3.94**
2.	Hb (g/dl)	6.84 ± 0.39	10 ± 0.41	4.34**
3.	PCV (%)	22.28 ± 1.34	31.96 ± 1.31	3.89**
4.	MCV (fL)	48.16 ± 1.48	44.61 ± 1.31	1.30NS
5.	MCH (pg)	14.42 ± 0.37	13.97 ± 0.42	0.64NS
6.	MCHC (%)	29.49 ± 0.26	31.31 ± 0.19	3.79**
7.	TLC (x10^9/L)	13.33 ± 1.20	9.28 ± 0.62	2.09*
8.	Lymphocytes (x10^9/L)	5.29 ± 0.65	5.02 ± 0.42	0.22 NS
9.	Monocytes (x10^9/L)	1.28 ± 0.32	0.36 ± 0.10	1.73 NS
10.	Eosinophils (x10^9/L)	0.83 ± 0.16	0.48 ± 0.06	1.53 NS
11.	Neutrophil (x10^9/L)	5.05 ± 0.67	3.55 ± 0.34	1.24 NS
12.	Platelets (x10^9/L)	163.78 ± 13.78	213.18 ± 10.69	2.93**

NS- Non-significant *Significant (P≤0.05) **Highly Significant (P≤0.01)

Table 5. Biochemical changes in theileriosis affected cattle (Mean ±SE).

Sr.No.	Parameter	Infected (n=40)	Healthy Control (n=17)	't' value
1	ALP (U/L)	273.68 ± 15.38	135.10 ± 13.57	2.98**
2	SGPT (ALT) (U/L)	49.32 ± 1.73	42.22 ± 1.94	1.37^{NS}
3	SGOT (AST) (U/L)	171.76 ± 8.02	72.04 ± 3.60	4.13**
4	Total Bilirubin (mg/dl)	3.25 ± 0.38	0.39 ± 0.02	2.53**
5	Direct Bilirubin (mg/dl)	1.57 ± 0.18	0.12 ± 0.02	2.62**
6	Indirect Bilirubin (mg/dl)	1.68 ± 0.20	0.27 ± 0.01	2.34*
7	Total Protein (g/dl)	5.09 ± 0.12	6.54 ± 0.10	4.13**
8	Albumin (g/dl)	2.40 ± 0.07	3.07 ± 0.05	3.11**
9	BUN (mg/dl)	48.49 ± 3.80	21.14 ± 0.70	2.43*
10	Creatinine (mg/dl)	2.86 ± 0.25	1.56 ± 0.07	2.06*
11	Calcium (mg/dl)	7.41 ± 0.27	10.21 ± 0.40	5.99**
12	Phosphorus (mg/dl)	4.72 ± 0.30	5.77 ± 0.11	2.50*
13	Magnesium (mg/dl)	1.94 ± 0.08	1.99 ± 0.07	0.43^{NS}
14	CK-MB (IU/L)	224.68 ± 41.19	65.16 ± 4.22	3.97**
15	CK-NAC (IU/L)	152.34 ± 33.89	43.12± 2.75	3.42**
16	ADA (IU/L)	159.85 ± 82.30	13.70 ± 0.95	2.49*

NS- Non-significant *Significant (P≤0.05) **Highly Significant (P≤0.01)

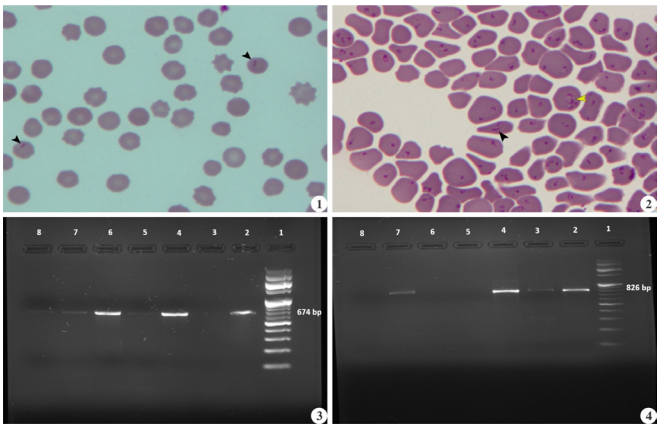

Fig.1. Blood Smear: *orientalis*: Presence of intra-erythrocytic rod or bayonet shaped *orientalis* piroplasm's (black arrowheads) (1000X, Giemsa Stain);**Fig.2.** Blood Smear: Mixed Infection: Note the presence of numerous intra-erythrocytic signet ring, pyriform and dot shaped *annulata* (yellow arrowhead) and rod or bayonet shaped *orientalis* (black arrowhead) piroplasm's (1000X, Giemsa Stain); **Fig. 3.** PCR amplification of MPSA (Tams1) gene of *annulata* from genomic DNA extracted from the blood samples of affected cattle. Lane 1: 100 bp DNA marker, Lanes 2-8: 674 bp amplicons of MPSP gene of *annulata*; **Fig.4.** PCR amplification of MPSP gene of *orientalis* from genomic DNA extracted from the blood samples of affected cattle. Lane 1: 100 bp DNA marker, Lanes 2-8: 826 bp amplicons of MPSP gene of *orientalis*.

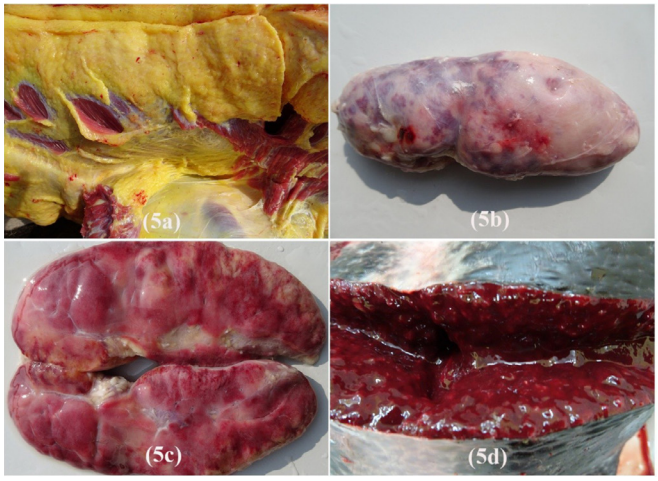

Fig. 5. Postmortem Lesions: Cattle: Theileriosis: **(5a)** Icteric discolouration of subcutaneous tissue; **(5b-5c)** Lymph node: Marked enlargement and haemorrhages in lymph nodes; **(5d)** Spleen: Semisolid or mushy appearance of splenic parenchyma.

significant decrease as compared to control animals (Table 5).

The external examination of carcasses of theileriosis affected cattle revealed presence of pale or papery white and icteric conjunctival mucous membranes, swollen lymph nodes and tick infestation. On incision, the subcutaneous tissue and fat showed marked dark yellow or icteric discolouration indicating jaundice (Fig. 5a). The affected lymph nodes appeared swollen, oedematous and

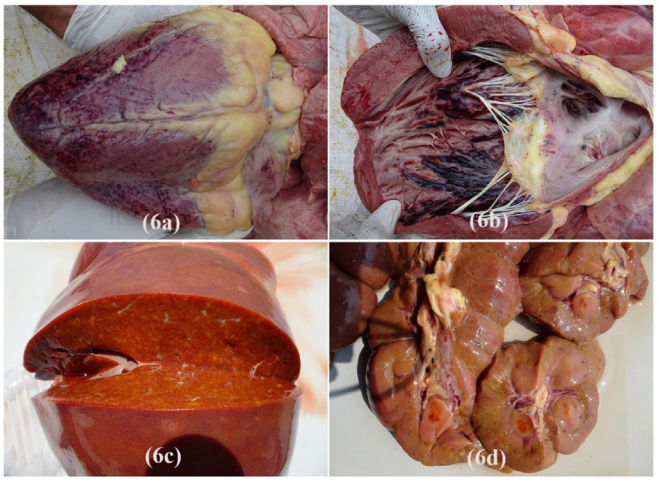

Fig. 6. Postmortem Lesions: Cattle: Theileriosis: **(6a)** Heart: Epicardial haemorrhages; **(6b)** Heart: Endocardial haemorrhages; **(6c)** Liver: Note the presence of marked icteric discolouration and hepatomegaly; **(6d)** Kidney: Note the presence of greenish yellow discoloration and presence of bile pigment aggregates in renal cortex.

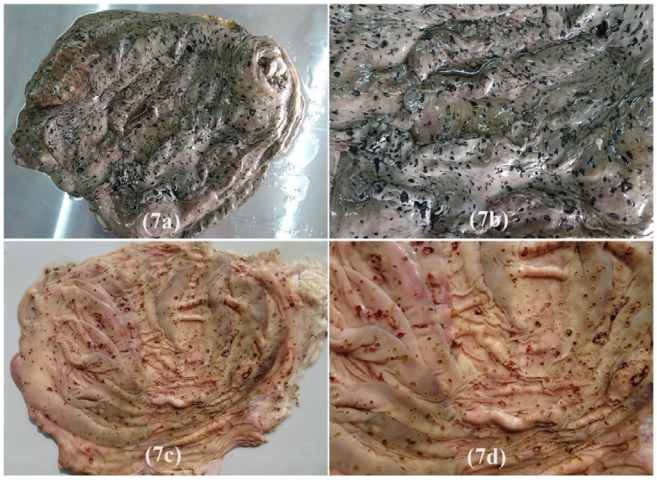

Fig. 7. Postmortem Lesions: Cattle: Theileriosis: **(a-d)** Abomasum: Note the presence of numerous punched out ulcers over the abomasal mucosa of affected cattle.

showed cortical haemorrhages intermixed with necrotic foci in few cases (Fig. 5b-5c). Marked splenomegaly was noticed in theileriosis affected cattle along with frequent presence of soft semisolid splenic pulp or parenchyma (Fig. 5d).

The gross lesions such as epicardial and endocardial haemorrhages and icteric discoloration pericardial fat were frequently noticed in affected cattle (Fig. 6a-6b). Liver of affected cattle frequently showed marked hepatomegaly along with dark icteric parenchymal discoloration indicating jaundice (Fig. 6c). The gross lesions such as yellowish green discoloration and presence of bile pigment aggregates in renal cortex were evident in kidneys of affected cattle (Fig. 6d). The gross examination of abomasum of affected cattle showed

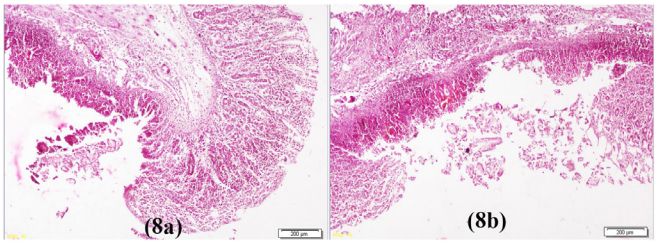

Fig. 8. Histopathological Lesions: Cattle: Theileriosis:(a-b) Abomasum: Mucosal ulcerations and presence of inflammatory response. (H & E Stain, a-b: bar=200 µm).

widespread mucosal punched out ulcers with necrotic centres and haemorrhagic borders as well as marked oedema of rugal folds (Fig. 7a-d).

Histopathological examination of abomasum -revealed multiple haemorrhagic ulcerations and marked necrosis of mucosal epithelium and heavy mononuclear cell infiltration were evident in abomasum of affected cattle. The denuded and necrosed mucosal epithelium frequently found trapped in fibrin and adhered to the native mucosal surface appearing as multiple deep eosinophilic masses. Marked lymphomononuclear cell infiltration was also frequently evident within underlying lamina propria, submucosa and around blood vessels (Fig. 8a-b).

Lymph nodes of affected cattle showed moderate lymphoid depletion, presence of fibrinoid material, cortical oedema and haemorrhages (Fig. 9a-b). In few cases, the marked proliferative changes (diffuse hyperplasia) of lymphoid follicles of cortex were noticed and cortex was replaced by sheets of lymphocytes and macrophages. Histopathological examination of spleen showed marked congestion, haemorrhages and hemosiderin deposition (presence of golden yellow to brown hemosiderin pigment scattered throughout the red and white pulp *i.e.* hemosiderosis) (Fig. 9c-d).

Marked widespread or diffuse hepatocellular vacuolar degeneration and cholestasis were evident in livers from affected cattle along with moderate infiltration of mononuclear cells within sinusoids and periportal areas. Marked tubular epithelial cells necrosis and mononuclear cell infiltration in interstitium was observed in kidneys of affected cattle. Marked mononuclear cell infiltration and myofibers degeneration in heart of affected cattle. Lungs revealed the presence of marked oedema and mononuclear cell infiltration within interstitium or alveolar septa, congestion and haemorrhages.

DISCUSSION

Effective disease control relies on accurate diagnosis. Traditionally, the microscopic examination of Giemsa-

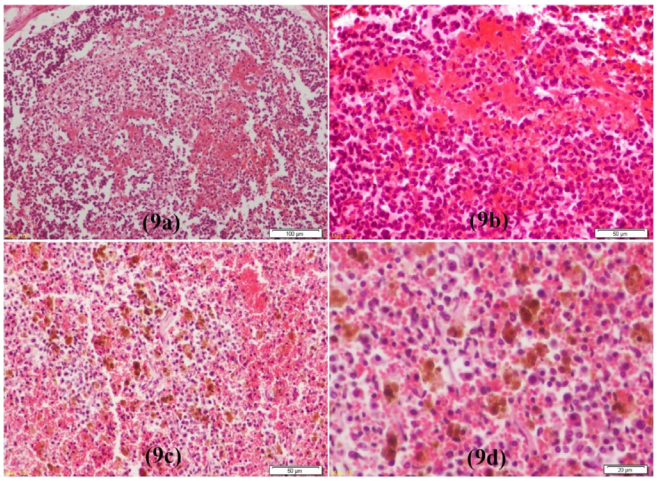

Fig. 9. Histopathological Lesions: Cattle: Theileriosis:**(9a-9b)** Lymph Node: Marked lymphoid depletion and presence of fibrinoid material and oedema; **(9c-9d)** Spleen: Presence of golden yellow to brown hemosiderin pigment scattered throughout the red and white pulp (hemosiderosis). (H & E Stain, a: bar=100 µm, b-c: bar=50 µm, d: bar=20 µm).

stained blood or lymph node smears has been the gold standard for field diagnosis of bovine theileriosis. While cost-effective and accessible, microscopy suffers from significant limitations, particularly low sensitivity in detecting carrier animals with low parasitemia and in many cases inability to reliably differentiate between morphologically similar species[6,35].

The advent of molecular diagnostic techniques, particularly Polymerase Chain Reaction (PCR) and its variants (nested PCR, real-time PCR, Loop-Mediated Isothermal Amplification), has revolutionized the detection of theileriosis. PCR assays targeting specific genes such as the Tams1 gene for T. annulata and the Major Piroplasm Surface Protein (MPSP) gene for T. orientalis offer superior sensitivity and specificity, enabling the detection of cryptic infections and the precise identification of genotypes[6,7,11,35].

Microscopic diagnostic features of *Theileria annulata* and *orientalis* which were recorded in blood smears of theileriosis affected cattle of present study were also found in consonance with earlier literature[7-8]. The findings of present study regarding molecular (PCR) detection of *annulata* and *orientalis* infection in cattle of present study were also found in consonance with studies of earlier authors[7,11-14].

The findings of the present study regarding the overall prevalence of theileriosis in cattle were largely consistent with previous reports[15]. Similar to the current study, earlier researchers also reported a higher prevalence of theileriosis in age groups older than 12 months of age[16-17]. The breed wise prevalence of theileriosis was highest in crossbred animals as compared to indigenous animals. This might be due to the stress due to high milk yield

potential, high tick infestation as compared to indigenous animals, breed resistance, genetic makeup and greater susceptibility of crossbred animals to the disease[18].

The higher prevalence of theileriosis in females than males recorded in current study also consistent with the earlier reports[19].The highest sex wise prevalence of theileriosis in female cattle may be associated to hormonal imbalances, which can lead to a compromised immune system and immunosuppression during advanced pregnancy and lactation in high yielding animals[11].

The clinical signs observed in theileriosis affected cattle of present study were also consistent with the clinical findings which were recorded in earlier studies[20-26]. The high fever in theileriosis is postulated due the liberation of endogenous pyrogens as a result of cellular lysis and high parasitaemia, which stimulates the thermoregulatory centre in the hypothalamus[20]. Furthermore, these earlier researchers[21-22] studied the expression of pro-inflammatory cytokines in cell lines experimentally infected with *annulata* and noted that the development of all the major clinical signs and pathology of acute theileriosis such as pyrexia, anaemia, anorexia, cachexia and disseminated haemorrhages, are significantly influenced by the cytokines produced by infected mononuclear cells, such as TNF-α, IL-1α, IL-1β and IL-6. Moreover, the earlier studies also stated that the numerous schizont production in lymphocytes results into the massive and uncontrolled proliferation of both specific and non-specific T lymphocytes, resulting in enlarged lymph nodes in *T. annulata* and *T. parva* infection[23-24].

The clinical signs such as presence of pale or icteric mucous membranes, icterus and haemoglobinuria as well as hematological changes in erythrocytic parameters which were observed in theileriosis affected cattle of present study may be attributed to anaemia due to haemolysis induced by merozoites of *Theileria spp*. The previous hematological investigations[25-26]on bovine theileriosis concluded that the several contributing factors including immune mediated haemolysis, excessive cytokines production and generation of reactive oxygen species as well as the removal of piroplasm infected erythrocyte by macrophages in the organs of the reticuloendothelial system contributes anaemia in theileriosis.

The other hematological alterations recorded in present study such as marked thrombocytopenia in cases of bovine theileriosis were also found in close proximity with hematological findings of previous studies[27-28]. These studies indicated that the thrombocytopenia observed in theileriosis is probably a result of increased destruction, consumption and degranulation of platelets in the peripheral blood and suppression of platelets release from the bone marrow into the blood stream by parasite and its product.

The serum biochemical changes observed in the present study were also found in consonance with findings of previous studies[29-30]. These earlier studies reported that thesignificant increase of CK-MB level in theileriosis affected cross breed cattle might be due to the severity of anaemia and parasitaemia contributing to the pathophysiology of myocardial damage. Moreover, the increased levels of adenosine deaminase (ADA) in tropical theileriosis in cattle are possibly due to involvement of cellular immune responses. Theileriosis is a progressive lymphoproliferative disease and the increase in the levels of ADA can attributed to lymphoproliferation of T-lymphocytes or mononuclear cell proliferation by schizont-infected cells, causing the increase in ADA levels in infected animals[31].

The serum biochemical alterations such as decreased levels of total protein, albumin, calcium and phosphorus in present study can be well correlated with the findings of earlier researchers stating that the decreased levels of calcium and phosphorus in bovine theileriosis can be linked to hypoproteinaemia, decreased dietary intake, gastrointestinal malfunction hepatic and renal damage[32-33].

More or less similar gross and histopathological lesions observed in present study were found in consonance with the findings of theileriosis in cattle and other animal species reported by previous researcher[7,11,21,32,34].

The earlier studies[21,34] on pathogenesis of gross and histopathological lesions in bovine theileriosis. These studies demonstrated that, the rapidly proliferating schizont infected mononuclear cells disseminates through the lymphoid tissues from the prescapular lymph node to distant lymph nodes and to the spleen and thymus. The parasitized mononuclear cells also spread rapidly into non-lymphoid organs e.g., liver, kidney, lung, abomasum, adrenal glands and pituitary gland and heart. These rapidly proliferating parasitized mononuclear phagocytes produces cytokines viz. TNF-α, IFN-γ and IL-2. These cytokines (TNF-alpha) disrupt the physiological integrity of the host; can also harm host by disrupting the regulation of the immune and endocrine systems. Furthermore, they stated that, these cytokines produced by parasitized mononuclear cells plays a major role in the development of clinical disease and lymphoid hyperplasia and tissue damage such as ulcerative lesions in theileriosis. The pathogenesis of gross and histopathological lesions observed in theileriosis affected cattle of present study can be correlated with the mechanism reported by these earlier workers.

In conclusion, the present study reports an overall

prevalence of 13.79% of theileriosis in the cattle population in and around Udgir city, Maharashtra state. Molecular techniques, particularly PCR, provide greater accuracy in identifying the specific *Theileria* species involved and are more reliable in detecting mixed infections.

Financial Support and Sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for Manuscript Preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 24-30, 2026: DOI: https://doi.org/10.56093/aq0b3a69

RESEARCH ARTICLE

Clinicopathological studies on respiratory affections in small ruminants with special reference to Mycoplasma species

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Received: 13.8.2025; Accepted: 12.9.2025

ABSTRACT

Pneumonia is a major health concern in small ruminants, significantly reducing productivity and causing substantial economic losses worldwide. Mycoplasma species are key pathogens implicated in respiratory infections of goats and sheep, acting either alone or in combination with other microbes. This study investigated haematological changes and identified major respiratory pathogens in clinically affected small ruminants. It also evaluated the pathological and immunohistochemical changes associated with *Mycoplasma* induced pneumonia in dead animals. A total of 50 animals (42 goats and 8 sheep) showing respiratory signs were examined. Blood and nasal swab samples were collected to assess haematological parameters and identify causative agents. Haematological analysis revealed significantly elevated total leukocyte counts and neutrophilia ($P<0.05$) in affected animals, along with a slight increase in lymphocytes, indicating an active inflammatory process. Bacterial culture of nasal swabs identified *Staphylococcus* spp. (50%) as the most common isolate, followed by *E. coli* (30%) and *Streptococcus* spp. (10%). Postmortem examination of 19 goats with respiratory illness revealed gross lung changes, including consolidation, mucopurulent discharge and tracheal congestion. Histopathological evaluation confirmed interstitial pneumonia in 11 (57.89%) animals, characterized by inflammatory infiltrates, alveolar septal thickening. The presence of mixed inflammatory cell populations suggested both acute and chronic phases of disease. Immunohistochemical analysis confirmed the presence of *Mycoplasma* antigens in all 11 histopathologically positive cases. In conclusion, *Mycoplasma* associated pneumonia, often compounded by secondary bacterial infections, plays a significant role in the respiratory disease complex of small ruminants. Early diagnosis, along with improved management and control measures is essential to reduce economic losses in small ruminant production systems.

Keywords: Mycoplasma species, Pneumonia, small ruminants

INTRODUCTION

Infiltration of inflammatory cells in lung parenchyma, alveoli and bronchioles are hallmarks of pneumonia, an acute, subacute or chronic infection of the lungs[1]. Pneumonia, the main health issue affecting small ruminants, can be brought on by *Mycoplasma* species either by itself or in combination with other microorganisms. In many regions of the world, pneumonia in small ruminants is a major hindrance to the development of this animal group and causes financial losses[2]. *Mycoplasmas* are found in nature in large quantities and can infect humans, animals and plants[3]. The infectious condition known as Mycoplasmosis is brought on by the tiniest, fastidious bacterium called *Mycoplasma*[4]. Extracellular parasites called *Mycoplasmas* frequently live as commensals on the mucous membranes of the upper respiratory tract in mammals. In small ruminants, they can lead to genital disorders, eye infections, respiratory conditions, arthritis and seldom, mastitis. Among other illnesses, respiratory conditions are prevalent in goats. Currently considered an emerging disease, Mycoplasmosis poses significant economic obstacles for farmers and small ruminant-rearing nations[5]. One of the main obstacles to the production of sheep and goats is poor management and husbandry techniques, as well as illnesses with a variety of aetiologies. Since air and blood are the primary means of transmission for respiratory infections, they are the most common among the various diseases that attack sheep and goats. The present study was undertaken to study the pathological changes induced by *Mycoplasma* in small ruminants causing pneumonia in dead animals and to study the haematological parameters along with to harbour main etiological agent causing respiratory affection in live animals.

How to cite this article : Bhullar, G.S., Singh, N.D., Filia, G., Gupta, J. and Leishangthem, G.D. 2026. Clinicopathological studies on respiratory affections in small ruminants with special reference to Mycoplasma species. Indian J. Vet. Pathol., 50(1) : 24-30.

MATERIALS AND METHODS

Sample collection from small ruminants suffering from respiratory affections

The goal of the current investigation was to determine the main causative agents of pneumonia in small ruminants. Blood Sample was collected from total of 50 animals (40 clinically sick and 10 healthy animals) to investigate comprehensive

haematology, including TLC, DLC etc., and Nasal swabs were collected from 40 clinically sick and 10 apparently healthy animals to identify the primary etiological agents in clinically unwell small ruminants brought to the hospital for treatment. According to IAEC No. (GADVASU/2024/IAEC73/13), the study was approved.

Blood sample collection

Blood was collected from the animals' jugular vein, which were then placed in tubes with 0.5 ml of ethylenediamine tetra acetic acid. The blood was slowly rotated in order to combine it. For the purpose of determining the differential leukocyte count, a fresh blood smear was produced on a clear, dry and non-oily slide and fixed with methanol for three to five minutes[6].

Isolation

Nasal and lung swabs taken from diseased animals during treatment were inoculated on BHI agar plates using the conventional streaking technique in a laminar flow. For a whole day, agar plates were incubated at 37^{O}C. Once more, pure and isolated single colonies of bacteria were obtained by subculturing on respective special media. Thin smears were made from pure isolated bacterial colonies and morphological identification was done by Gram staining.

Sample collection

From January 2024 to March 2025, 19 goats with a history of respiratory disease performed necropsied at the Department of Veterinary Pathology's post-mortem hall at Guru Angad Dev Veterinary and Animal Science University in Ludhiana, India. Following a comprehensive internal and exterior examination, 10% neutral buffered formalin was used to obtain representative tissue samples from the bronchial and mediastinal lymph nodes as well as the afflicted lungs. Additionally, Leishman stain was used to stain cytosmears that were taken from the damaged lung tissues.

Histopathology

Tissue samples were further cut after the first fixation for at least 24 hours, and they were further treated with benzene, water, graded alcohol, xylene, a xylene-benzene combination and melted paraffin. Sections of 3-4 µm were cut after embedding in paraffin blocks. After being moved to glass slides, the thin paraffin slices were stained using standard haematoxylin and eosin (H&E)[7].

Immunohistochemistry

Sections of paraffin tissue (4-5 microns) were placed on slides coated using APES, incubated at 37^{O}C, and then washed three times in 1X PBS (pH 7.2) for five minutes. After 15 minutes of deparaffinization in xylene 1 and 2, the mixture was hydrated with distilled water. After adding citrate buffer (pH 6.0), sections were subjected to antigen retrieval using the EZ Retriever IR System for three minutes at 92^{O}C and seven minutes at 70^{O}C. Sections were treated with 3% H_2O_2 in 100% methanol for 20 minutes after chilling for at least 45 minutes. After 20 minutes of incubation, two drops of 2.5% Normal Horse Serum (NHS) were administered, and any extra NHS was tapped off. Negative controls were given NHS in PBS, while the primary antibody (1:200 dilution in NHS) was incubated for the whole night at 4^{O}C in a humidified room. PBS washing was followed by an hour-long application of HRP conjugated goat anti-mouse IgGs (1:500 dilution). The sections were counter stained with Gill's haematoxylin for one minute after being developed with DAB and washed in double distilled water to stop the reaction. Sections were then cleaned in xylene, dehydrated in increasing alcohol grades and mounted using DPX.

Statistical analysis

The haematological data generated was subjected to paired 't' test using GraphPad Prism (Version 9.3.1.471).

RESULTS

Live animals

Haematological analysis

Haematological analysis was performed on blood samples taken from 50 animals (40 with respiratory diseases and 10 healthy); 42 of the 50 samples were from goats and 8 were from sheep. As previously mentioned, 50 animals' nasal swabs were also gathered in order to identify the primary causative agents of respiratory disorders in small ruminants out of which 40 animals were clinically ill and 10 healthy animals. There was no appreciable difference in the haematological parameters of haemoglobin (HB), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), MCH and MCV between sick and healthy sheep and goats. Sheep and goats with respiratory disorders have higher White Blood Cell (WBC) counts. All of these pathological states demonstrated a significant rise in relative neutrophil levels in all of the respiratory ill animals. In all of these clinical scenarios, sheep and goats showed no discernible changes in their levels of monocytes, eosinophils or basophils. Leucocytes (neutrophils) were shown to be significantly higher ($P<0.05$) in sheep and goats with pathological diseases such as pneumonia, abscess and haemorrhage. Similarly, Table 1 indicates that the absolute neutrophil count was much higher in clinically unwell small ruminants than in healthy ones.

Isolation of bacteria

Nasal samples were streaked on Brain Heart Infusion agar. Plates were examined for bacterial growth following a 24 hour incubation period. Isolated colonies were stained with the Gram stain. Gram staining revealed that *Streptococci* species were present as purple coloured cocci shaped chains and the *Staphylococcus* species (Table 2) were present as clusters resembling grapes (Fig. 1A).

Table 1. Haematological parameters of clinically normal and diseased animals (n=50)

Parameters	Control	Diseased animals
Hb(g%)	8.100±0.3861[a]	7.915±0.1818[a]
PCV (%)	41.08±2.081[a]	38.99±1.185[a]
RBC (106/cu.mm)	13.91±0.7899[a]	13.81±0.5211[a]
MCV (fl)	29.73±0.6388[a]	28.68±0.2903[a]
MCH	5.778±0.08784[a]	5.478±0.04613[a]
MCHC	19.35±0.1004[a]	19.28±0.1261[a]
WBC	10.61±0.9849[a]	16.98±1.036[b]
Absolute count of neutrophils	4270±5971[a]	10091±875.3[b]
Absolute count of lymphocyte	6110±703.2[a]	6662±503.8[a]

b-Data represents Mean± S.E.M. differs significantly at p value ≤ 0.05.

Table 2. Table depicting bacterial isolates cultured from nasal swabs of clinically ill animals (n=50)

Serial No.	Bacteria isolated from live animals	Number of animals	Percentage
1	*Staphylococcus* species	25	50%
2	*E. coli* species	15	30%
3	*Streptococci* species	5	10%
5	No growth	10	20%
	Total	50	100%

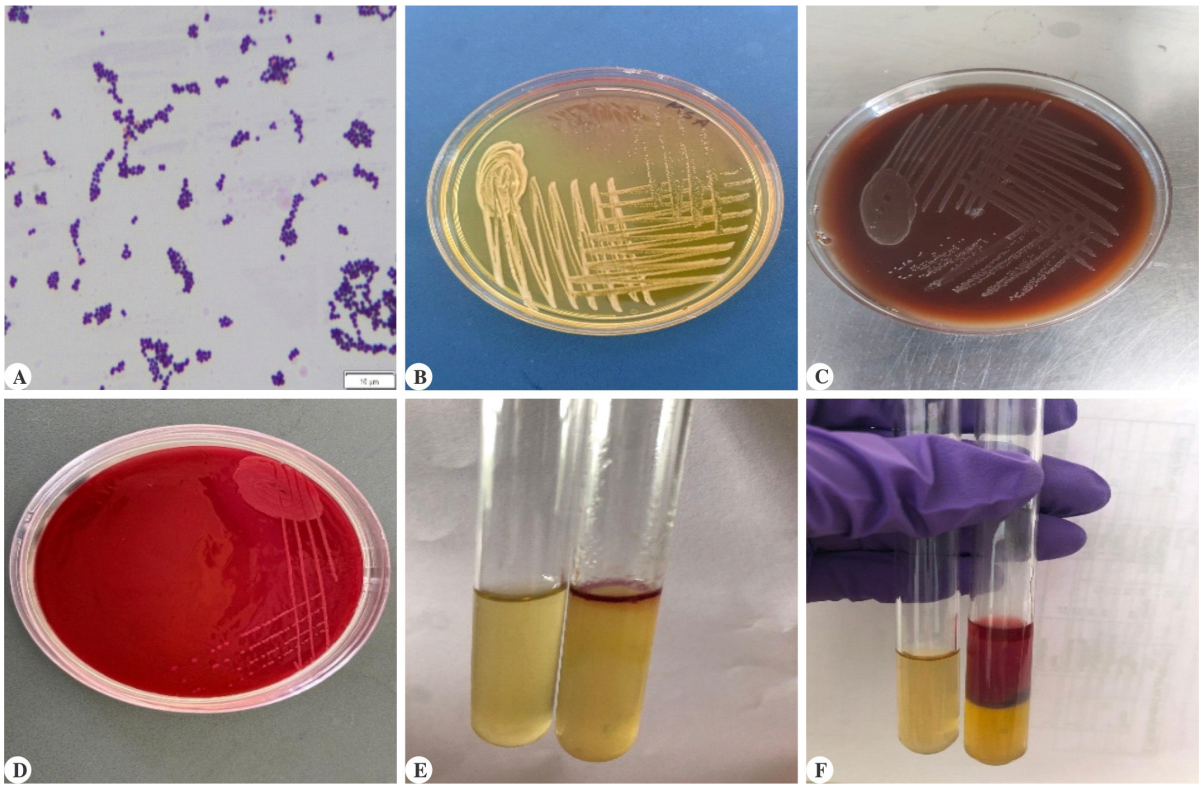

Fig. 1A. Gram stain showing *Staphylococcus aureus*, gram positive in a typical grape shaped (Gram stain x10 µm). **B.** Mannitol salt agar showing typical yellow-coloured colonies of *Staphylococcus aureus*. **C.** Showing colourless colonies of *Streptococci* species on Edward agar. **D.** MacConkey lactose agar showing lactose fermenter colonies of pink colour. **E.** Showing an indole-positive reaction typical for *E. coli*. **F.** Showing methyl red positive reaction typical for *E. coli*.

The catalase test was used to distinguish between *Streptococci* and *Staphylococcus* species, and the coagulase test was used to distinguish between *Staphylococcus aureus* and other *Staphylococcus* species. Of these, *Staphylococcus aureus* exhibited a positive reaction, while other *Staphylococcus* species showed a negative reaction. Coagulase positive *Staphylococci*, such as *Staphylococcus aureus*, produced yellow colonies (Fig. 1B) on Mannitol Salt Agar, whereas *Staphylococcus* species were further streaked on their specific medium, namely Baird Parker media, on which colonies appeared black with clear zones surrounding them. Additionally, *Streptococci* species were streaked on their particular medium, such as blood agar, which displayed white colonies with a haemolysis zone and Edward's agar likewise displayed colourless colonies with a visible haemolysis zone (Fig. 1C). Gram staining made gram negative bacteria look blue. Eosin methylene blue agar (EMB) and MacConkey lactose agar (MLA) were streaked with additional gram negative bacteria. Colonies in MLA had a pink colour, indicating that the bacteria were lactose fermenters (Fig. 1D). These lactose fermenting colonies had a distinctive green metallic sheen that is typical of *Escherichia coli* when streaked further on EMB agar. Additionally, the Indole, Methyl Red, Voges Proskauer, and Citrate test (ImViC) were used to validate it (Fig. 1E & F).

Postmortem examination

11 (57.89%) of the 19 lung samples collected from necropsied animals were tested positive for Mycoplasma species. Grossly, mucopurulent discharge was observed inside the nasal cavity. Six out of eleven small ruminants (54.54%) had mild hyperaemia (Fig. 2A), four out of eleven instances (36.36%) had moderate hyperaemia and one out of eleven (9.09%) had severe hyperaemia. There was also mild to severe tracheal mucosal congestion. Mononuclear cells, primarily lymphocytes and a small number of macrophages were seen in the impression smear upon cytological analysis (Fig. 2B). Histopathological analysis identified the primary features of interstitial pneumonia linked to Mycoplasma infection. The affected lungs were heavy, expanded, light in colour (with a more noticeable pale colour) and rubbery in texture rather than collapsing (Fig. 2C). The lung's pleural surface showed distinct rib imprints (Fig. 2D). In many cases, the rapid drainage of frothy and/or bloody exudate from the lung's cut surface was a sign of oedema and congestion. Patchy, dark red, discoloured patches that were more widely dispersed across the lungs showed pulmonary consolidation. There were times when the caudo dorsal area of the lungs was reddish brown, meaty and sometimes browned lobular (Fig. 2E). Upon opening the lungs, the interlobular septa (marbling) expanded and held a yellowish jelly like

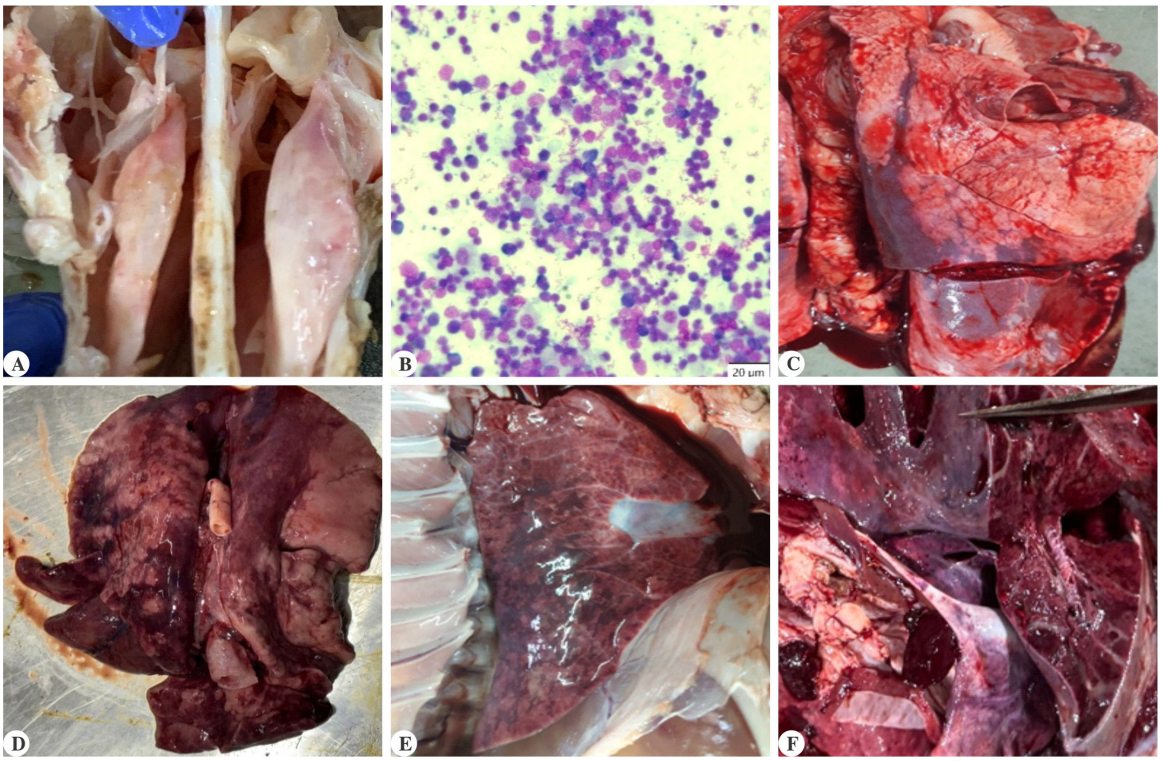

Fig. 2A. Nasal turbinate showing very mild congestion. **B.** Impression smear showing the presence of mononuclear cells (MNCs). Note the presence of some binucleate cells and conjoined cells giving the appearance of syncytia (Leishman x20 µm). **C.** Lungs showing pale and enlarged lungs. **D.** The lung showing irregular areas of consolidation with rib impression. **E.** Lung showing reddish brown colour in caudo dorsal lobe. **F.** Cut surface of lung showing typical marble pattern of Mycoplasmosis.

substance with different levels of lobule consolidation (Fig. 2F).

Microscopic examination

Inflammatory cell infiltration under a microscope mostly consists of a mixed cell population with hyperplastic alterations in the nasal mucosa lining epithelium (Fig. 3A). The lymph node showed the development of syncytia (Fig. 3B) and the presence of binucleate cells. During necropsy, interstitial pneumonia was discovered in 11 (57.89%) of the 19 cases. Four out of seven cases (57.14%) had mononuclear cells (primarily lymphocytes and few macrophages) that suggested chronic interstitial pneumonia, one out of seven cases (14.28%) had neutrophils (Fig. 3C), which suggested acute interstitial pneumonia and two (28.57%) out of seven cases had both mononuclear (MNCs) and neutrophils/polymorphonuclear (PMNCs) cells (Fig. 3D), which suggested a mixed infection. In these cases, the histopathological results showed dilated and enlarged alveolar capillaries and vasculature (Fig. 3E), along with varied degrees of thickened interlobular septa caused by oedema, fibrin deposition, syncytia formation and a significant infiltration of inflammatory cells, primarily MNCs. The interalveolar septa thickened as a result of the infiltration of inflammatory cells and the hyperplasia and hypertrophy of type II pneumocytes. Desquamation of necrotic epithelial cells of the bronchioles inside the lumen, peribronchiolar lymphoid cell infiltration, multifocal nodules of mononuclear cell infiltration and mononuclear cell aggregation in the bloodstream were all observed in the lung tissues exhibiting the chronic stage of pneumonia in *Mycoplasma* positive samples.

Immunohistochemistry

Immunohistochemical staining revealed positive immunoreactions in 11 (57.89%) of the 19 histopathologically indicated *Mycoplasma* cases. Both the lumen of the lung's blood vessels and the cytoplasm of pulmonary intravascular macrophages in the interalveolar septa displayed positivity. *Mycoplasma* antigen was detected in the pleura, interlobular septa, extracellular space and the cytoplasm of macrophages (Fig. 3F). The necro suppurative exudate that obstructed the bronchiolar lumina and was occasionally observed along the surface of the bronchiolar epithelial cells and the cytoplasm of inflammatory cells implicated in the lesion was related to the immunoreactivity.

DISCUSSION

Leucocytes (neutrophils) were shown to be significantly higher (P<0.05) in sheep and goats with

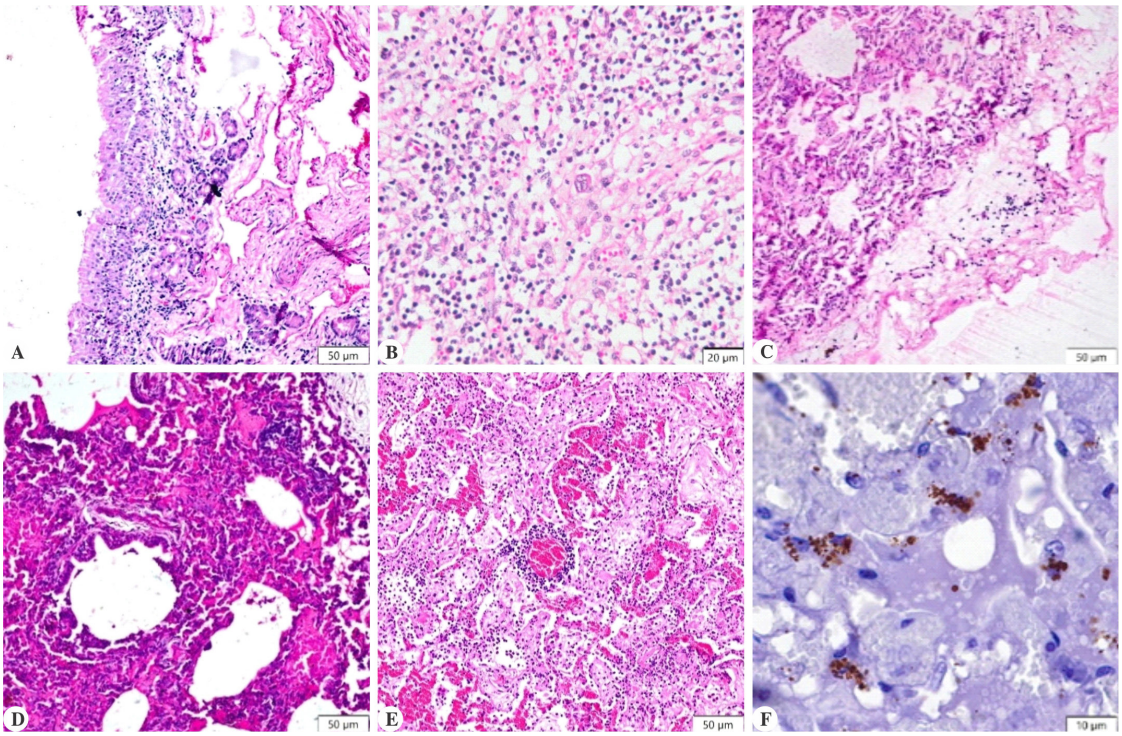

Fig. 3A. Revealed hyperplasia of epithelium along with mixed cell population infiltration (H&E x50 µm). **B.** Lymph node showing typical syncytia formation and lymphoid depletion (H&E x20 µm). **C.** Lungs showing infiltration of cells in the interstitium, giving a thickened appearance along with infiltration (H&E x20 µm). **D.** Showing infiltration of mixed cell population of inflammatory cells in the interstitium, suggestive of interstitial pneumonia along with syncytia (H&E x50 µm). **E.** Showing serous fluid infiltration in alveoli and diffuse infiltration of mixed cell population and the presence of perivascular infiltration (H&E x50 µm). **F.** Immunolocalization of *Mycoplasma* in the interstitium of the tissue and in inflammatory cells (IHC x10 µm).

pathological diseases such as pneumonia, abscess and haemorrhage. These results are in accordance with research from studies[8,9] which revealed several lung degenerative diseases. In studies[10,11], an increase in leukocytes was found and this was also evident in the current investigation. Additionally, [12]observed a noteworthy reduction in lymphocytes in sheep and goats with respiratory ailments. As a defense mechanism against the etiological agents that cause the body's inflammatory processes, particularly in the spleen, liver, kidneys and lung, where the inflammation prompts the bone marrow to produce a lot of white blood cells (WBC), they did observe a notable increase in neutrophils and a decrease in lymphocytes[13].

The most frequent bacterium responsible for pneumonia in sheep and goats was *Staphylococcus aureus*[14]. However, the most frequent bacterium that caused respiratory problems in small ruminants was *E. coli*, according to a study done by[15]. Other bacteria, including *Klebsiella* and *Streptococci* species, were also reported by him. *Pasteurella multocida* and *E. coli* species were the most often detected bacteria in the respiratory tracts of ill small ruminants, ranking second and third, respectively[16]. However, the geographic variation of the place from where the samples were taken, the mixed bacterial population in animals and the immunological condition of the animal[14] could all be responsible for a variation in the isolation percentage of various etiological agents.

Tracheal mucosal oedema and congestion were indicated by lesions that resembled froth in the trachea and other related organs[17]. The stroma, or supporting tissue, grows as inflammatory cells reach the lungs and in certain cases, cranioventral congestion[18] causes the lungs to appear mottled. The interlobular septa (marbling) grew larger and held a yellowish jelly like fluid with different levels of lobule consolidation[19] when the lungs were cut open. In the pulmonary lobules, compensatory emphysema from alveolar wall rupture and atelectasis from significant thickening of the interalveolar septa were frequently seen. Additionally, some of the animals exhibited many hemorrhagic regions. Pale lungs, especially when the alveolar walls are fibrosed, are caused by severe alveolar capillary obliteration (a reduced blood tissue ratio).

Type II pneumonocytes, which are essentially progenitor cells that differentiate, take the place of necrotic type I pneumonocytes. This causes the alveolar walls to thicken. The reason the lungs don't collapse regularly when the thorax is opened, why they feel rubbery to the touch and why the lung's sliced surface seems "meaty"[20] can all be explained by this mechanism. The complicated aetiology of interstitial pneumonia may be caused by hematogenous damage to the alveolar capillary endothelium or alveolar basement membrane. Alveolar and interlobar septal deposits of antigen antibody complexes (type III hypersensitivity) are created when inhaled antigens and circulating antibodies mix. This results in thickening (marbling), a series of inflammatory responses and damage[20] of the animals had interstitial pneumonia, fibrous pleuritis and excess serosanguinous pleural fluid. The animals with more chronic illnesses showed lung adhesion to the rib cage because to *Mycoplasma* species, which is similar to the findings of[21]. Hydropic degeneration, necrosis and in certain cases, severe hemorrhagic pneumonia[22] were observed in the lining of the bronchi. Acute lesions with a bad prognosis are frequently the outcome of septicemia in these illnesses, which is caused by *Mycoplasma* growth and bloodstream dispersion[21]. The majority of *Mycoplasma* species proliferate and disperse via blood vessels, leading to the formation of microthrombi[23]. The lungs' stoma or supporting tissue receives blood from their blood vessels, so when etiological agents cause microthrombi to form in the tissue, inflammatory cells are drawn to the area. This leads to inflammation of the stroma or supporting tissue, which in turn causes interstitial pneumonia. The thickening of the interlobar and interalveolar septa, which results in interstitial pneumonia characteristic of *Mycoplasma*, is caused by the increased attraction of inflammatory cells to the site, i.e., the interstitium as stated in[21,23,24].

CONCLUSION

In conclusion, *Mycoplasma* associated pneumonia, often compounded by secondary bacterial infections, plays a significant role in the respiratory disease complex of small ruminants. Early diagnosis, along with improved management and control measures, is essential to reduce economic losses in small ruminant production systems.

ACKNOWLEDGEMENT

The author would like to thank the Incharge, Animal Disease Research Centre, GADVASU, Ludhiana for PCR work. The authors are also thankful to Dean Post Graduate Studies, GADVASU and Professor cum Head, Department of Veterinary Pathology, GADVASU, Ludhiana for providing necessary facilities and funds to carry out the research work.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 31-35, 2026: DOI: https://doi.org/10.56093/40trrs12

RESEARCH ARTICLE

Comprehensive evaluation of auricular squamous cell carcinoma in dogs: Clinical and pathological perspectives

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Received: 12.10.25; Accepted: 19.11.25

ABSTRACT

Squamous cell carcinoma (SCC) is one of the most frequently encountered malignant cutaneous neoplasms in dogs particularly affecting sun-exposed regions such as the pinna. The present study was conducted over a period of one year to evaluate the prevalence, haematological, biochemical and histopathological characteristics of auricular SCC in 786 dogs of different breeds of canine dermatological cases presented to the Teaching Veterinary Clinical Complex, Bihar Veterinary College, BASU, Patna. Twenty-two cases were recorded having squamous cell carcinoma with a mean age of 9.1 ± 2.3 years. The overall prevalence rate of auricular SCC during the study period was 2.8% among canine dermatological cases. Breed-wise prevalence indicated higher incidence in German Shepherds (32%), Labrador Retrievers (27%), Spitz (18%) and non-descript dogs (23%) with a higher occurrence in males. Clinically, affected dogs exhibited ulcerated, proliferative or crusted growths on the pinna, foul-smelling discharge, head shaking, pain and pruritus in chronic cases. Haematological analysis revealed moderate anaemia, leucocytosis and neutrophilia indicative of chronic inflammatory response and tumour-associated infection. Biochemical evaluation demonstrated elevated liver enzymes (ALT, AST) and increased total protein and globulin levels suggestive of systemic inflammatory and hepatic stress responses. Histopathological examination confirmed the diagnosis revealing invasive nests and cords of neoplastic squamous epithelial cells with keratin pearl formation, cellular pleomorphism, mitotic figures and stromal desmoplasia consistent with well to moderately differentiated SCC. Auricular squamous cell carcinoma in dogs is a relatively common malignant neoplasm with breed predisposition and distinct clinico-pathological alterations. This study highlights the clinicopathological significance of auricular squamous cell carcinoma in dogs emphasizing the importance of early diagnosis, surgical management and awareness among veterinarians and dog owners particularly in predisposed breeds such as German Shepherds and Labradors exposed to prolonged sunlight. Early recognition through clinical and histopathological correlation is crucial for timely management and to prevent local invasiveness and recurrence.

Keywords: Biochemistry and histopathology, ear, prevalence, haematology, squamous cell carcinoma

INTRODUCTION

Squamous cell carcinoma (SCC) is one of the most common malignant cutaneous tumours in dogs arising from the epidermal keratinocytes and frequently affecting regions exposed to chronic ultraviolet (UV) radiation such as the nasal planum, eyelids and pinnae[5,14]. It constitutes a significant dermatological and oncological concern in canines particularly in middle-aged to geriatric animals due to its locally invasive nature, potential for tissue destruction and tendency for recurrence despite low metastatic rates[6,18].

In dogs, SCC presents in the skin at slightly pigmented or hairy sites[13]; the digits, representing 25% to 52 % of all neoplasms that occur at this location[12]; the nasal planum which may be associated with cutaneous erythematosus lupus, pemephigus and vitiligo; the oral mucosa constituting the most common oral neoplasia in dogs[13] and rarely the eye[12]. In a retrospective study of third eyelid neoplasms in dogs and cats, 145 dogs were evaluated and only one (0.8%) was diagnosed with SCC of the third eyelid, highlighting the rarity of this presentation in dogs[4].

Squamous cell carcinoma (SCC) is an uncommon but important cutaneous neoplasm in dogs representing roughly 4–5% of canine cutaneous tumours in large retrospective surveys[17]. Anatomical distribution differs between species and studies. Tumours involving the pinna or external ear (auricular/ pinnal SCC) are reported in the veterinary literature (case reports and retrospective reviews of ear neoplasms) but ear tumours overall are uncommon and pinnal SCC is usually described as isolated cases or small series rather than large population percentages[7].

The ear pinna is among the most commonly affected sites

How to cite this article: Sinha, V.K., Kumar, K. Kumar, D. and Kumar, R. 2026. Comprehensive evaluation of auricular squamous cell carcinoma in dogs: Clinical and pathological perspectives. Indian J. Vet. Pathol., 50(1) : 31-35.

because of its sparse hair coverage and high sun exposure especially in lightly pigmented and short-coated breeds[1]. Chronic solar exposure, repeated trauma and papillomavirus infection have been suggested as major predisposing factors in the development of auricular SCC[8,11]. Environmental and geographic factors also play a crucial role with increased incidence observed in tropical and subtropical regions where sunlight intensity is high[15].

Clinically, affected dogs present with ulcerated, proliferative or crusted lesions that may be painful or bleeding and in chronic cases, necrotic with foul odour and discharge [16,18]. The condition often leads to significant discomfort, otitis externa and secondary bacterial infections compromising animal welfare. Early and accurate diagnosis is therefore essential to prevent local invasion into deeper tissues and recurrence following surgery.

Haematological and biochemical alterations in affected dogs often reflect chronic inflammation, secondary infection and occasionally hepatic stress due to systemic inflammatory mediators[3]. Histopathological examination remains the gold standard for confirmation typically revealing irregular nests, cords or sheets of malignant squamous epithelial cells, keratin pearl formation, nuclear pleomorphism and mitotic figures consistent with well to moderately differentiated SCC[6,10].

The incidence of SCC in dogs has been reported to range between 4% and 19% of all skin tumours with a predilection for German Shepherds, Labradors, Boxers and Spitz breeds[8,14]. Despite its frequent occurrence, regional studies in India remain limited particularly those focusing on auricular forms with clinicopathological correlations.

The present study was therefore undertaken to evaluate the prevalence, breed-wise and age-wise distribution along with the haematological, biochemical and histopathological characteristics of auricular squamous cell carcinoma in dogs presented to the Department of Pathology, Bihar Veterinary College, Patna, over a one-year period. The findings are expected to enhance understanding of the epidemiology and pathological behaviour of SCC in dogs contributing to improved diagnostic and therapeutic approaches in veterinary oncology.

MATERIALS AND METHODS

The present study was conducted on clinical cases of auricular squamous cell carcinoma (SCC) in dogs that were presented to the Department of Veterinary Surgery and Radiology, Teaching Veterinary Clinical Complex, Bihar Veterinary College, Patna, during a period of one year. A total of 786 dogs of different breeds, ages and sexes were examined. Out of 786, 22 dogs were selected for study for further process. The animals were brought with a history of ulcerated or proliferative growths on the ear pinna, crusting, bleeding, foul-smelling discharge

Table 1. Haematological profiles of 22 dogs (Breed wise) suffering from Squamous cell carcinoma.

Parameter	Labrador Retriever (n=6)	German Shepherd (n=7)	Spitz (n=4)	Non-descript (n=5)	Reference Range (canine)
RBC ($\times10^{12}$/L)	5.8 ± 0.5	5.6 ± 0.6	5.9 ± 0.4	5.5 ± 0.6	5.5 - 8.5
Hb (g/dL)	12.7 ± 1.3	12.5 ± 1.4	12.9 ± 1.2	12.4 ± 1.3	12 - 18
PCV (%)	38 ± 3.2	37 ± 3.8	39 ± 2.7	37 ± 3.9	37 - 55
WBC ($\times10^{9}$/L)	12.8 ± 2.4	13.5 ± 3.1	12.6 ± 1.6	13.3 ± 3.0	6 - 17
Neutrophils (%)	65 ± 5	67 ± 7	64 ± 5	66 ± 6	60 - 70
Lymphocytes (%)	28 ± 4	27 ± 5	28 ± 3	27 ± 5	20 - 30
Monocytes (%)	4 ± 1	4 ± 1	4 ± 1	4 ± 1	3 - 10
Eosinophils (%)	3 ± 1	3 ± 1	3 ± 1	3 ± 1	2 - 10
Platelets ($\times10^{9}$/L)	280 ± 30	275 ± 40	290 ± 25	278 ± 35	200 - 500

Table 2. Biochemical profiles of 22 dogs (Breed wise) suffering from Squamous cell carcinoma.

Parameter	Labrador Retriever (n=6)	German Shepherd (n=7)	Spitz (n=4)	Non-descript (n=5)	Reference Range (canine)
ALT (U/L)	48 ± 12	52 ± 15	46 ± 10	50 ± 13	10 - 60
AST (U/L)	40 ± 10	42 ± 12	38 ± 9	41 ± 11	15 - 45
ALP (U/L)	110 ± 25	120 ± 30	105 ± 20	115 ± 28	20 - 150
Total Protein (g/dL)	6.8 ± 0.5	7.0 ± 0.6	6.9 ± 0.4	6.7 ± 0.5	5.5 - 7.5
Albumin (g/dL)	3.2 ± 0.3	3.3 ± 0.4	3.2 ± 0.2	3.1 ± 0.3	2.5 - 4.0
Globulin (g/dL)	3.6 ± 0.4	3.7 ± 0.5	3.7 ± 0.3	3.6 ± 0.4	2.5 - 4.0
Urea (mg/dL)	32 ± 5	34 ± 6	33 ± 4	32 ± 5	20 - 40
Creatinine (mg/dL)	1.2 ± 0.2	1.3 ± 0.3	1.2 ± 0.1	1.2 ± 0.2	0.5 - 1.5

and in some cases, head shaking and scratching due to discomfort occurs. The duration of lesions as reported by owners ranged from one month to eight months prior to presentation. Some owners also noticed loss of appetite, irritability and occasional ear tilting.

On clinical examination, the affected dogs were generally alert and responsive though some showed signs of pain and pruritus upon palpation of the ear. Lesions were mostly ulcerated, proliferative or nodular varying in size from 1 cm to 6 cm and occasionally associated with serosanguinous exudation. Regional lymphadenopathy was palpable in a few chronic cases. Physiological parameters such as rectal temperature, pulse rate and respiration rate remained within normal limits in most animals.

For diagnostic evaluation, haematological and biochemical analyses were performed using standard protocols. Blood samples were collected aseptically from the cephalic vein into EDTA and plain vacutainers. Haematological parameters included haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC). Biochemical assays measured serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, globulin, blood urea nitrogen (BUN) and creatinine using an automatic biochemical analyser. Haematological findings showed mild to moderate anaemia, leucocytosis and neutrophilia while biochemical profiles revealed elevated ALT and AST with normal renal parameters (Tables 1 & 2).

Dogs with confirmed or suspected SCC underwent surgical excision of the auricular mass under general anaesthesia. Animals were pre-medicated with inj. atropine sulphate (0.04 mg/kg body weight, i.m.) and inj. xylazine (1 mg/kg body weight, i.m.) followed by induction with inj. ketamine (5 mg/kg body weight, i.v.). After achieving adequate anaesthesia, the animal was positioned in lateral recumbency and the affected ear was prepared aseptically. Draping of surgical site of ear tumour in dog was done (Fig. 1). Linear incision was made over the affected auricular tissue having tumour mass (Fig. 2) and the mass was carefully excised using scissors and tissue forceps (Fig. 3). Bleeding vessels were ligated with absorbable suture material (Polyglycolic acid 2/0) and the incision was closed with nylon 2/0 (Fig. 4).

The excised tumour specimens were immediately fixed in 10% neutral buffered formalin and submitted to the Department of Veterinary Pathology, Bihar Veterinary College, BASU for gross, cytological and histopathological evaluation. Grossly, the masses were examined for size, colour, consistency and ulceration. Tissue samples were routinely processed by paraffin embedding, sectioned at 4–5 µm thickness and stained with Haematoxylin and Eosin (H&E) for microscopic evaluation.

Postoperatively, dogs were treated with inj. ceftriaxone (25 mg/kg body weight, i.m.) once daily for five days and inj. meloxicam (0.2 mg/kg body weight, i.m.) for three days to control pain and inflammation. The surgical site was cleaned with povidone-iodine ointment twice daily for one week and sutures were removed after 10–12 days.

All procedures were carried out in compliance with the Institutional Animal Ethics Committee (IAEC) guidelines, Bihar Veterinary College, BASU, Patna (Approval No.: IAEC/BVC/2025/29).

RESULTS AND DISCUSSION

All affected dogs resumed normal feeding and activity within 3–7 days post-surgery. The surgical wounds healed uneventfully and sutures were removed on day 10–12 without complications. On follow-up examination after two months, no local recurrence or evidence of metastatic spread was observed in any of the treated cases indicating a favourable postoperative outcome.

Grossly, the excised auricular masses were irregular, firm to hard and ulcerated with raised and proliferative margins. The cut surface appeared whitish to grayish-white occasionally showing yellow necrotic foci and areas of haemorrhage. The tumour size varied from 1.5 cm to 6.2 cm in diameter. The lesions were mostly unilateral and the underlying cartilage was thickened in chronic cases though invasion beyond the pinna was not observed.

Histopathologically, the cells exhibited abundant eosinophilic cytoplasm, prominent intercellular bridges and keratin pearl formation which are characteristic features of squamous differentiation. It demonstrated moderate cellular pleomorphism, hyperchromatic nuclei, occasional mitotic figures and focal keratinization (Fig. 5). Histopathological examination of tumour sections also shows nests and cords of neoplastic epithelial cells infiltrating the dermis (Fig. 6). The stroma showed fibroblastic proliferation and lymphoplasmacytic infiltration around tumour lobules indicative of a chronic inflammatory response. Based on these findings, the lesion was diagnosed as well to moderately differentiated squamous cell carcinoma.

Squamous cell carcinoma is one of the most frequently diagnosed malignant cutaneous neoplasms in dogs accounting for 4–19% of all skin tumours [1,8]. The mean age of affected dogs in the present study was 9.1 ± 2.3 years which agrees with the previous findings who reported a higher occurrence in middle-aged to

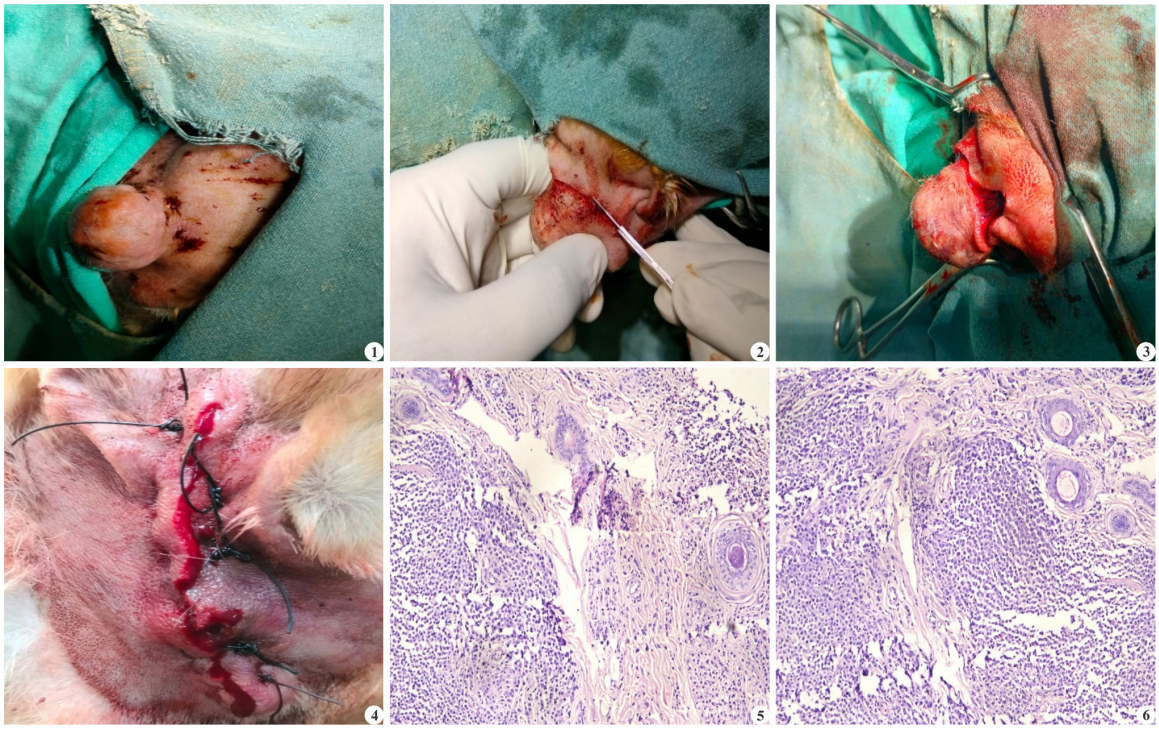

Fig. 1. Image showing draping of surgical site of ear tumour in dog; **Fig. 2.** Linear incision over the affected auricular tissue showing surgical approach to the tumour mass; **Fig. 3.** Intraoperative view showing scissors and forceps employed for precise dissection of auricular tumour; **Fig. 4.** Operative site showing apposition of skin margins with interrupted sutures after tumour removal from the ear; **Fig. 5.** Histological section of canine oral tumour (H&E, 100x) showing well-differentiated squamous cell carcinoma characterized by concentric keratin pearl formation surrounded by malignant epithelial cells; **Fig. 6.** Histological section of ear of dog (H&E, 100x) showing nests and cords of neoplastic epithelial cells infiltrating the dermis.

geriatric animals[8,14]. Breed-wise prevalence indicated a higher incidence in German Shepherds (32%) followed by Labrador Retrievers (27%), Spitz (18%) and non-descript dogs (23%) suggesting breed predisposition and increased solar exposure as important contributing factors. Previous studies have also reported breed-related clustering of cutaneous SCC with higher overall prevalence of SCC among short-haired, lightly pigmented breeds and dogs with chronic sun exposure. Additionally, broader epidemiological surveys have shown that squamous cell carcinoma accounts for 5–7% of all canine cutaneous tumours with commonly affected sites including the ventral abdomen, limbs, nasal planum, eyelids and pinnae supporting the multifocal nature and variable anatomical distribution of SCC in dogs. Similar observations have been reported by earlier authors[1,15].

A total of 138 dogs with a history of neoplastic growths were evaluated from nearby veterinary hospitals between August 2004 and December 2006 at the Department of Pathology, Veterinary College, Hebbal, Bangalore. Out of which, 17 cases were confirmed of of squamous cell carcinoma (SCC). Out of SCC-affected dogs, eight were males and nine were females. The highest occurrence was observed in nondescript dogs (8 cases; 47.1%) followed by Dobermans (2 cases; 11.8%). German Shepherd, Dalmatian, Spitz, Golden Retriever, Cocker Spaniel, Labrador and Poodle contributed one case each (approximately 5.9% per breed) [2].

Study further supported a breed-specific predisposition for squamous cell carcinoma in dogs. In their detailed analysis of 79 dogs with digital squamous cell carcinoma, they constituted nearly one-third of all cases indicating a strong breed over-representation. Other frequently affected breeds in the cohort included Labrador Retrievers, Rottweilers, Poodles and other large, dark-coated breeds highlighting the influence of both genetic and phenotypic factors on SCC susceptibility. The authors suggested that pigmentation, body size and breed-associated keratinisation patterns may contribute to this clustering. Although this study focused on digital SCC, the consistent over-representation of certain breeds across SCC types supports the broader concept of breed-related risk, reinforcing patterns observed in the present investigation[9].

Haematological examination revealed mild to moderate anaemia, leucocytosis and neutrophilia consistent with a chronic inflammatory condition. These findings are comparable to those of previously documented similar alterations in dogs with cutaneous

neoplasms[3]. Biochemical analysis showed elevated ALT and AST levels and mild increase in ALP and globulin indicative of liver stress and systemic inflammatory response. Comparable biochemical deviations have been recorded in cases of chronic cutaneous SCC previously [8,16].

Histopathological features observed in this study namely keratin pearl formation, cellular pleomorphism and stromal desmoplasia closely resemble the previous descriptions[6,18]. The absence of vascular invasion and metastasis in all cases supports the notion that auricular SCC in dogs is typically locally invasive but rarely metastatic as also concluded in past[1].

The favourable postoperative recovery and absence of recurrence following complete surgical excision reaffirm that early detection and wide surgical resection remain the treatment of choice for auricular SCC in dogs. Adjuvant therapy may be considered in extensive or recurrent cases. The findings of the present study highlight the need for early clinical recognition especially in sunlight-exposed breeds and stress the importance of routine ear examination in geriatric dogs for timely intervention.

CONCLUSION

Auricular squamous cell carcinoma in dogs is an important cutaneous malignancy, particularly affecting middle-aged to older animals with prolonged exposure to sunlight. The condition holds considerable clinical and pathological significance due to its locally invasive nature and potential for tissue destruction and recurrence. Early diagnosis through clinical, haematological, biochemical and histopathological evaluation followed by prompt surgical excision offers an excellent prognosis. The present study emphasizes the influence of breed and age predisposition and underlines the necessity of accurate histopathological confirmation and periodic monitoring for effective management and prevention of recurrence.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Vice Chancellor and the Dean of Bihar Veterinary College, Bihar Animal Sciences University, Patna, for providing the necessary facilities to carry out this work.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI) Assisted technology for manuscript preparation: The authors confirm that there was no use of AI – Assisted Technology for assisting in the writing of the manuscript and no images were manipulated using AI

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Indian J. Vet. Pathol., 50(1) : 36-39, 2026: DOI: https://doi.org/10.56093/cr49qj17

RESEARCH ARTICLE

Canine Parvovirus-2a infection in a juvenile pup: Bone marrow findings and clinical implications

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Received: 02.11.25; Accepted: 21.11.25

ABSTRACT

A 2 month old non descriptive pup was presented to the Emergency and Critical Care Unit (ECCU) at Madras Veterinary College Teaching Hospital with a 4-day clinical history of bloody diarrhoea, vomition and neurological manifestations suggestive of severe systemic involvement. Haematological analysis revealed anaemia and profound leukopenia, indicative of marked immunosuppression. Necropsy findings demonstrated extensive hemorrhagic enteritis and multi-organ pathology, including fatty enlarged liver and epicardial pallor. Histopatholgical examination revealed hepatic fatty degeneration, myocardial fibre degeneration, and intestinal crypt cell necrosis. Bone marrow examination showed severe hypocellularity with depletion of myeloid and erythroid cell lines that lead to leukopenia. Molecular detection and characterization of the VP2 gene confirmed infection with the CPV-2a variant, genetically related to strains reported from India, Bangladesh, and Nigeria. The study highlights the importance of bone marrow evaluation in diagnosing and managing CPV-2 infection, and underscores the value of integrating molecular and pathological findings for accurate diagnosis and improved treatment outcomes.

Keywords: Bone marrow, Canine Parvovirus, Colony-Stimulating Factors, Hypocellularity, Leukopenia, Phylogeny, VP2 Gene

INTRODUCTION

Canine parvovirus type 2 (CPV-2) is a significant cause of illness and mortality in young dogs, with a history of evolution and adaptation since its discovery in 1978[1]. Classified within the family Parvoviridae, CPV-2 is a single-stranded DNA virus with a 5.2 kb genome encoding two structural proteins (VP1 and VP2) and two non-structural proteins (NS1 and NS2)[2]. The VP2 protein is crucial for receptor recruitment, host-specificity, and antigenicity[3]. The virus being reported to have evolved from feline panleukopenia virus (FPV), CPV-2 shares over 98% nucleotide similarity with FPV but has six amino acid changes in VP2 that enable it to infect dogs while losing its capacity to infect cats[4]. CPV-2 has three variants: CPV-2a (426Asn), CPV-2b (426Asp), and CPV-2c (426Glu), classified based on the amino acid configuration of the capsid protein.

In India, CPV-2 infection was first documented in 1982[5], followed by the detection of all three variants - CPV-2a, CPV-2b, and CPV-2c across various regions, with CPV-2c being the least frequently detected[6]. The virus primarily replicates in highly mitotically active tissues such as bone marrow, lymphoid tissue, and gastrointestinal tract epithelium, making young growing dogs more susceptible to infection. The destruction of hematopoietic progenitor cells in bone marrow and lymphoproliferative organs leads to notable hematobiochemical alterations. This study aims to elucidate the presence of parvovirus in bone marrow and its molecular differentiation, addressing the lack of comprehensive knowledge on the entire genome of CPV-2 in circulation in India.

How to cite this article: Thilahayswari, J., Nagarajan, K., Hemalatha, S. and Chandrasekar, M. Canine Parvovirus-2a infection in a juvenile pup: Bone marrow findings and clinical implications. Indian J. Vet. Pathol., 50(1) : 36-39.

MATERIALS AND METHODS

A 2-month-old female pup, weighing 1.5 kg, was presented to the Emergency and Critical Care Unit (ECCU) at Madras Veterinary College Teaching Hospital with a 4-day clinical history of bloody diarrhoea and vomition. Born to a vaccinated dam, the pup was part of a litter of five that had received a single vaccination against canine distemper, adenovirus type-2, leptospirosis, parainfluenza, and parvovirus. Notably, one week after vaccination, four of the five littermates developed lethargy, vomition, and diarrhoea, and despite treatment, all four pups succumbed to the illness. Upon presentation, the last surviving pup was found to be dull, recumbent, and hypothermic, with foul-smelling bloody faeces adherent to the anal region. Laboratory findings revealed severe panleukopenia, microcytic anaemia, and hypoalbuminemia that indicated a critical condition.

The pup underwent intensive care, including stabilization on a heating pad, administration of IV fluids comprising Ringer's Lactate with 5% dextrose supplementation, and a regimen of medications consisting of Ceftriaxone (20 mg/kg) to combat bacterial infections, Ondansetron (0.5 mg/kg) to prevent vomition, and Pantaprazole (1 mg/kg) to protect the gastrointestinal tract was followed. A worse, marked decline in white blood cell count and onset of neurological signs, including vocalization, cervical hyperesthesia, seizures and nystagmusdevelopedthat led to collapse and deathof the pup.A detailed necropsy was conducted, and bone marrow aspirates were collected in a sterile manner for laboratory investigation. Representative tissue samples and cut section of the femur with bone marrow were collectedin 10% neutral buffered formalin. Tissuesamples were routinely processed, bone tissue decalcified and 3-5 micron thick tissue sections were stained with Haematoxylin and eosin[7].

Bone marrow tissue sample of about 25 mg was used for the extraction of genomic DNA, using commercially standardized DNeasy Blood and Tissue Kit (QIAGEN, Germany). The CPV genome was confirmed by Polymerase Chain Reaction (PCR), targeting a portion of the VP2 gene. The amplification was carried out under standard conditions using the specific primer set: Forward Primer (CPV-F: AAG ACG TGC AAG CGA GTC C) and Reverse Primer (CPV-R: GAG CGA AGA TAA GCA GCG TAA). The resulting PCR product was then subjected to electrophoresis and analyzed on a 1.5% agarose gel. The final analytical stages involved nucleotide sequencing of the purified VP2 gene product. The sequence data were used for a detailed comparison against VP2 reference sequences retrieved from the National Centre for Biotechnology Information (NCBI) database, utilizing MEGA12 software to construct phylogenetic trees.

RESULTS

A detailed necropsy revealed a carcass poor in condition with pallor of visible mucous membranes. Liver appeared enlarged, yellow with rounded borders (Fig. 1). Lungs were pale with watery fluid flowing out on incision. Epicardium revealed patchy areas of pale discolouration (Fig. 2). The kidneys appeared pale. Large roundworms, approximately 5-8 cm in diameter, in the lumen of intestine was observed (Fig. 3). The mucosa of intestine was dark red with blood mixed contents. The stomach revealed serosal haemorrhage with blood tinged contents. Additionally, faecal examination confirmed the presence of *Toxocara canis* eggs .

Microscopically, liver shared fatty degeneration of hepatocytes, characterized by intracytoplasmic clear vacuoles leading to cytoplasmic rarefaction and peripheral displacement of nuclei, indicating secondary hypoxic injury (Fig. 5). The kidneys showed glomerular atrophy (Fig. 4) and mild tubular degeneration. The myocardium revealed degeneration of muscle fibres with loss of cross striations. Affected cardiac muscle fibers appear swollen, fragmented, and hypereosinophilic with mild mononuclear cell infiltration. Lungs revealed edema of alveoli. The spleen revealed marked lymphoid depletion in the white pulp. The intestine showed

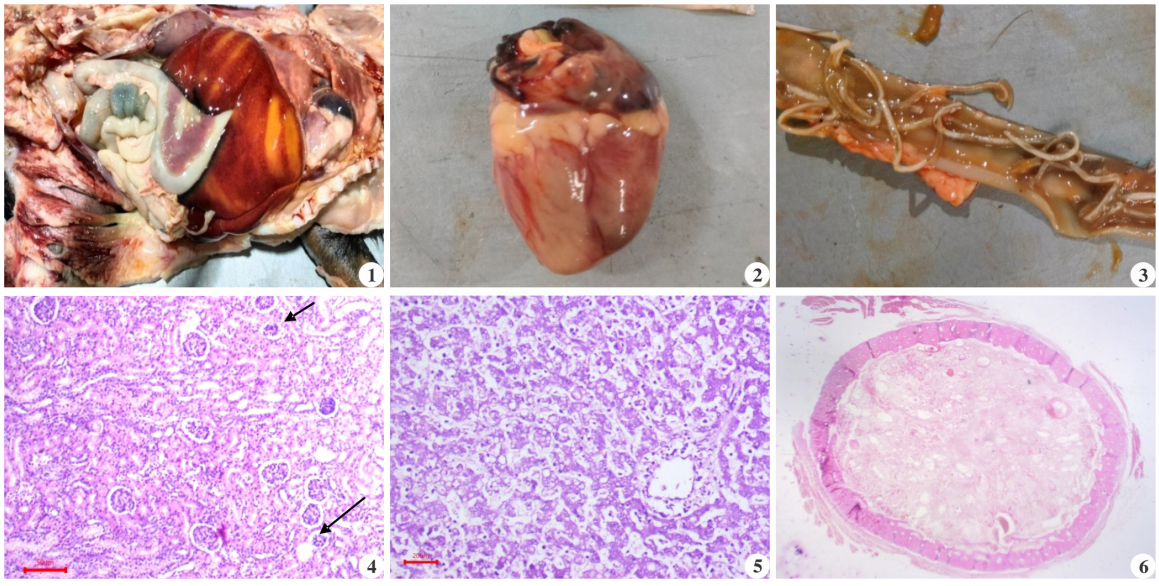

Fig. 1: Liver- enlarged with rib impressions; soft and rounded borders; Fig.2: Heart- pallor of epicardium; Fig.3: Intestine- Lumen occluded with Toxocara canis; Fig.4: Kidney- Glomerular atrophy; 100X; Fig.5:Liver-Fatty change in hepatocytes; H&E stain;200X; Fig.6: Cross section of femur with hypocellular bone marrow inside; 12.5X-H&E

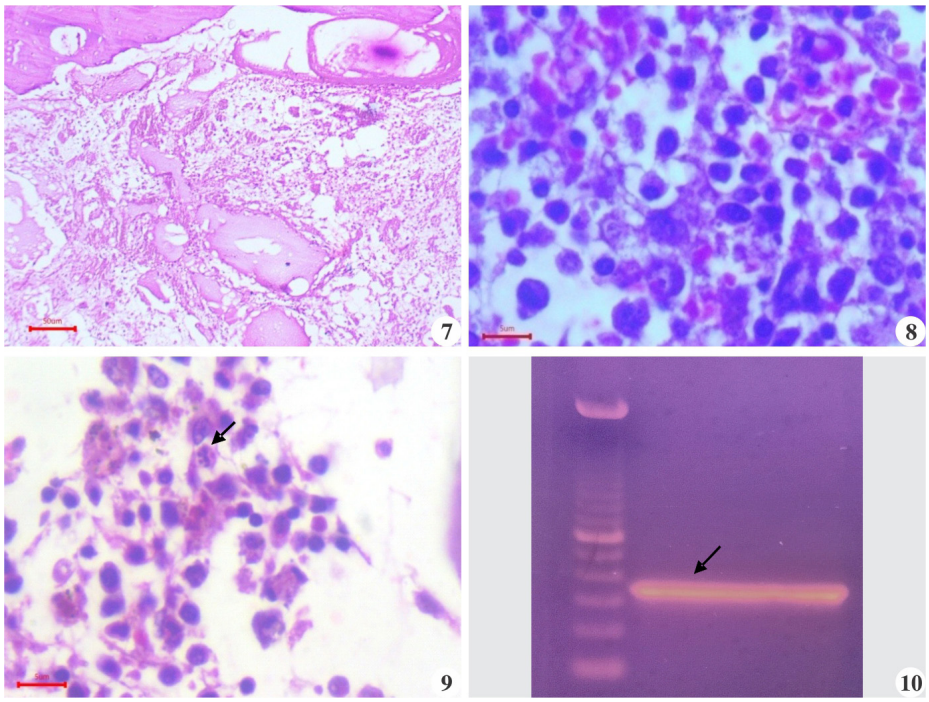

Fig.7. Bone marrow- hypocellularity with adipose tissue and stroma; 100X-H&E; **Fig.8.** Bone marrow- A pool of erythroid blast cells without maturation; 1000X-H&E; **Fig.9.** Bone marrow- Dysplastic neutrophil with fragmented nuclei (black arrow); 1000X; **Fig. 10:** PCR- A lane showing a positive thick band at 377 bp

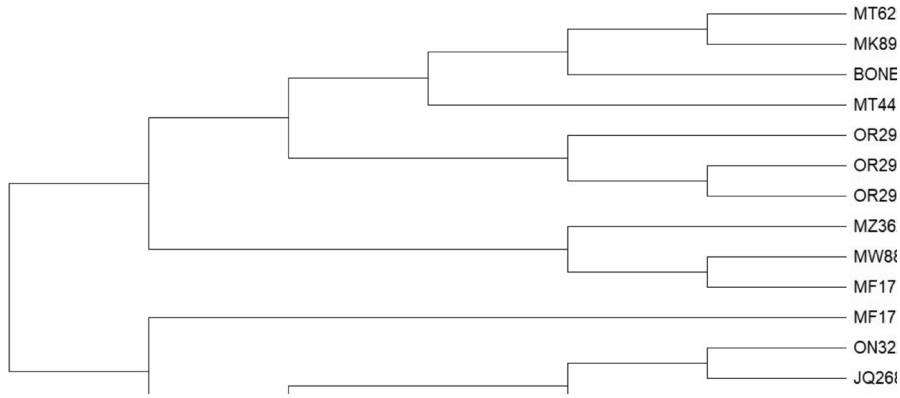

Fig. 11. The phylogeny was inferred using the Maximum Likelihood method and Tamura-Nei (1993) model [10] of nucleotide substitutions and the tree with the highest log likelihood (-556.54) is shown. The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree [11] and a Maximum Parsimony (MP) tree. The NJ tree was generated using a matrix of pairwise distances computed using the Tamura-Nei (1993) model [10]. The MP tree had the shortest length among 10 MP tree searches, each performed with a randomly generated starting tree. The analytical procedure encompassed 18 nucleotide sequences with 339 positions in the final dataset. Evolutionary analyses were conducted in MEGA12 [12] utilizing up to 3 parallel computing threads.

extensive sloughing of the mucosal epithelium with hyperplasia of intestinal crypt epithelial cell. The stomach revealed mild multifocal necrosis of crypt epithelium.

Histopathological examination of bone marrow revealed marked hypocellularity. The marrow spaces were largely replaced by adipose tissue and eosinophilic proteinaceous material i.e., 20% cells and 80% stroma and fat (Figs. 6, 7), with pronounced depletion of the myeloid series, particularly granulocytic precursors. Cytologically, erythropoiesis was minimal with an increased proportion of blast cells (Fig. 8). Occasional dysplastic changes in neutrophils (Fig. 9) were evident, indicating disrupted hematopoiesis. These alterations collectively contributed to severe leukopenia, neutropenia, and immunosuppression characteristic of the disease.

The presence of the CPV in the bone marrow was confirmed by PCR (Fig. 10). Subsequent nucleotide sequencing and comparative analysis, allowed for the accurate typing of the CPV variant. The evolutionary relationship and clustering of the Chennai isolate relative to global and regional strains (CPV-2a, 2b, 2c) were confirmed thereby establishing its regional significance within the phylogenetic lineage (Fig. 11).

DISCUSSION

In this study, we have documented severe bone-marrow hypoplasia in a young pup infected with CPV-2 (confirmed by PCR and VP2 sequencing). The histological (hypocellularity, myeloid depletion) and cytological (erythroid blast-cell increase, dysplastic neutrophils) findings indicate the suppression of haematopoiesis alongside intestinal and lymphoid organs. The findings substantiate the significance of bone-marrow evaluation and its significant prognostic and therapeutic implications in CPV-enteritis.

The CPV preferentially infects highly mitotic cells of intestinal crypt epithelium,

lymphoid tissue and bone-marrow progenitor cells. The destruction of hematopoietic progenitor cells in bone marrow leads to leukopenia, neutropenia and lymphopenia, which are recognized markers of severity in CPV infection[8]. The cytological findings in the case studied (marked myeloidcells depletion, adipose/ stroma replacement, and dysplastic neutrophils) align with earlier reports of bone-marrow alterations associated with CPV infection— for example, vacuolated myeloid precursors and bizarre toxic neutrophil forms.

Bone marrow cytological studies are of prognostic significance and are an indicator for the effective immune response[9]. In the present case, the severe bone marrow suppression of haemopoeisiscorresponded with rapid clinical deterioration and death, reinforcing the link between marrow damage and outcome.

Since the general clinical examination consists of peripheralblood smear examination and Complete blood counts (RBC, Hemoglobin, PCV, WBC, Neutrophils, Lymphocytes, Monocytes, Eosinophils, Platelets), the inclusion of bone marrow cytology/biopsy may refine the diagnosis and prognosisin CPV infections. For example, if marrow reveals near-complete myeloid progenitor loss, the expected neutrophil recovery may be delayed or absent, signalling a guarded to grave prognosis. In addition, marrow evaluation allows exclusion of concurrent processes (e.g., marrow aplasia of other aetiology, neoplasia) in critical cases.

The observed clustering of the bone marrow CPV isolate within the CPV-2a clade confirms that its evolution is driven by mutations in the VP2 gene. The placement implies that the isolate retains the antigenic and genetic characteristics typical of CPV-2a, which result in differences in pathogenicity and vaccine responsiveness compared to the CPV-2b and CPV-2c variants. Consequently, accurate diagnosis necessitates the integration of clinical, pathological, and molecular findings for reliable confirmation. Finally, effective disease prevention and control rely on the critical recognition of factors contributing to vaccine failure, such as improper vaccination timing, interference by maternally derived antibodies, and, more rarely, vaccine virus reversion.

In present case study, the acute canine parvovirus enteritis that led to systemic shock, immunosuppression, and multi-organ damage was confirmed by histopathological, molecular findings of the viral etiology, with evidence of severe bone marrow hypocellularity underlying the clinical immunosuppression. This case study therefore highlights the prognostic significance of bone marrow cytological studies in CPV infection and highlights the urgent need for integrated diagnostic and preventive approaches—combining pathological surveillance, molecular typing (confirming regional significance of CPV-2a), and aggressive supportive care to effectively reduce morbidity and mortality in affected animals.

ACKNOWLEDGEMENT

The authors are grateful for the support received from the Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai, for providing the necessary facilities and infrastructure to complete this case study.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI) Assisted technology for manuscript preparation: The authors confirm that there was no use of AI – Assisted Technology for assisting in the writing of the manuscript and no images were manipulated using AI

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Indian J. Vet. Pathol., 50(1) : 40-43, 2026: DOI: https://doi.org/10.56093/f5pjfn68

RESEARCH ARTICLE

Histopathological findings in a cat with feline panleukopenia

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Received: 17.9.25; Accepted: 14.11.25

ABSTRACT

Feline panleukopenia (FPL) is a highly contagious and often fatal viral disease of cats, characterized by viral tropism for rapidly dividing cells. This study aimed to document the histopathological changes in various organs of a cat affected with feline panleukopenia virus (FPV). Necropsy was performed on one of the sixty-three confirmed FPL cases presented to the Department of Veterinary Medicine, KVAFSU, Hebbal, Bengaluru. The cat, which succumbed during therapy, was subjected to post-mortem examination and the diagnosis was confirmed by PCR, antigen ELISA and pathognomonic lesions. Sixteen tissue samples were collected and processed for histopathology. Gross lesions included emaciation, segmental enteritis with necrotic mucosa, pulmonary congestion, hepatic hemorrhages, cortical necrosis of kidneys and lymphoid depletion in the spleen. Microscopically, epithelial necrosis, villous atrophy, crypt depletion, goblet cell hyperplasia, lymphoid depletion in spleen and mesenteric nodes, bone marrow appeared pale and hypoplastic and extra-intestinal changes in the liver, lungs, kidneys, heart and cerebellum were observed. These findings confirm the predilection of FPV for mitotically active cells in the intestines, bone marrow and lymphoid tissues, leading to severe immunosuppression and systemic organ damage. The results underscore the importance of strengthening vaccination strategies beyond 12 weeks of age for effective prevention.

Keywords: Feline panleukopenia, histopathology, lymphoid depletion, necropsy

INTRODUCTION

Feline panleukopenia (FPL), caused by feline panleukopenia virus (FPV), is one of the most lethal infectious diseases of cats, with a high morbidity and mortality in unvaccinated populations. The virus replicates in rapidly dividing cells such as intestinal crypts, bone marrow progenitors, lymphoid tissues and the fetal/neonatal cerebellum, resulting in severe enteritis, leukopenia, immunosuppression and neurological deficits in neonates[1-3]. Although clinical and diagnostic aspects are well-documented, detailed histopathological descriptions from naturally infected cats in India remain scarce. This case study aimed to characterize the histopathological lesions in different organs of a cat that succumbed to FPL.

How to cite this article : Apoorva H.J., Lathamani V.S., Girish B.C., Sudharani R. and Veena M.P. 2026. Histopathological findings in a cat with feline panleukopenia. Indian J. Vet. Pathol., 50(1) : 40-43.

MATERIALS AND METHODS

One domestic shorthair cat, confirmed positive for FPV by PCR and antigen ELISA (Fig. 1), which succumbed during hospitalization, was subjected to necropsy. Sixteen tissue samples which included the gastrointestinal tract, mesenteric lymph node, spleen, thymus, processing of bone marrow, liver, lungs, kidneys, heart and cerebellum, were collected and fixed in 10% neutral buffered formalin. After 48 hours, tissues were trimmed (3–5 mm), dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. Sections of 4–5 μm thickness were cut using a rotary microtome, mounted on Mayer's albumin-coated slides and stained with hematoxylin and eosin (H&E) following standard protocol[4]. Gross and microscopic lesions were examined under a light microscope and compared with earlier reports on feline panleukopenia.

RESULTS

The gross pathology of the carcass exhibited poor body condition, pale mucous membranes and dehydration (Fig. 2). The intestines were gas-filled, congested and hemorrhagic, with segmental necrosis and fibrinous exudates (Fig. 3). The spleen was markedly reduced in size with pale parenchyma. Lungs showed congestion with petechiae, kidneys revealed cortical necrosis and the liver was friable with multifocal hemorrhages (Fig. 4).

Histopathological Findings

The esophagus (Fig. 5) showed squamous epithelial hyperplasia along with epithelial necrosis, reflecting degenerative and proliferative changes. The stomach (Fig. 6a) exhibited goblet cell hyperplasia, mucosal erosion and accumulation of cell debris

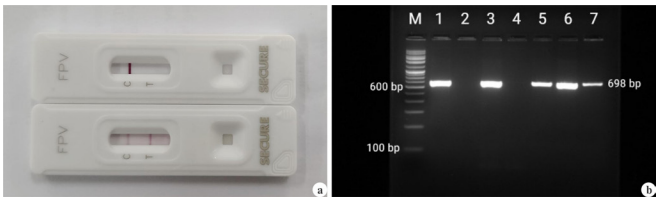

Fig. 1. FPV positive confirmation **a.** FPV-Ag snap test (ELISA) showing positive results. **b.** PCR showing positive with amplicon size of 698bp, Lane M-100bp ladder, Lane 1-positive control, Lane 2-negative control, Lane 3,5,6 & 7-positive clinical samples, Lane 4-negative sample

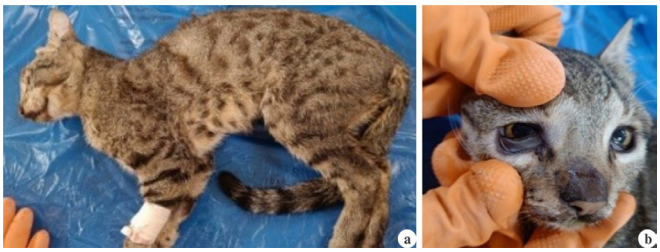

Fig. 2. Low body condition carcass **a.** Severely dehydrated **b.** Pale mucus membranes & sunken eyeballs

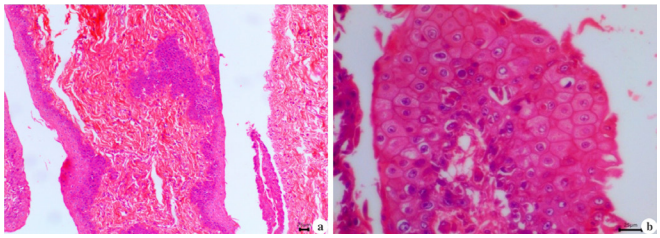

Fig. 5. Section of esophagus showing **a.** Hyperplasia of squamous epithelia **b.** Epithelial necrosis, H&E, (100X & 400X)

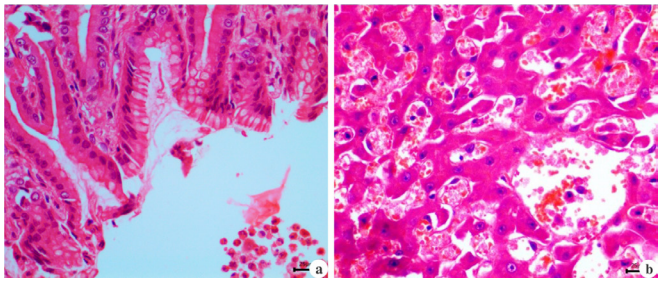

Fig. 6. a. Section of stomach showing goblet hyperplasia, erosion of mucosa and cell debris in lumen H & E, 400X **b.** Section of liver showing sinusoidal dilatation and congestion, H & E, 400X

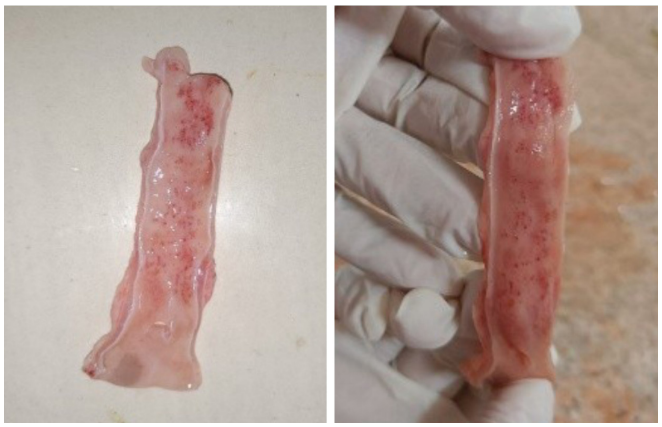

Fig. 3. Segmental enteritis in SI- reddened, thickened and edematous, petechial hemorrhages on serosal surfaces

within the lumen, suggestive of severe mucosal injury associated with FPL infection. Hepatic lesions (Fig. 6b) included sinusoidal congestion, Kupffer cell hyperplasia, focal hepatocellular degeneration and portal mononuclear infiltration[5]. The different parts of small intestines and large intestines including duodenum, jejunum, ileum, cecum and colon (Figs. 7, 8) revealed crypt epithelial necrosis, villous blunting and fusion, epithelial denudation, goblet cell hyperplasia and Peyer's patch depletion, along with severe mucosal hemorrhage accompanied by neutrophilic and macrophagic infiltration [2]. The mesenteric lymph nodes (Fig. 9a) showed lymphoid depletion, follicular necrosis, sinus histiocytosis and plasma cell infiltration, reflecting marked immunosuppression[1]. The heart (Fig. 10a) presented focal myocardial hemorrhages; although myocarditis was not grossly evident, microscopic lesions indicated possible subclinical cardiac involvement[4]. The cerebellum (Fig. 10b) showed neuronal degeneration and hypocellularity, consistent with FPV-induced cerebellar hypoplasia in perinatal infections[1]. The spleen (Fig. 9b) exhibited white pulp atrophy, lymphoid necrosis, cortical depletion and hemorrhage[5]. Renal tissues (Fig. 10c) revealed tubular degeneration, necrosis and hyaline casts within the renal tubules, with associated interstitial congestion[2]. Bone marrow examination (Fig. 9c) demonstrated severe hypocellularity with aplasia of

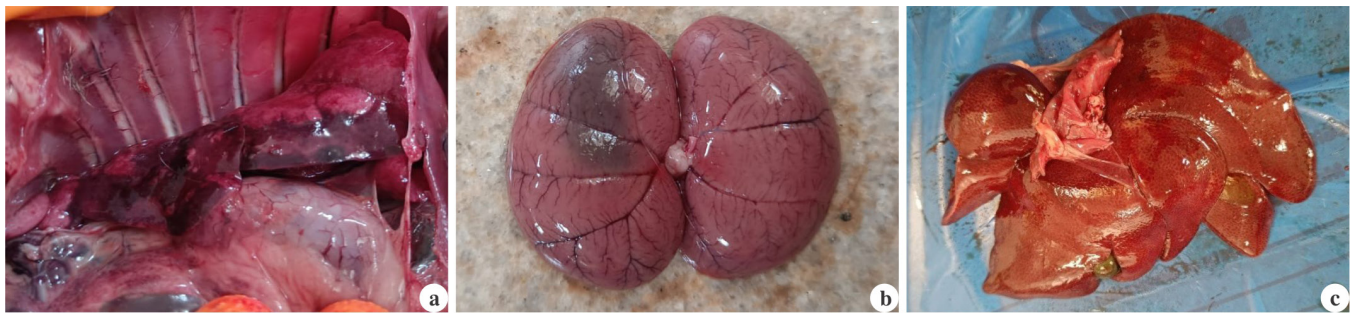

Fig. 4. Gross pathology of Lungs, Kidneys and Liver – **a.** Lungs-hemorrhages with congestion **b.** Kidneys showing cortical necrosis, discoloration & normal medullary region **c.** Liver- hemorrhages and necrosis

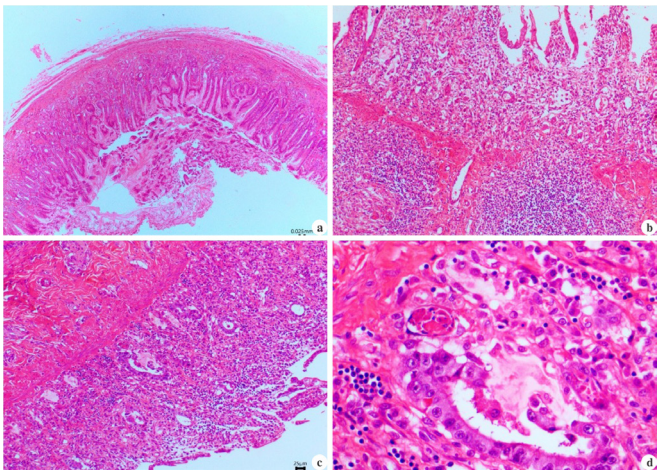

Fig. 7. Histopathological lesions observed in the small intestinal parts of FPV affected cat-**a.** Section of duodenum showing denudation of epithelia, goblet hyperplasia, fusion of villi, H & E, 40X **b.** Section of jejunum depleted payer's patches with granuloma and fusion of villi necrosis of epithelia, H & E, 100X **c.** Section of ileum showing crypt epithelial necrosis & fusion of villi necrosis of epithelia of villi with infiltration of mononuclear cells, H & E, 100X and 400X

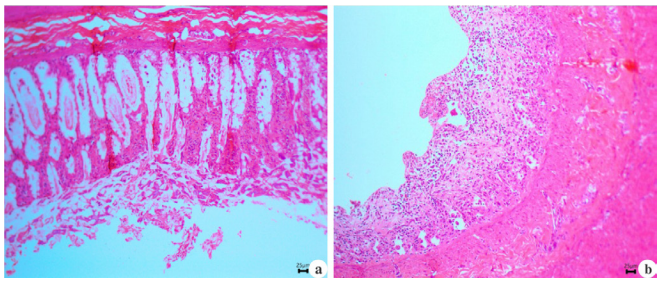

Fig. 8. Histopathological lesions observed in the large intestinal parts of FPV affected cat-**a.** Section of caecum showing dilation of glands, necrosis & sloughing of lining cells **b.** Section of colon showing Inflammatory changes in mucosa (H & E, 10X)

myeloid and erythroid series, adipose replacement and reduced mitotic activity, consistent with panleukopenia[5]. Pulmonary changes (Fig. 10d) were characterized by interstitial pneumonia with alveolar edema, congestion and bacterial aggregates, most likely secondary to neutropenia[7].

DISCUSSION

The histopathological findings confirm FPV's strong tropism for mitotically active tissues, especially intestinal crypts, bone marrow precursors and lymphoid follicles. Intestinal lesions such as villous blunting, crypt necrosis and epithelial sloughing accounted for the severe diarrhea and malabsorption observed clinically, while bone marrow aplasia and lymphoid depletion explained the profound leukopenia and immunosuppression[2,3].

Secondary bacterial infections, as evident in pulmonary pneumonia, likely exacerbated the clinical

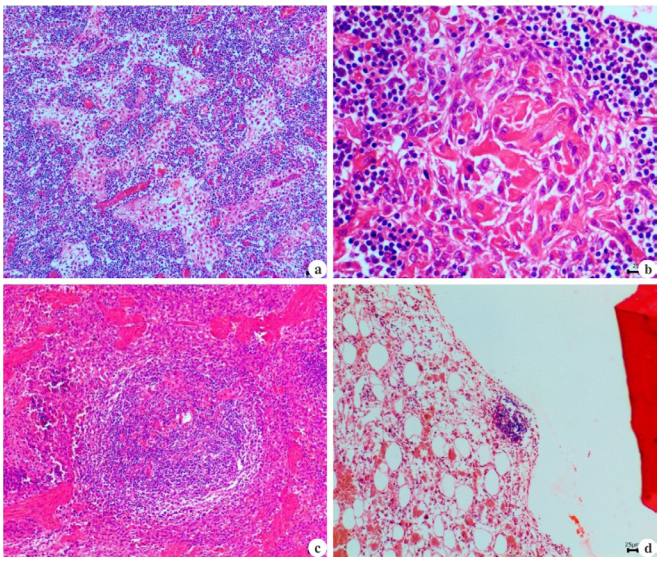

Fig. 9. Histopathological lesions showing immunosuppression in FPV affected cat-**a.** Section of mesenteric lymph node showing hypocellularity, infiltration of plasma cells and granuloma formation within follicle (H & E, 100X and 400X) **b.** Section of spleen showing decreased cellularity, H & E, 100X **c.** Section of bone marrow showing severe hypocellularity, note foci of lymphoid aggregation, H & E,10X

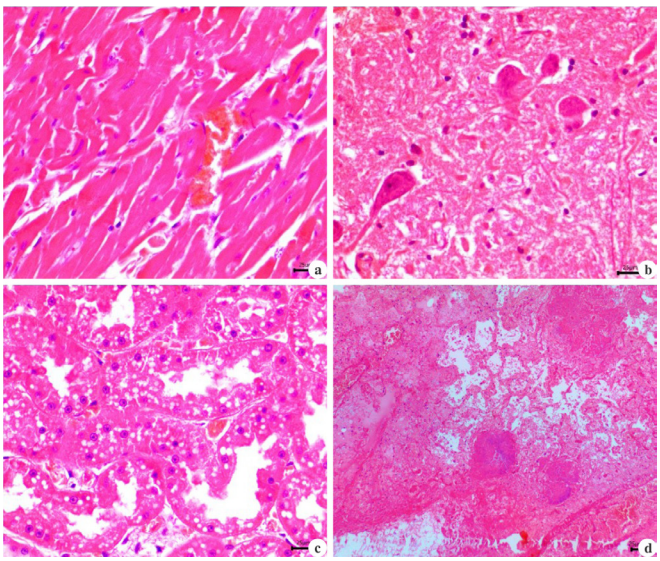

Fig. 10. Histopathological lesions in vital organs due to FPV **a.** Section of heart showing focal hemorrhages, H & E, 400X **b.** Section of cerebellum showing neuronal degeneration and decreased cellularity, H & E, 400X **c.** Section of kidney showing vacuolar degeneration in tubular epithelia, H & E, 400X **d.** Section of lung showing severe pneumonia with secondary bacterial aggregation and edema, H & E, 100X

deterioration[5]. Neural involvement in the cerebellum highlighted the risk of congenital cerebellar hypoplasia in kittens infected in utero[1]. In our case cardiac damages were minimal due to the acute stage of the disease but the literature suggests FPV DNA and myocardial inflammation can occur in some advanced cases[6,7].

The pathological changes in this cat mirrored those reported globally[8-10] and reinforced the importance of complete vaccination schedules beyond 12 weeks of age to ensure adequate protection.

CONCLUSION

Histopathological analysis of feline panleukopenia in this case confirmed characteristic lesions in the gastrointestinal tract, bone marrow and lymphoid organs, along with multi-organ involvement including liver, lungs, kidneys, cerebellum and heart. These changes reflect viral replication in rapidly dividing cells and the systemic consequences of immunosuppression. Strengthening vaccination protocols and early supportive interventions remain essential to reduce mortality in cats with FPL.

ACKNOWLEDGEMENT

The authors express their sincere gratitude to the Dean and the faculty of the Department of Veterinary Medicine and Veterinary Pathology, Veterinary College, Bengaluru, for their valuable support and guidance throughout the course of this case study.

Financial Support & Sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 44-47, 2026: DOI: https://doi.org/10.56093/bdhj7n10

RESEARCH ARTICLE

Hepato-nephropathy associated with aflatoxicosis complicated with inclusion body hepatitis in Broilers

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Received: 19.8.2025; Accepted: 12.9.2025

ABSTRACT

Poultry continues to be one of the fastest growing segments of the agricultural sector in India today. However, this sector faces greater degree of economic losses due to mycotoxicosis. The present study investigated the aflatoxicosis complicated with inclusion body hepatitis. A total of 64 birds from 10 different broiler farms were presented for necropsy. The carcasses revealed large areas of cooked or parboiled appearance in the pectoral muscles. The liver showed diffuse swelling with multifocal, small to large, pale whitish necrotic stripes/patches. In many birds, liver appeared yellowish and fragile and in few cases, focal hemorrhages were also observed. Kidneys were diffusely swollen, necrotic and mottled. The cardiac muscles showed multifocal to diffuse whitish areas. Hydropericardium was recorded in carcasses from 2 flocks. The spleen was diffusely swollen, pale and focally congested. The histopathological examination of liver revealed moderate to severe multifocal round vacuoles in cytoplasm, multifocal areas of necrosis leading disruption of hepatic cord arrangement, individualization of hepatocytes and multifocal areas of infiltration predominantly by PMNs with a few MNCs. In 2 out of 10 flocks, the liver also revealed typical intranuclear inclusion bodies in addition to above changes. Kidneys showed mild to moderate degenerative and necrotic changes with tubular swelling, thickening of glomerular basement membranes and increased number of mesangial cells. The cardiac muscles exhibited interstitial infiltration. Spleen showed multifocal areas of lymphoid depletion and associated reticulo endothelial cell hyperplasia. Bursa of fabricius showed moderate to severe lymphoid depletion. DNA was extracted from liver and spleen samples and the conventional PCR targeting hexon gene specific for FAdV yielded expected product of 897 bp in 3 flocks. Toxicological analysis of feed samples confirmed the presence of toxic levels of aflatoxin (AFB1-181, 196 and 241 ppb, AFB2-15, 15 and 20 ppb) from 3 different farms including those 2 farms showing intranuclear inclusions in hepatocytes and PCR positivity for FAdV. The gross and histopathological lesions, toxicological analysis and PCR confirmed the occurrence of aflatoxicosis and IBH together.

Keywords: Aflatoxin, fowl adenovirus, hepato-nephropathology

INTRODUCTION

Poultry farming plays a vital role in the global food industry, with broilers forming a significant portion of meat production due to their rapid growth rate and feed efficiency. However, one of the persistent challenges in broiler production is the contamination of feed with mycotoxins, particularly aflatoxins. Aflatoxins are important mycotoxin which are hepatotoxic, mutagenic and carcinogenic compounds produced mainly by *Aspergillus* spp., which are more prevalent in tropical regions. Among the aflatoxins, aflatoxin B1 is considered as a most potent hepatotoxic agent and induces high morbidity, mortality and production losses. Extended and improper storage of chicken feed leads to increased aflatoxin production. The clinical symptoms of aflatoxicosis include fatigue, loss of appetite, reduced growth rate, microbial stress, economic losses and toxicity[1]. In field conditions, most of the time, high levels of mycotoxins in feed result in immunosuppression that exposes birds to infectious diseases. Fowl adenoviruses are common pathogens secondary to mycotoxicosis. IBH is the most common concomitant disease along with aflatoxin B1 when present in feed at higher than permissible levels, *i.e.* 20 ppb[2].

Fowl adenovirus (FAdV) infection in broilers is a disease that leads to significant economic losses for poultry farmers due to increased mortality and decreased productivity from poor chicken performance. FAdVs cause various diseases, including inclusion body hepatitis (IBH), hydropericardium syndrome (HPS), respiratory infections, gizzard erosions, arthritis and pancreatitis[3]. In addition to its direct impact, the virus acts as an immunosuppressive agent, this can result in secondary complications. Although most FAdVs are considered non-pathogenic due to their widespread presence

How to cite this article : Chandan, H.U., Dhaygude, V.S., Mote, C.S., Tumlam, U.M., Bhandekar, S., Dethe, P. and Sonawane, G.S. 2026. Hepato-nephropathy associated with aflatoxicosis complicated with inclusion body hepatitis in Broilers. Indian J. Vet. Pathol., 50(1) : 44-47.

in poultry flocks, certain Group I FAdVs, such as serotypes 4 and 8, are pathogenic and linked to IBH in chickens. While it was previously thought that immunesuppression caused by pre-infection or concurrent infection with Infectious Bursal Disease Virus (IBDV) or Chicken Infectious Anemia virus (CIAV) contributed to IBH, recent studies suggest that these infections or other immunosuppressive factors may not be necessary for the onset of IBH caused by FAdV[4]. This indicates that FAdV is emerging as a primary pathogen capable of causing significant diseases, with or without predisposing factors.

The concurrent impact of aflatoxicosis and IBH in broilers has a synergistic and devastating effect on health and productivity. Field data from India underscore that FAdV particularly serotypes 11 and 8b is rapidly becoming a primary threat to flock health, independent of secondary immunosuppressive triggers. However, this investigation reports concurrent occurrence of inclusion body hepatitis and aflatoxicosis in commercial broiler chickens.

MATERIALS AND METHODS

The current study examined aflatoxicosis complicated by inclusion body hepatitis (IBH) in spontaneous field cases. A total of 64 birds from 10 different broiler farms were brought to the Department of Veterinary Pathology, KNPCVS, Shirwal, for necropsy during September and October, 2024.

Necropsy

Necropsy examinations were performed on the deceased birds and the gross findings were documented. Representative tissue samples were collected and preserved in 10% neutral buffered formalin for histopathological analysis and some stored at -80^{O}C for molecular studies. Feed samples were collected for toxicological analysis.

Histopathology

The tissue samples were fixed in 10% neutral buffered formalin and processed using the routine paraffin-embedding technique. In brief, 4 µm thick sections were deparaffinized and stained using the Hematoxylin and Eosin staining method for detailed microscopic examination.

DNA extraction & polymerase chain reaction (PCR)

The tissue samples stored at -80^{O}C were processed for DNA extraction using the DNeasy Blood and Tissue Kit, according to the manufacturer's instructions. PCR was carried out using primers targeting the hexon gene of FAdV Group I (Forward primer: 5'-CAARTTCAGRCAGACGGT-3'; Reverse primer: 5'-TAGTGATGMCGSGACATCAT-3'). The PCR reaction mixture of 25 µl consisted of 3.0 µL DNA (5 ng/µL), 12.5 µL DreamTaq PCR Master Mix (Thermo Scientific, USA), 1.0 µL forward primer (10 pmol/µL), 1.0 µL reverse primer (10 pmol/µL) and 7.5 µL nuclease free water. The amplification conditions included an initial denaturation at 95^{O}C for 15 minutes, followed by 35 cycles of 94^{O}C for 45 seconds, 57^{O}C for 45 seconds, 72^{O}C for 45 seconds and a final extension at 72^{O}C for 10 minutes.

The PCR products were then analyzed using electrophoresis on a 1.5% agarose gel (in 0.5 Tris-Borate EDTA buffer), stained with ethidium bromide (0.5 µg/mL). After the dye had migrated sufficiently, the gel was visualized under ultraviolet light (302 nm) and documented using Alpha Imager software. The relative size of the amplified product was determined by comparing it with a standard DNA molecular weight marker run along side the PCR products (Thermo Scientific, USA).

Toxicological analysis

Feed samples were collected from different flocks. Aflatoxin B1, Aflatoxin B2, Aflatoxin G1, Aflatoxin G2, Ochratoxin, T-2 toxin, Citrinin, Zearalenone were estimated in the feed samples by the method described by Romer (1975)[5]. The toxins were extracted with acetone, treated with cupric carbonate and ferric gel to eliminate fluorescent materials other than aflatoxin, then washed with acid and alkali and extracted with chloroform, dried, rediluted with chloroform and spotted in an activated thin layer chromatography (TLC) plate with standards and ascertained the concentration by visual comparison method in a UV viewing cabinet[6].

RESULTS

Gross and Histopathological findings

A detailed necropsies showed large areas of cooked or partially cooked appearance in pectoral muscles (Fig. 1). The liver exhibited diffuse swelling with multifocal, small to large, pale, whitish necrotic streaks or patches on the surface. In several birds, the liver appeared yellowish (fatty) and fragile with focal hemorrhages (Fig. 2). The kidneys were consistently swollen, necrotic and mottled (Fig. 3). The cardiac muscles showed areas of whitish discoloration both multifocally and diffusely. Hydropericardium was recorded in carcasses of three flocks. The spleens were enlarged, pale and occasionally congested in focal areas (Fig. 4).

The histopathological examination of the liver revealed moderate to severe multifocal round vacuoles (clear cell areas) in the cytoplasm (Fig. 5), along with multifocal necrotic areas causing disruption of the hepatic cord arrangement, individualization of hepatocytes and multifocal infiltration primarily by polymorphonuclear cells (PMNs). Focal congestion and mild periarteriolar infiltration of MNCs were also observed (Fig. 5). In two out of the ten flocks, the liver showed typical intranuclear

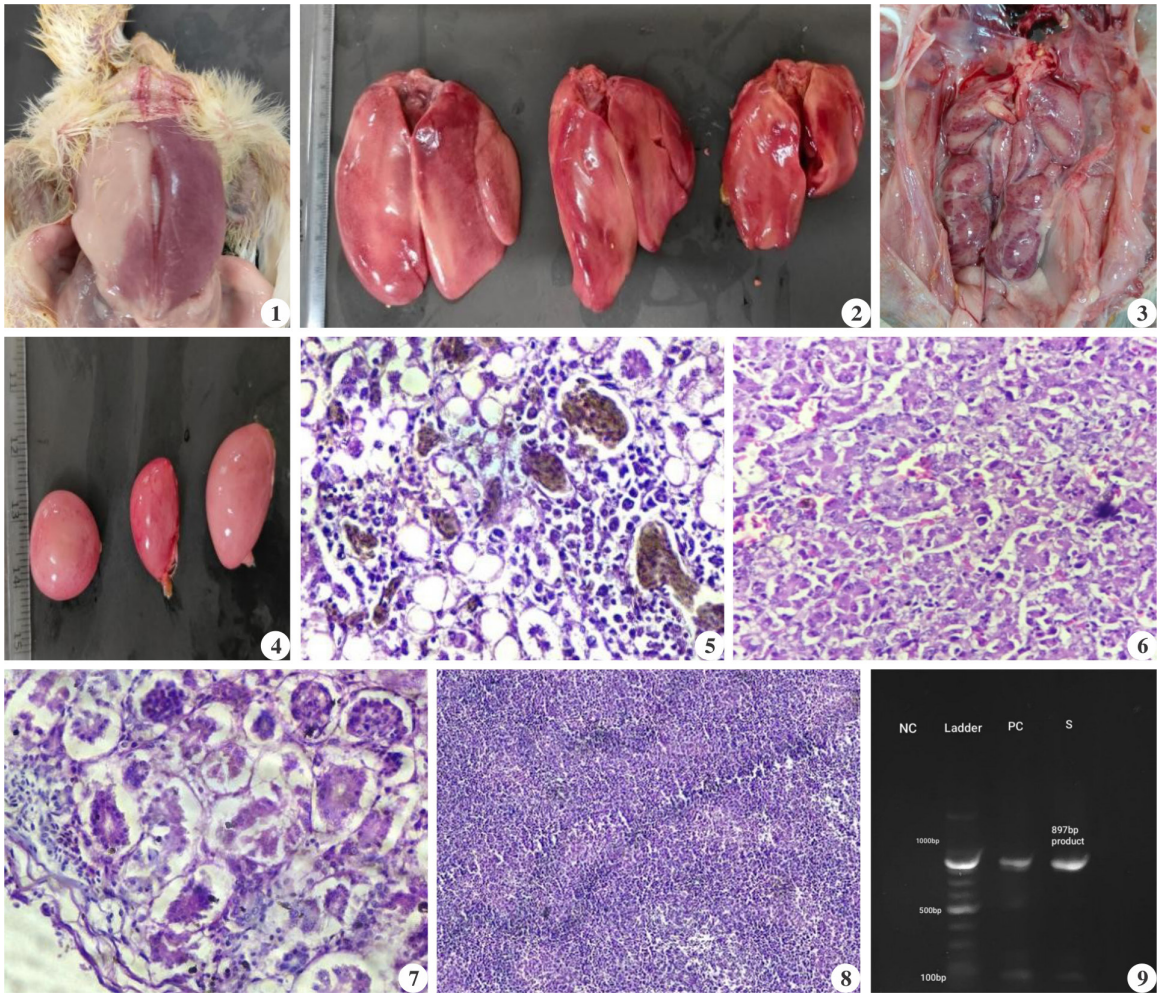

Fig. 1. Cooked or parboiled large area in pectoral muscles; **Fig. 2.** The liver showed diffuse swelling, multifocal small to large pale whitish necrotic stripes/patches; **Fig. 3.** Kidneys were diffusely swollen, congested, necrotic and mottled; **Fig. 4.** Spleen were diffusely swollen, pale and focally congested; **Fig. 5.** Liver: Moderate to severe multifocal round vacuoles (fatty change) in cytoplasm & focal congested areas (H&E x400); **Fig. 6.** Liver: Degenerative & necrobiotic changes in hepatocytes & basophilic intranuclear inclusion bodies (H&E x400). **Fig. 7.** Kidney: Tubular degeneration, necrosis and infiltration (H&E x400); **Fig. 8.** Spleen: Multifocal areas of lymphoid depletion and reticulo endothelial cell hyperplasia (H&E x100); **Fig. 9.** Agarose gel electrophoresis showing PCR amplification of "hexon" gene (897 bp) (1000 bp ladder, NC: Negative control, PC: Positive control, S: Sample).

Flock no.	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin	T-2 toxin	Citrinin	Zearalenone
1	181ppb	15ppb	ND	ND	ND	ND	ND	ND
2	196ppb	15ppb	ND	ND	ND	ND	ND	ND
3	241ppb	20ppb	ND	ND	ND	ND	ND	ND

ND: Not Detected

inclusion bodies in addition to the other changes (Fig. 6). The kidneys exhibited mild to moderate degenerative and necrotic changes, including tubular swelling and increased number of mesangial cells (Fig. 7). Cardiac muscles showed interstitial infiltration. The spleen displayed multifocal lymphoid depletion along with reticulo endothelial cell hyperplasia (Fig. 8).

Genome detection studies

DNA was extracted from liver and spleen samples and conventional PCR targeting the hexon gene specific to FAdV produced the expected 897 bp product in three flocks (Fig. 9).

Toxicological analysis of feed samples

Toxicological analysis confirmed the presence of toxic levels of aflatoxin, with AFB1 levels of 181, 196 and 241 ppb and AFB2 levels of 15, 15 and 20 ppb, in feed samples from three different farms which were also found positive for IBH histopathologically and confirmed by PCR. The

other toxins like Aflatoxin G1, Aflatoxin G2, Ochratoxin, T-2 toxin, Citrinin, Zearalenone were not detected in any of the tested flocks.

DISCUSSION

The simultaneous detection of high aflatoxin levels and histopathological lesions typical of IBH (e.g., intranuclear inclusion bodies) along with PCR confirmation suggests a synergistic role of aflatoxins and FAdV in exacerbating hepatic damage and immunosuppression. The observation of cooked or partially cooked pectoral muscles is unusual in aflatoxicosis + IBH, since this appearance is classically associated with heat stroke or deep pectoral myopathy due to ischemic necrosis. In this case, the presence of aflatoxin induced hepatic dysfunction and IBH associated circulatory disturbance may have predisposed to severe ischemic myopathy, producing the "cooked" appearance. Reduced detoxification capacity of the liver could exacerbate muscle hypoxia, leading to protein denaturation in muscles. The hepatic failure and anemia in IBH could impair oxygen delivery, contributing to muscular ischemia and the cooked like gross change. Macroscopic and microscopic examination of the liver showed hallmark changes of both aflatoxicosis (fatty change, fragility, hemorrhages) and IBH (intranuclear inclusion bodies, necrosis, PMN infiltration). This dual pathology confirms co-morbidity and emphasizes the need for differential diagnosis. Aflatoxin B1 levels in all three affected flocks (181-241 ppb) exceeded acceptable limits, indicating feed contamination and an important predisposing factor. The absence of other mycotoxins supports the conclusion that AFB1 was the primary toxin involved. Aflatoxins are known to suppress the immune system, likely facilitating FAdV replication and worsening the clinical severity of IBH. This highlights the importance of toxin control in preventing viral opportunism. Lesions in kidneys, cardiac muscles and spleen, along with hydropericardium and cooked muscle appearance were pointing to systemic toxicity[7]. These findings align with both aflatoxin poisoning and systemic IBH, making diagnosis more complex. PCR amplification of the hexon gene results revealed the presence of a DNA band measuring 897 base pairs (bp) and same findings were correlated with the previous findings[8] confirmed the presence of FAdV in affected tissues, validating the IBH diagnosis and its co-occurrence with aflatoxicosis. Aflatoxins cause oxidative stress, impair detoxification and filtration processes and suppress immunity, while FAdV-induced IBH exacerbates hepatic injury through viral replication and inclusion body formation. The combined effect leads to marked histopathological changes, immunosuppression and increased mortality, highlighting the need for early diagnosis, mycotoxin control and FAdV monitoring in broiler production. The presence of aflatoxins in feed not only impacts poultry health but also poses a potential public health risk *via* the food chain. Economic losses due to mortality and reduced productivity are significant, particularly in commercial poultry operations. Regular testing of feed for aflatoxins and implementation of mycotoxin binders or detoxifiers is essential in disease prevention, especially in areas with known FAdV prevalence. A comprehensive approach including biosecurity, virological monitoring, feed hygiene and farmer education is required to prevent recurrence of such compounded outbreaks.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 48-56, 2026: DOI: https://doi.org/10.56093/pykd1c30

RESEARCH ARTICLE

Hepatoprotective effect of microalgal *Spirulina* extract fortified with finger millet and *Moringa* leaves on arsenic induced hepatotoxicity in Wistar rats

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Received: 31.10.25, Accepted: 18.11.25

ABSTRACT

The present study was conducted to assess the hepatoprotective effects of microalgal *Spirulina* extract fortified with finger millet and *Moringa* leaves (MSFM) in male Wistar rat model of sodium arsenite induced hepatotoxicity. The study consisted of six groups (Group I to VI), comprising of six rats in each group. Group I (normal control) rats received normal saline orally daily for 60 days. All the rats in Group II (disease control) to VI rats received sodium arsenite @ 10mg/kg body weight orally daily for 60 days. In addition to sodium arsenite, Group III (reference control) rats received Telmisartan @ 10mg/kg body weight orally for 60 days; while Group IV, V and VI rats received MSFM@100mg/kg, @200mg/kg and @400mg/kg body weight orally daily for 60 days, respectively. Sodium arsenite exposure in Group II rats resulted in significant alterations in body weight, hematological, biochemical, antioxidant, lipid peroxidation and pathomorphological parameters of liver compared to the normal control rat. Telmisartan administration partially alleviated these changes. MSFM supplementation demonstrated a dose-dependent protective effect, evident through marked improvements in hematological and serological parameters, antioxidant status, reduction of lipid peroxidation and histopathological restoration when compared to Group II rats. Among the treatment groups, MSFM at 400 mg/kg bodyweight offered the highest degree of protection, surpassing Telmisartan, while 200 mg/kg provided moderate protection. These findings highlight the potential of *Spirulina*, *Moringa* and finger millet based MSFM formulation as a natural therapeutic strategy for mitigating sodium arsenite induced hepatotoxicity.

Key words: Arsenite, Finger millet, hepatotoxicity, microalgal *Spirulina* extract, *Moringa*

INTRODUCTION

Water is vital for all living organisms, yet its quality and availability are increasingly threatened by growing populations and rising demand for clean water in domestic and economic activities. However, river systems are often heavily polluted with heavy metals from domestic, industrial, mining, and agricultural effluents[1].

Heavy metals are major environmental pollutants due to their high toxicity, persistence, and bio-accumulative nature. Although some occur naturally, human activities have significantly increased their environmental presence. Interaction with air, water, and soil enhances their toxicity, leading to entry into the food chain and subsequent exposure in humans and animals[2]. Chronic exposure disrupts essential biochemical processes and damages vital organs such as the liver, heart, brain, and kidneys. Their non-biodegradable nature further contributes to long-term health and environmental risks[3].

Amongst the heavy metal toxicity, arsenic toxicity especially has been a significant concern in areas where the ground water level of arsenic is much high[4]. Long-term consumption of arsenic contaminated water can lead to arsenicosis, a form of chronic arsenic poisoning affecting both humans and animals. To safeguard public health, the World Health Organization has set the maximum allowable limit for arsenic in drinking water at 0.01 mg/L. Water with arsenic concentrations above this level is deemed unsafe[5].

Recent findings support that oxidative stress, through elevated reactive oxygen species and increases lipid peroxidation, disrupts normal tissue respiration, and stimulates the release of

How to cite this article: Golla H., Shilpa V.T., Girish B.C., Anjan Kumar K.R., Ramesh D., Rajashailesha N.M. and Pramod S.N. Hepatoprotective effect of microalgal *Spirulina* extract fortified with finger millet and *Moringa* leaves on arsenic induced hepatotoxicity in Wistar rats. Indian J. Vet. Pathol., 50(1) : 48-56.

pro-inflammatory cytokines, playing a key role in the development of arsenic-induced organ toxicity[6].

Spirulina is a microscopic and filamentous cyanobacterium that belongs to family *Oscillatoriaceae* and has a long history of use as food and food supplement. Multiple studies have demonstrated that *Spirulina* possesses a wide range of health-promoting properties, including anti-inflammatory, anticancer, antibacterial, antioxidant, anti-anemic potential and immune-modulatory effects[7].

Moringa's antioxidant and anti-inflammatory effects are attributed to its rich bioactive composition, including phenolic acids, flavonoids, alkaloids, phytosterols, vitamins, and minerals, supporting its traditional use against heavy metal toxicity[8]. Similarly, finger millet exhibits strong antioxidant potential due to its high levels of enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and non-enzymatic (glutathione, vitamins E and C) antioxidants[9].

Spirulina, *Moringa* leaves, and finger millet are recognized for their exceptional phytonutrient, vitamin, and mineral composition, offering high-energy nutrition with a low glycemic index. These super foods possess strong antioxidant, anti-inflammatory, and immune-enhancing properties that contribute to overall health improvement**.** Fortification of microalgal *Spirulina* extract with *Moringa* leaves and finger millet is expected to enhance its therapeutic potential by developing a synergistic, nutrient-dense formulation with improved functional and protective properties. Hence, the present study was designed to assess the hepato-protective effects of *Spirulina* extract fortified with finger millet and *Moringa* leaves against arsenic-induced toxicity in Wistar rats.

MATERIALS AND METHODS

Animals: The research was carried out on adult healthy male Wistar albino rats. Adult male Wistar rats, each weighing between 150 to 200 grams, were obtained from the Chromed Biosciences Private limited Labs Plot No. C- 38, KIADB, Industrial area, Hirehalli, Mydala, Tumkur, Karnataka-572168 (Reg. No. 2171/PO/RcBiBt/S/22/CCSEA). All the rats were acclimatized to standard laboratory conditions at Small Animal House facility, Hassan for seven days prior to initiation of the experiment and maintained at 25±2°C housing temperature and relative humidity of 50 to 70 per cent and to laboratory conditions of 12-hour light/dark cycle throughout the study period and provided with regular standard pellet diet along with free access to deionized drinking water *ad libitum* throughout the course of the experiment. All the protocols were adhered as per the guidelines of the Committee for Control and Supervision of Experiments on Animals for care and use of laboratory animals and were approved by the Institutional Animal Ethics Committee at the Veterinary College, Hassan (HVC/IAEC/11/2025).

Preparation of test item and mode of administration: Sodium arsenite was obtained from Jai Maruthi Scientific Bangalore, manufactured by NICE Chemicals Private Limited, Kochi. It was administered orally at a dose of 10 mg/kg body weight, in normal saline, daily for 60 days. MSFM in powder form was received from Department of Studies in Food Technology, Davangere University, and Karnataka. It was used at the dose rates of 100, 200, and 400 mg/kg body weight. Telmisartan procured from Intas Pharmaceutical Limited, Ahmedabad, India was administered orally daily at a dose of 10 mg/kg body weight.

Experimental protocol: The study consisted of six groups (Group I to VI), comprising of six rats in each group. Group I (normal control) rats received normal saline orally daily for 60 days. All the rats in Group II (disease control) to VI rats received sodium arsenite@10mg/kg body weight orally daily for 60 days. In addition to sodium arsenite, Group III (reference control) rats received Telmisartan@10mg/kg body weight orally for 60 days; while Groups IV, V and VI rats received MSFM@100mg/kg, @200mg/kg and @400mg/kg body weight orally daily for 60 days, respectively.

Parameters assessed: Body weight (g) were assessed every week. All animals were examined twice daily for any signs of clinical abnormalities or symptoms. At the end of the study, all rats were ethically euthanized by administering an intramuscular overdose of Ketamine and Xylazine. Blood samples were drawn from the retro-orbital plexus and collected into EDTA vials for hematological analysis and serum vials for biochemical evaluation.

Estimation of antioxidant enzymes: The representative tissue samples from liver were dissected and washed with Normal saline to remove any tissue debris and blood clots. The collected liver samples were homogenized in a solution of ice-cold 0.1M Phosphate buffered saline (pH 7.4) at 4ºC and centrifuged for 10 minutes at 15,000 rpm. The supernatants were stored at -80ºC for analysis of superoxide dismutase, catalase, and thiobarbituric acid reactive substances.

Histopathological analysis: Liver tissue from rats of all the groups was subjected to histopathological studies. The tissue was fixed using 10 per cent Neutral Buffered Formalin solution and sections were prepared using paraffin blocks and stained with hematoxylin and eosin stain and Masson's Trichrome stain[10].

Histopathological scoring for liver: The liver samples were examined in random microscopic areas semi-quantitatively under high power fields and the number of changes were assessed by the counting twenty non overlapped fields for the same slide of each animal. The extent of damage and the severity of lesions in the liver were assessed semi-quantitatively[11] with slight modification (Table 1).

Statistical analysis: Statistical analysis of the data collected for various parameters was done using one-way ANOVA with Post-hoc test (Tukey's test) [12] using the software SPSS, version-16.0.

Table 1. Histopathological scoring system for liver

Parameters	Score 0	Score 1	Score 2	Score 3	Score 4
Vacuolar degeneration	Absent	Minimal (1-10 %)	Mild (11-30 %)	Moderate (31-60 %)	Severe (61-100 %)
Sinusoidal dilatation	Absent	Minimal (1-10 %)	Mild (11-30 %)	Moderate (31-60 %)	Severe (61-100 %)
Bile duct epithelium proliferation	Absent	Minimal (1-4 bile duct showing epithelial hyperplasia)	Mild (4-8 bile duct showing epithelial hyperplasia)	(8-12 bile duct showing epithelial hyperplasia)	(>12 bile duct showing epithelial hyperplasia)
New bile duct formation	Absent	Minimal (1-5 number)	Mild (5-10 number)	Moderate (10-15 number)	Severe (>15 number)
Inflammatory cell infiltration	Absent	Minimal (1-10 %)	Mild (11-30 %)	Moderate (31-60 %)	Severe (61-100 %)
Central and portal vein dilatation and congestion	Absent	Minimal (1-5 number)	Mild (5-10 number)	Moderate (10-15 number)	Severe (>15 number)
Thickening of blood vessels	Absent	Minimal (0-1 number)	Mild (2-4 number)	Moderate (4-6 number)	Severe (>8 number)

RESULTS

General observation: Group I rats remained healthy and active throughout the experiment. Group II rats exhibited clinical signs such as reduced feed intake, reduced body weight, ruffled hairs, dehydration, restlessness and were difficult to handle. The rats of Group III to VI manifested similar clinical signs as that of disease control rats, but with reduced intensity and frequency.

Body weight: The mean body weights in grams with standard error of mean at different time intervals of 0 day and 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th week of the experiment have been presented in Table 2. Group I rats remained healthy and active throughout the period of experiment and demonstrated steady and progressive enhancement in their body weight over the course of experiment. Body weight of Group II rats were decreased significantly from 3rd week to 8th week in comparison to Group I rats. Group III rats showed a significant and consistent increase in body weight from the 4th to 8th week compared to Group II. MSFM-treated rats (Groups IV to VI) exhibited a consistent and significant ($p<0.05$) increase in body weight throughout the study compared to Group II. Also, Group VI showed improvement comparable to Group I normal control rats throughout the experiment period.

Hematological parameters: The mean values of hematological parameters with standard error of mean on 60th day of the experiment is presented in Table 3. The

Table 2. The mean (± SE) body weight (g) values of rats of different experimental groups in the study at weekly interval

Groups	Day 0	1st Week	2nd Week	3rd Week	4th Week	5th Week	6th Week	7th Week	8th Week
Group I (NC)	170.83 ±2.83	181.67 ± 2.23^{a}	200.17 ± 3.08	223.17 ± 2.09	243.67 ± 3.13	268.50 ±3.86	276.33 ± 3.79	282.83 ± 4.04	294.50 ± 3.35
Group II (DC)	172.67 ± 3.90	179.17 ±3.72	191.33 ± 2.51^{a}	202.00 ± 2.91^{b}	212.00 ± 3.30	221.00 ± 3.13	229.00 ± 4.62	240.83 ± 4.44	250.33 ± 2.99
Group III (RC)	171.17 ± 4.61	180.50 ±4.57	193.17 ± 3.84^{a}	212.33 ± 3.26^{abc}	233.33 ± 1.78	255.50 ± 1.45	264.83 ± 1.92^{a}	273.83 ± 1.08^{a}	277.67 ± 1.15
Group IV (MSFM@100)	169.67 ± 2.38	180.17 ±1.82	191.17 ± 0.95^{a}	207.00 ± 1.95^{bc}	232.00 ± 1.64	254.00 ± 1.37	265.67 ± 1.38^{a}	275.17 ± 1.51^{a}	278.00 ± 1.72
Group V (MSFM@200)	169.50 ± 4.21	181.50 ±2.16	195.33 ± 2.29^{a}	212.33 ± 3.42^{abc}	240.00 ± 0.89^{ac}	260.50 ± 1.65^{ac}	269.67 ± 1.67^{a}	280.33 ± 1.93^{a}	282.50 ± 2.23
Group VI (MSFM@400)	170.67 ± 4.52	182.00 ±2.14	198.50 ± 2.04^{a}	216.50 ± 4.46^{ab}	246.50 ± 1.12^{a}	263.83 ± 3.61^{ac}	275.33 ± 3.61^{a}	278.50 ± 4.36^{a}	286.50 ± 4.18^{ac}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at $p < 0.05$ (n=6)

Table 3. The mean (±SE) values of various haematological parameters of rats in different groups on final day (60th day) of the study

GROUPS	TEC (106/µL)	TLC (103/µL)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (%)
Group I (NC)	7.33±0.16^{a}	8.35±0.08^{a}	14.63±0.41^{a}	41.88±0.71^{aa}	51.51±1.36^{a}	17.01±0.53^{c}	32.76±0.83^{a}
Group II (DC)	5.18±0.21^{b}	5.30±0.38^{b}	10.36±0.45^{d}	30.38±0.74^{c}	63.75±1.64^{b}	25.13±1.44^{a}	26.20±0.88^{b}
Group III (RC)	6.98±0.13^{ac}	7.01±0.32^{c}	12.64±0.47^{bc}	36.71±0.47^{ab}	53.25±1.58^{a}	21.63±1.08^{ab}	31.04±0.72^{a}
Group IV (MSFM@100)	6.57±0.22^{c}	7.40±0.18^{ac}	11.52±0.32^{c}	36.05±1.02^{b}	53.82±1.18^{a}	22.48±1.03^{ab}	30.16±0.52^{a}
Group V (MSFM@200)	7.01±0.08^{ac}	7.64±0.22^{ac}	12.99±0.45^{abc}	39.38±1.80^{ab}	52.75 ±1.08^{a}	20.92 ± 1.14^{abc}	31.26± 0.85^{a}
Group VI (MSFM@400)	7.24±0.13^{ac}	7.85±0.31^{ac}	14.09±0.30^{ab}	40.66±1.33^{ab}	51.76 ± 0.71^{a}	18.67±0.69^{bc}	33.06 ± 0.66^{a}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

mean values of all the hematological parameters such as Total erythrocyte count (TEC), Haemoglobin (Hb), Packed Cell Volume (PCV), Total leucocyte count (TLC) and Mean Corpuscular Haemoglobin concentration (MCHC) of Group II rats were significantly (p<0.05) decreased when compared to Group I rats, whereas Mean corpuscular volume (MCV) and Mean Corpuscular Haemoglobin (MCH) were significantly (p<0.05) elevated in Group II rats compared to Group I. Group III rats exhibited significantly(p<0.05) higher mean values of TEC, Hb, PCV, TLC, and MCHC, along with a significantly (p<0.05) lower MCV compared to Group II, while the mean MCH value remained comparable. The MSFM-treated groups (Group IV to VI) showed significant (p<0.05) improvement in TEC, TLC, and PCV values relative to Group II. Hb and MCHC levels were also significantly elevated in Groups IV to VI and were comparable to those of the Group I. The mean MCH value showed a significant (p<0.05) reduction in Group VI and was relatively lower in Groups IV and V compared to Group II, while MCV values were significantly (p<0.05) decreased across all MSFM-treated groups.

Table 4. The mean (± SE) values of various serum biochemical parameters of rats different groups on the final day (60th day) of the study

GROUPS	ALT (IU/L)	ALP (IU/L)
Group I (NC)	41.88 ± 0.42^{d}	107.54 ± 1.08^{b}
Group II (DC)	71.22 ± 1.16^{a}	131.04 ± 2.49^{a}
Group III (RC)	48.56 ± 1.51^{c}	109.93 ± 1.86^{b}
Group IV (MSFM@100)	57.65 ± 1.83^{b}	124.85 ± 1.33^{a}
Group V (MSFM@200)	49.04 ± 1.59^{c}	111.52 ± 1.20^{b}
Group VI (MSFM@400)	46.24 ± 1.53^{cd}	108.57 ± 1.29^{b}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

Serum Biochemistry: The mean values with standard error of mean on 60th day of the experiment is presented in Table 4.

Alanine transaminase (ALT): There was a significant (p<0.05) increase in serum ALT levels of Group II rats by 70.05% than Group I rats. The mean values of serum ALT levels of Group III to VI rats were significantly decreased (p<0.05) than Group II rats.

Table 5. The mean (± SE) values of TBARS of rats in different groups on the final day (60th day) of the study.

Groups	TBARS (nmol MDA/g of Liver tissue)
Group I	7.17±0.08^{b}
Group II	16.76±0.64^{a}
Group III	11.39±0.37^{c}
Group IV (MSFM@100)	12.32±0.29^{c}
Group V (MSFM@200)	10.72±0.35^{c}
Group VI (MSFM@400)	7.50±0.26^{b}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

Table 6. The mean (± SE) values of Catalase and Superoxide dismutase enzyme activity of rats in different groups on the final day (60th day) of the study.

Groups	Superoxide dismutase (U/mg of protein)	Catalase (µM of H_2O_2 decomposed /min)
Group I (NC)	8.50±0.32^{a}	47.67±0.35^{a}
Group II (DC)	5.55±0.21^{b}	33.75±0.45^{d}
Group III (RC)	6.89±0.37^{ab}	39.12±0.35^{c}
Group IV(MSFM@100)	7.64±0.29^{ab}	39.62±0.37^{c}
Group V(MSFM@200)	8.21±0.30^{a}	43.70±0.55^{b}
Group VI(MSFM@400)	8.37±0.18^{a}	46.70±0.97^{a}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

Table 7. The mean (±SE) HP score of liver of rats in different groups on final day (60th day) of the study

Groups	Cellular swelling to vacuolar degeneration	Sinusoidal dilatation	Bile duct epithelium proliferation	New bile duct formation	Inflammatory cell infiltration	Central and portal vein dilatation and congestion	Thickening of blood vessels
Group I	0.37±0.08^{a}	0.27±0.02^{a}	0	0.10±0.05^{a}	0.23±0.02^{a}	0.07±0.02^{a}	0
Group II (DC)	3.55±0.02^{b}	3.23±0.15^{b}	2.52±0.17^{b}	3.42±0.20^{b}	2.90±0.09^{b}	3.05±0.05^{b}	2.74±0.04^{b}
Group III (RC)	2.97±0.07^{c}	2.83±0.14^{bc}	2.15±0.05^{c}	3.02±0.07^{bc}	2.57±0.75^{bc}	2.62±0.04^{c}	2.00±0.09^{c}
Group IV (MSFM@100)	2.92±0.11^{c}	2.85±0.20^{bc}	1.10±0.04^{d}	2.70±0.19^{c}	2.62±0.10^{bc}	2.57±0.07^{c}	1.92±0.62^{c}
Group V (MSFM@200)	2.63±0.02^{cd}	2.41±0.22^{c}	1.10±0.04^{d}	2.75±0.02^{c}	2.52±0.11^{bc}	2.02±0.04^{d}	1.22±0.04^{d}
Group VI (MSFM@400)	2.38±0.07^{d}	2.16±0.21^{c}	1.02±0.02^{d}	2.57±0.06^{c}	2.27±0.08^{c}	1.62±0.06^{e}	1.05±0.02^{d}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at $p < 0.05$ (n=6)

Alkaline phosphatase (ALP): There was a significant (p<0.05) increase in serum ALP levels of Group II rats by 21.84% than Group I rats. The mean values of serum ALP levels of Group III to VI rats were significantly decreased (p<0.05) than Group II rats.

Lipid peroxidation assay as an oxidative stress marker: The mean values of liver tissue Thiobarbituric acid reactive substances (TBARS) in nmol MDA/g of tissue levels with standard error of mean on 60th day of the experiment have been presented in Table 5. There was a significant (p<0.05) increase in TBARS levels of Group II rats in comparison to Group I rats. Among the treatment groups (III to V), there was no significant difference in mean values. The decrease in the values in Group III to VI were statistically significant (p<0.05) in comparison to Group II rats and values of Group VI rats were comparable to that of Group I rats.

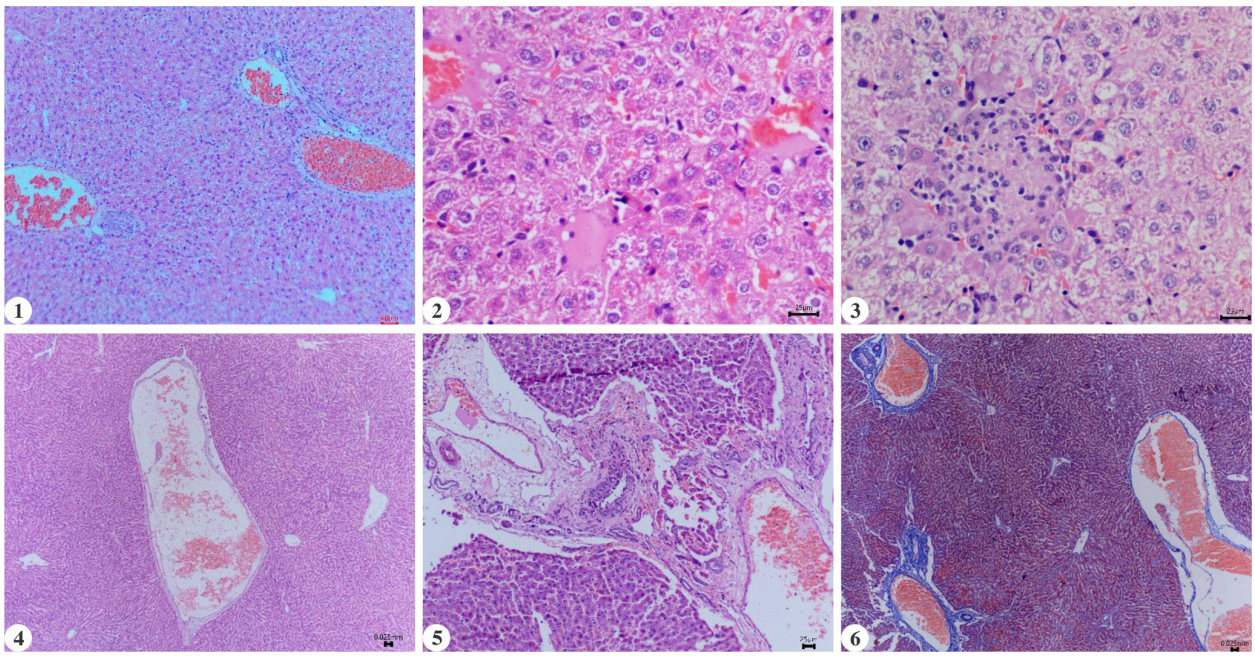

Fig. 1. Section of liver from Group II disease control rat showing vacuolar degeneration in hepatocytes along with dilated and congested portal veins H&E X 100; **Fig. 2.** Section of liver from disease control (Group II) rat showing degenerated hepatocytes with oedema and haemorrhage H&E X 400; **Fig. 3.** Section of liver from disease control (Group II) rat showing a necrotic foci with inflammatory cell infiltration H&E X 400; **Fig. 4.** Section of liver from disease control (Group II) rat showing severe dilated central vein with thickening of wall H&E X 40; **Fig. 5.** Section of liver from disease control (Group II) rat showing dilated portal vein, proliferation of bile duct and peri-portal fibrosis H&E X 100; **Fig 6.** Section of liver from disease control (Group II) rat showing dilated central and portal vein and peri-portal fibrosis MT X 40

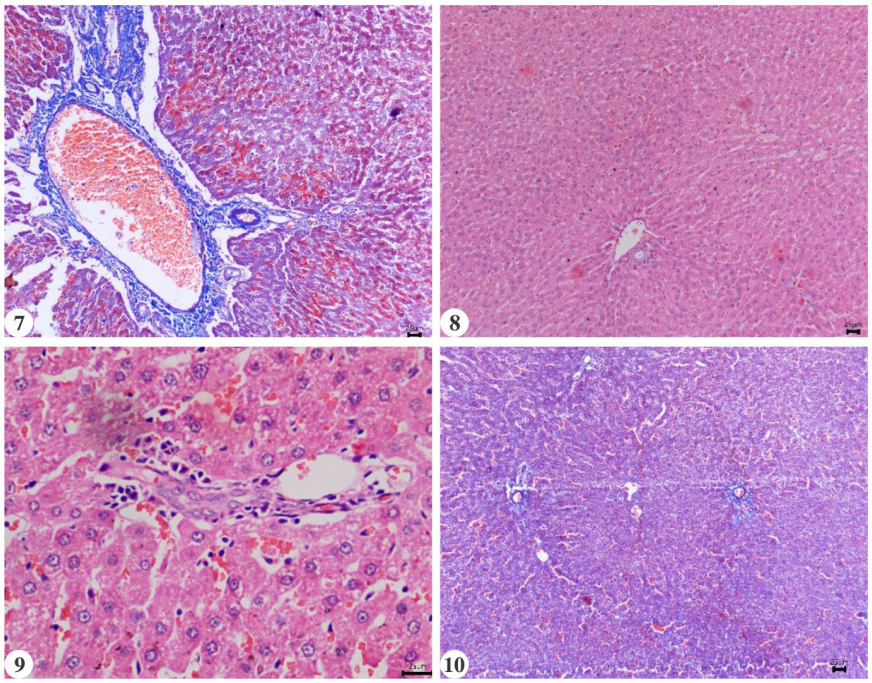

Fig. 7. Section of liver from disease control (Group II) rat showing dilated portal vein, proliferation of bile duct and peri-portal fibrosis. MT X 100; **Fig. 8**. Section of liver from Group VI (MSFM@400) rat showing improvement in hepatocytes and normal portal triad compared to Group II rats. H&E X 100; **Fig. 9.** Section of liver from Group VI (MSFM@400) rat showing improvement in hepatic architecture with minimal peri-portal inflammation compared to Group II rats. H &E X 400; **Fig. 10.** Section of liver from Group VI (MSFM@400) rat showing normal architecture of portal triad. MT X 100

Antioxidant enzymes: The mean values of liver tissues with standard error of mean on 60th day of the experiment have been presented in Table 6.

Catalase (CAT): There was a significant (p<0.05) decrease in catalase enzyme activity of Group II rats in comparison to Group I rats. All the treatment groups were significantly (p<0.05) higher than Group II rats. Group VI showed a significant (p<0.05) increase in CAT activity compared to all other treatment groups (III to V) and were comparable to that of Group I rats.

Superoxide dismutase (SOD): There was a significant (p<0.05) decrease in superoxide dismutase enzyme activity of Group II rats in comparison to Group I rats. Among the MSFM treated groups, there were no significant difference in mean values of SOD activity and values of Group V and VI were comparable to that of Group I rats.

Histopathology: The liver of the disease control group (Group II) rats showed severe loss of normal architecture of hepatocytes with degenerative changes ranging from cellular swelling to vacuolar degeneration throughout the hepatic parenchyma. Individual hepatic cell necrosis with cytoplasmic eosinophilia and pyknotic nuclei was noted in multiple areas with infiltration of inflammatory cells. Occasional fatty changes along with apoptotic cells were evident. A moderate to severe degree of congestion of central and portal vein were observed. The central vein and portal vein diameter was increased compared to normal control rats. The bile duct epithelium showed severe proliferation with new bile duct formation in few triads. The portal triad showed mild inflammation with fibroplasia (Fig 1 to 7).

The liver of treatment group rats (IV to VI) showed mild to moderate

Table 8. The mean (±SE) HP score of liver of rats in different groups on final day (60th day) of the study

Groups	Cellular swelling to vacuolar degeneration	Sinusoidal dilatation	Bile duct epithelium proliferation	New bile duct formation	Inflammatory cell infiltration	Central and portal vein dilatation and congestion	Thickening of blood vessels
Group I	0.37±0.08^{a}	0.27±0.02^{a}	0	0.10±0.05^{a}	0.23±0.02^{a}	0.07±0.02^{a}	0
Group II (DC)	3.55±0.02^{b}	3.23±0.15^{b}	2.52±0.17^{b}	3.42±0.20^{b}	2.90±0.09^{b}	3.05±0.05^{b}	2.74±0.04^{b}
Group III (RC)	2.97±0.07^{c}	2.83±0.14^{bc}	2.15±0.05^{c}	3.02±0.07^{bc}	2.57±0.75^{bc}	2.62±0.04^{c}	2.00±0.09^{c}
Group IV (MSFM@100)	2.92±0.11^{c}	2.85±0.20^{bc}	1.10±0.04^{d}	2.70±0.19^{c}	2.62±0.10^{bc}	2.57±0.07^{c}	1.92±0.62^{c}
Group V (MSFM@200)	2.63±0.02^{cd}	2.41±0.22^{c}	1.10±0.04^{d}	2.75±0.02^{c}	2.52±0.11^{bc}	2.02±0.04^{d}	1.22±0.04^{d}
Group VI (MSFM@400)	2.38±0.07^{d}	2.16±0.21^{c}	1.02±0.02^{d}	2.57±0.06^{c}	2.27±0.08^{c}	1.62±0.06^{e}	1.05±0.02^{d}

One-way ANOVA with Tukey's post hoc test (SPSS)
Mean ± SE values within a column with different superscripts differ significantly at p < 0.05 (n=6)

improvement when compared to Group II rats characterised by decreased degenerative changes in hepatocytes, reduced dilatation and congestion of veins, with mild proliferation of bile duct epithelium (Fig 8 to 10).

Histopathological score of liver

The mean HP severity score values of liver with standard error of mean are presented in Table 7. The mean HP score of Group II disease control rats for each of the recorded lesion were significantly (p <0.05) higher than mean HP score of Group I normal control rats. The mean HP score of Group III to VI were significantly (p <0.05) lower than mean HP score of Group II disease control rats.

DISCUSSION

In the present study, sodium arsenite administration resulted in reduced feed intake, decreased body weight, ruffled hair coat and mild hair loss. These clinical manifestations are consistent with earlier finding who also observed similar signs in arsenic-exposed rats[13]. Our findings of sodium arsenite induced reduction in body weight are in accordance with reports of previous workers[14-15]. The progressive loss of body weight gain in sodium arsenite administration group may be because of gradual onset of anorexia due to toxaemia[16]. Sodium arsenite induced hepatotoxicity related decrease in hematological parameters like TEC, Hb, PCV, TLC, MCHC and elevated MCV, MCH levels were similar to those previously reported[11,17]. The reduction in PCV, Hb, RBC, and MCHC levels observed in arsenic alone exposed groups may be mechanistically linked to impaired heme biosynthesis. Arsenic has been reported to inhibit the activity of aminolevulinic acid dehydratase, a critical enzyme in the porphyrin synthesis pathway, thereby disrupting heme formation and consequently diminishing erythropoiesis and hemoglobin synthesis[18]. Arsenic participates in cellular redox cycling, promoting the formation of ROS, which in turn inflict oxidative stress on erythrocytes. This oxidative damage compromises membrane integrity, leads to the generation of methemoglobin, and triggers erythrocyte lysis and cell death[19]. The significant reduction in TLC levels can be attributed to the direct cytotoxic effects of arsenic bone marrow leading to suppressed hematopoiesis and resulting in erythrocytopenia and leukopenia[20]. An elevation in MCV and MCH observed following arsenic exposure is generally suggestive of a macrocytic response associated with anemia[21]. Reduced MCHC in arsenic toxicity group results from a combination of impaired heme biosynthesis, oxidative stress-induced Hb degradation, and macrocytic anaemia, leading to a decrease in the concentration of Hb per unit volume of erythrocyte[18].

A significant elevation in the mean serum levels of ALT and ALP was recorded in the present study, which is consistent with the earlier observations[22-23]. Arsenic-induced hepatic toxicity is primarily attributed to oxidative stress resulting from excessive production of reactive oxygen species (ROS), which leads to structural and functional impairment of hepatocytes. The consequent damage to hepatocellular membranes causes the leakage of intracellular enzymes such as ALT and ALP into the bloodstream, thereby reflecting hepatic injury[24].

In the present study, a significant elevation in hepatic TBARS levels accompanied by a marked reduction in CAT and SOD enzyme activities was observed, indicating increased lipid peroxidation and oxidative stress in rats. These findings are consistent with earlier reports[15,25]. The pronounced rise in MDA levels in the arsenic-exposed group reflects enhanced lipid peroxidation resulting from excessive generation of reactive oxygen and nitrogen species. Arsenic promotes ROS production by disrupting mitochondrial electron transport, activating NADPH oxidase, promoting oxidative metabolism of its intermediates, and releasing redox-active iron from ferritin. The observed decline in enzymatic antioxidants such as SOD and CAT may be attributed to direct inhibition of their activity or depletion of essential cofactors by arsenic and its metabolites[26].

The examination of liver tissue from rats in the disease control group revealed extensive damage characterized by loss of architecture of hepatocytes with degenerative changes from cellular swelling to vacuolar degeneration throughout the hepatic parenchyma. Individual hepatic cell necrosis with cytoplasmic eosinophila and pyknotic nuclei was noted in multiple areas with infiltration of inflammatory cells. A moderate to severe degree of congestion of central and portal vein were observed. These observations were consistent with previous studies[27-28]. These histopathological alterations primarily result from sodium arsenite induced oxidative stress and inflammatory injury by production of ROS. The resultant oxidative burden leads to structural and functional damage followed by degenerative changes and necrosis of hepatocytes and inflammatory changes[29]. The observed dilatation and congestion of hepatic central and portal veins in disease group may be attributed to arsenic-induced portal hypertension, which are associated with overexpression of endothelins and vascular endothelial growth factor-B[30]. Arsenic-induced hepatic fibroplasia and fibrosis may arise from multiple mechanisms, including alterations in histone acetylation, oxidative stress, apoptosis, DNA methylation, and other epigenetic modifications. Additionally, microRNA-21 mediates abnormal hepatocyte–hepatic stellate cell interactions *via* the hypoxia inducible factor-1α/Vascular endothelial growth factor signalling pathway, further contributing to fibroplasia and fibrosis[31].

Improvement in treatment groups (Group IV to VI) may be attributed to the antioxidant, anti-inflammatory

anti-apoptotic properties of MSFM. *Spirulina* possesses a potent antioxidant profile comprising β-carotene, tocopherol, chlorophyll, phycobiliproteins such as phycocyanin, cryptoxanthin, and allophycocyanin, which collectively contribute to effective free radical scavenging and attenuation of oxidative stress[32]. Among these, phycocyanin a major biliprotein containing the tetrapyrrole pigment phyco-cyanobilin plays a central role in *Spirulina's* antioxidant potential. It not only exhibits strong free radical quenching activity but also demonstrates notable metal-binding and chelating properties, thereby aiding in the detoxification and elimination of heavy metals from the body. C-phycocyanin also inhibits phosphorylation of *p38MAPK*, thereby regulating cytokine synthesis which in turn reduces the inflammation[33].

Finger millet is a good source of phytochemicals mainly phenolic and flavonoids compounds. The polyphenols consist of hydroxybenzoic (protocatechuic, p-hydroxybenzoic) acids, hydroxyl-cinnamic (p-coumaric, ferulic, syringic) acids, flavonoids (quercetin, apigenin, catechin, epicatechin), and pro-anthocyanidins. All these can act as reducing agents (free radical terminators), metal chelators, and singlet oxygen quenchers[34]. The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, β-sitosterol, caffeoylquinic acid and kaempferol which contribute to its antioxidant and anti-inflammatory property[35].

The current research showed that MSFM exhibited significant, dose-dependent protection against sodium arsenite-induced hepatotoxicity, with higher doses (200 and 400 mg/kg body weight) showing enhanced efficacy. The improvement in biochemical, antioxidant, and histopathological parameters highlights its potential as a supportive intervention for arsenic toxicity. However, further mechanistic and clinical studies are needed to confirm its therapeutic applicability.

***Financial support & sponsorship*:** None

***Conflicts of Interest*:** None

***Use of Artificial Intelligence (AI) - Assisted Technology for manuscript preparation*:** The authors confirm that there was no use of AI- assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 57-60, 2026: DOI: https://doi.org/10.56093/fx8he559

SHORT COMMUNICATION

Mycobacterium orygis associated generalised tuberculosis in Indian Cattle

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Received: 15.5.2025; Accepted: 1.1.2026

ABSTRACT

The present report describes two cases of generalised tuberculosis in cattle caused by *Mycobacterium orygis*. The affected cattle, both adult female cows from an organized dairy farm in Bareilly, Uttar Pradesh exhibited severe clinical signs including weakness, lethargy and respiratory distress and succumbed to the disease despite treatment efforts. Necropsy examination revealed marked emaciation, a rough body coat and pale mucous membranes. Gross lesions consisted of variably sized caseo-calcified nodules in multiple organs, including the lungs, lymph nodes (prescapular, mediastinal, mesenteric), kidneys, udder, uterus, meninges and brain. Histopathological examination revealed diffuse caseo-calcified granulomas in above mentioned organs. Duplicate sections of various organs were positive for acid-fast bacilli on Zeihl-Neelsen staining. Generalized tuberculosis caused by *M. orygis* was diagnosed based on pathomorphology, Ziehl-Neelsen staining, culture isolation and multiplex PCR. This report highlights the broader implications of mycobacterial infections in livestock and the potential risks to public health.

Keywords: Cattle, *Mycobacterium orygis*, region of difference, tuberculosis

Tuberculosis (TB), a deadly airborne disease affecting livestock, wildlife and humans worldwide is caused by the Mycobacterium tuberculosis complex (MTBC), which includes species such as *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. caprae*, *M. canettii*, *M. tuberculosis*, *M. microti* and *M. orygis*[1]. In India, Mycobacterium bovis has long been considered the primary cause of tuberculosis in approximately 21.8 million cattle affected. However, recent reports of *Mycobacterium orygis* induced TB in humans, cattle and occasionally wild animals challenge this assumption[2,3]. In this case, the infection was initially attributed to *M. bovis* due to its historical association with zoonotic TB. However, subsequent molecular characterization confirmed *M. orygis* as the actual causative agent. This misdiagnosis highlights the need to reconsider *M. bovis* as the sole proxy for zoonotic TB, particularly in South Asia, where recent molecular epidemiological studies including one from southern India found no evidence of wildtype *M. bovis*[1-3]. These findings emphasize the need to update diagnostic criteria and expand the definition of zoonotic tuberculosis to include *M. orygis*. To break the transmission linkage between humans, livestock and wild animals, timely diagnosis and appropriate preventive measures are important[3,4]. However, diagnosing bovine tuberculosis and differentiating species within the *M. tuberculosis* complex remains challenging, as traditional methods such as history, radiology, smear microscopy, physical examination and histomorphology rely on acid-fast staining but cannot identify specific Mycobacterium species[1,5]. PCR (Polymerase Chain Reaction) has emerged as a rapid and reliable tool for detecting Mycobacterium genomic DNA, offering superior accuracy compared to traditional methods and specifically identifying and differentiating specific bacteria from other *M. tuberculosis* complex members[6,7]. This report describes the pathological features of tuberculosis in cattle caused by *M. orygis* and confirms the etiological agent through molecular identification techniques.

Two adult Tharparkar cattle from an organized farm in Bareilly presented with clinical signs including weakness, anorexia, progressive emaciation and respiratory distress. Despite treatment, both animals ultimately succumbed to the disease. A detailed postmortem examination was performed on both carcasses and gross lesions were recorded. Peripheral lymph nodes were inspected and impression smears were prepared for acid-fast staining. The representative tissues pieces of visceral organs like lungs, lymph nodes, liver, intestine, spleen, kidney, heart, ovaries, uterus, adrenal glands and brain were collected and fixed in 10% neutral buffered formalin (NBF) for routine histopathological examination. Tissue samples were also collected and stored on ice for bacterial isolation and molecular studies. Smears

How to cite this article : Thilageshwaran, S., Karikalan, M., Deekshita, V., Chatla, A., Pawde, A.M. and Sharma, A.K. 2025. *Mycobacterium orygis* associated generalised tuberculosis in Indian Cattle. Indian J. Vet. Pathol., 50(1) : 57-60.

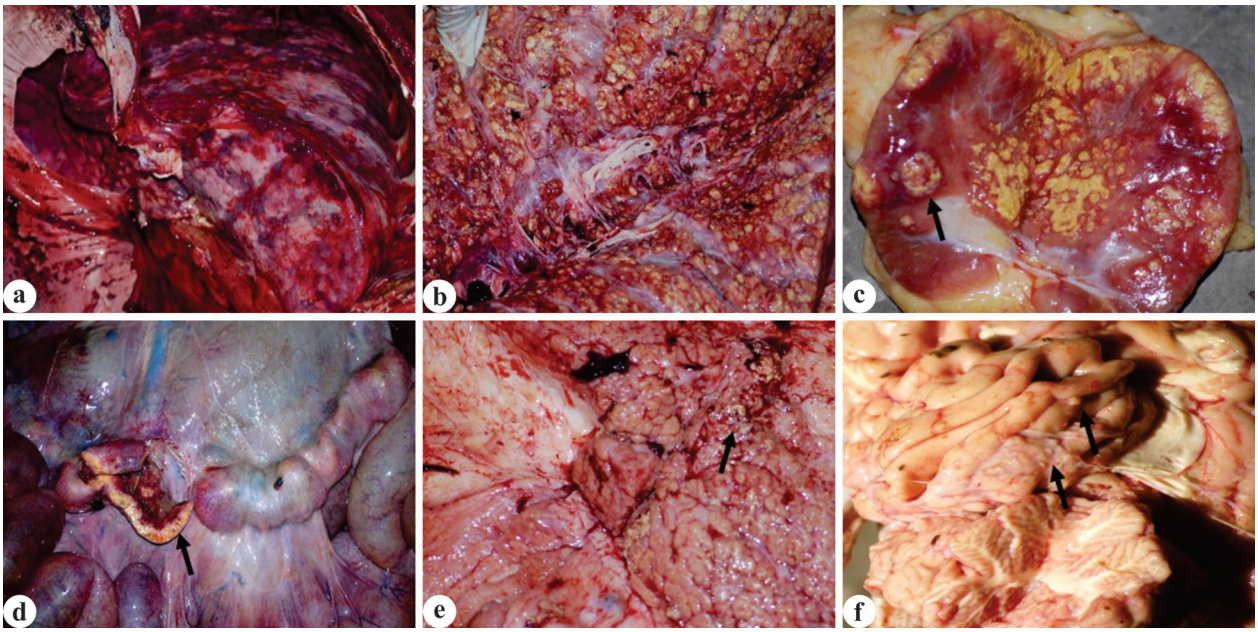

Fig. 1a. Multifocal to coalescing, poorly demarcated, variably sized, pale yellowish caseous nodules on both the surface and cut surface of the lung parenchyma, accompanied by severe consolidation. **b.** Dissected nodules in the lung revealing caseo-calcified, pale-white material. **c & d.** Prescapular and mesenteric lymph nodes exhibiting enlargement with gritty, caseous nodules visible on the cut surface (arrow). **e.** Hard, indurated udder showing tiny caseous nodules (arrow). **f.** Meninges displaying tuberculous nodules (arrow).

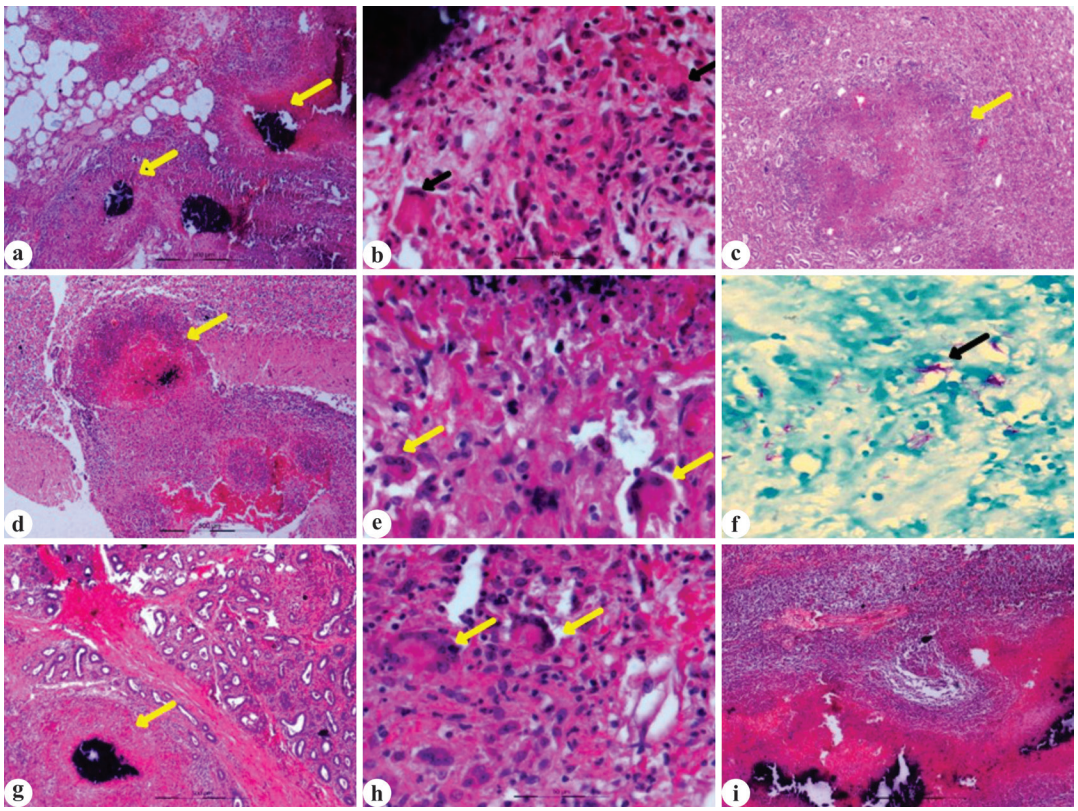

Fig. 2a & b. Lung sections showing multiple granulomatous nodules with central caseo-necrotic areas surrounded by a mixed population of inflammatory cells, including a few multinucleated giant cells (arrow) around the caseo-granulomatous lesions (H&E x100, x400). **c.** Kidney sections exhibiting caseous granulomas (arrow) encircled by a severe inflammatory reaction (H&E x100). **d & e.** Meninges around the cerebellum showing tuberculous granulomas surrounded by multinucleated giant cells (arrow) (H&E x100, x400). **f.** Cerebellum showing few acid-fast bacilli (arrow) in the caseous material (ZN x1000). **g & h.** Mammary gland sections revealing caseous granulomas with multinucleated giant cell formation (arrow) (H&E x100, x400). **i.** Lymph node sections displaying small tuberculous granulomas in the medulla (H&E x100).

from lymph nodes, lung and lesions in other organs were stained with Ziehl-Neelsen (ZN) to detect acid-fast Mycobacteria. Lowenstein-Jensen (L-J) medium containing glycerol and pyruvate were used for bacterial cultural isolation[2]. DNA was extracted from both cultures and tissues and subjected to multiplex PCR. Primers used for amplification of MTBC targeting 16S rRNA, MPB70 gene[6] and specific conventional PCR for species differentiation targeting region of difference (RD9 and RD12) were used[1].

Necropsy examination revealed similar findings in both cases, including pale mucous membranes and a debilitated body condition with a rough hair coat. Internal examination revealed multifocal to coalescing, caseous nodules, varying in size, on the surface and cut surface of the lung parenchyma, accompanied by severe consolidation (Fig. 1a). Upon dissection, these nodules contained caseo-calcified, pale-white material (Fig. 1b). The mesenteric lymph nodes exhibited enlargement with gritty, caseous nodules on the cut surface (Fig. 1c & d) and the hard, indurated udders were partially necrotic and ulcerated (Fig. 1e). The meninges exhibited tuberculous nodules (Fig. 1f), while the uterus showed granulomas in both the endometrium and myometrium. Histopathological examination of the lung sections revealed multiple granulomatous nodules with central caseo-necrotic areas that were surrounded by a mixed population of inflammatory cells, along with a very few multinucleated giant cells around the caseo-granulomatous lesions (Fig. 2a & b). The kidney sections exhibited caseous granulomas surrounded by a severe inflammatory reaction (Fig. 2c). The meninges around the cerebellum contained tuberculous granulomas encircled by multinucleated giant cells (Fig. 2d & e) and the sections of the mammary gland showed caseous granulomas with multinucleated giant cell formation (Fig. 2f & h). Similarly, lymph node sections showed tiny tuberculous granulomas in the medulla (Fig. 2i). The duplicate sections from the lungs, lymph node, brain and mammary gland showed acid-fast bacilli on ZN staining (Fig. 2f). The presence of MTBC was confirmed by culturing the sample on Lowenstein-Jensen (LJ) medium containing sodium pyruvate, which yielded moist, granular colonies. Acid-fast staining of the cultured sample revealed clusters of acid-fast bacilli. Isolated DNA samples were successfully amplified for the 16S rRNA and *mpb70* genes, yielding amplicon sizes of 1030 bp and 372 bp, respectively, which are specific to *Mycobacterium tuberculosis* complex (MTBC) organisms (Fig.3). Additionally, *M. orygis* was identified using specific PCR targeting regions of difference (RD9 and RD12) showed a positive amplification with an amplicon size of 410 bp for RD9 and 264 bp for RD12, indicating infection caused by *M. orygis*.

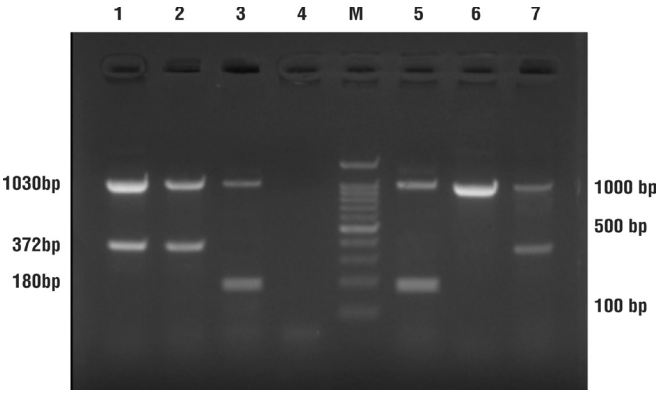

Fig. 3. Agarose gel electrophoresis: Amplification of 16S rRNA and MPB70 gene segment to differentiate MTBC organisms from NTM in tissue samples by conventional multiplex PCR. Lane M: 100 bp DNA ladder. Lane1-2: Sample of cattle lung showing both 372 bp and 1030 bp amplicon specific for MTBC organisms; Lane 3: Positive control showing both 180 bp and 1030 bp specific for *M. avium*; Lane 4:Negative control; Lane 5: Positive control of *M. avium*; Lane 6: Positive control of NTM; Lane 7:Positive control of MTBC

Tuberculosis continues to be a significant airborne disease, affecting humans, domestic and wild animals and birds and causing substantial morbidity and mortality[8-11]. This report describes two cases of generalized tuberculosis in adult Tharparkar cattle caused by *M. orygis*, a member of the *Mycobacterium tuberculosis* complex that is increasingly being recognized. However, an increasing number of cases have now been reported in humans, domestic animals and wild animals[1-3]. This study highlights the capability of *M. orygis* to cause systemic disease in cattle, marked by extensive granulomatous lesions in multiple organs, including the lungs, lymph nodes, udder, uterus, adrenal glands and brain. These findings underscore the severity and widespread pathological impact of *M. orygis* infections in bovine hosts. Histopathological evaluation revealed characteristic granulomas, while advanced molecular tools, including PCR targeting specific regions of difference, confirmed the pathogen, overcoming the limitations of traditional diagnostic methods[10,11]. The finding of this study highlight the growing importance of *M. orygis* as a pathogen in livestock health, particularly in the context of bovine tuberculosis[1,2,8,11]. Its zoonotic potential raises concerns about interspecies transmission, further complicating public health and veterinary control measures. These findings align with prior studies, reinforcing *M. orygis* role in systemic tuberculosis outbreaks in cattle and highlighting its diagnostic challenges. This case report underscores the need for robust surveillance systems, rapid and precise diagnostics and effective intervention strategies to curb the spread of *M. orygis* induced tuberculosis. Such efforts are vital to safeguard livestock, prevent zoonotic transmission and protect public health.

ACKNOWLEDGEMENT

The authors are thankful to the Director, ICAR-IVRI for providing necessary facilities to conduct the study.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 61-63, 2026: DOI: https://doi.org/10.56093/ky5z6y74

SHORT COMMUNICATION

Parakeratosis: Hypozincemia and its therapeutic management in captive Asian Elephants (*Elephas maximus)*

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Received: 21.7.2025; Accepted: 1.9.2025

ABSTRACT

Three captive Asian elephants presented with chronic skin lesions characterized by thickened, crusty and scaly growth on the ears and limbs. Clinical examination, blood sampling, mineral profiling and skin biopsy were carried out to investigate the underlying cause. Histologically skin biopsy revealed hyperkeratosis and marked parakeratosis. Serum analysis showed reduced zinc levels. Based on the clinical signs, histopathology and biochemical findings, zinc deficiency was identified as a contributing factor.

Keywords: Asian elephant, hypozincemia, keratinization, parakeratosis, skin

India has a long and intriguing history of domesticating elephants from the wild. The religion, mythology and cultural legacy of India have been intricately linked to captive Asian elephants for years[1]. As they are voracious feeders due to its large digestive tract, they need enormous source of feed for its growth and maintenance. Elephants are prone to a variety of infectious and non-infectious diseases, but recognizing that they are even sick may be difficult and challenging[2]. Elephants often do not manifest clinical signs of illness until disease is well advanced. Such masking of clinical signs makes identifying and treating diseases in elephants is very challenging for veterinarians and zoo managers. Among these, micro-mineral deficiencies, though often overlooked compared to more acute infections or major metabolic imbalances can have insidious yet significant long-term effects on health and physiology[3]. Iron, zinc, copper, selenium and other trace minerals are essential for immunological function, enzyme functioning, antioxidant defence systems and epithelial integrity. In elephants, deficits in these micronutrients may not show up as obvious symptoms at first, but they can progressively affect the function of the skin barrier, wound healing, infection resistance and reproductive performance[4]. Among those, zinc is a vital trace mineral for many different kinds of metabolic, enzymatic and regulatory functions, including growth, immunological response and tissue integrity Zinc also form a component of several enzymatic systems[5]. Its role in cell division, keratinisation, gene expression and protein synthesis accentuates its significance in both healthy and diseased conditions[6,7]. Although zinc deficiency is often subclinical, it can cause a variety of disorders that impact the skin, reproductive organs, gastrointestinal tract and immune system. These disorders usually show signs such as dermatitis, growth retardation, alopecia, impaired wound healing and increased susceptibility to infections[3,8]. In captive Asian elephants, trace mineral imbalances such as zinc deficiency may go unrecognized due to a lack of standardized reference ranges and species-specific diagnostic tools. The present study reports the occurrence of parakeratosis in Asian elephants with its diagnostic approach.

How to cite this article : Tamileniyan, E., Prasath, N.B., Veeraselvam, M., Jeevitha, M. and Latchumikanthan, A. 2026. Parakeratosis: Hypozincemia and its therapeutic management in captive Asian Elephants *(Elephas maximus)*. Indian J. Vet. Pathol., 50(1) : 61-63.

Three elephants of different age group, sex and setting (Table 1) presented with chronic dermatological lesions since two months were examined as a part of health assessment. Cutaneous lesions were predominantly distributed over the caudal pinna (Fig. 1), forelimbs and caudal

Table 1. Details of Elephants under study

ELEPHANT	AGE (YEARS)	SEX	LOCATION
E1	61	Male	Mudumalai Tiger Reserve, Udhagamandalam
E2	45	Male	Mudumalai Tiger Reserve, Udhagamandalam
E3	35	Female	Private Elephant, Trichy

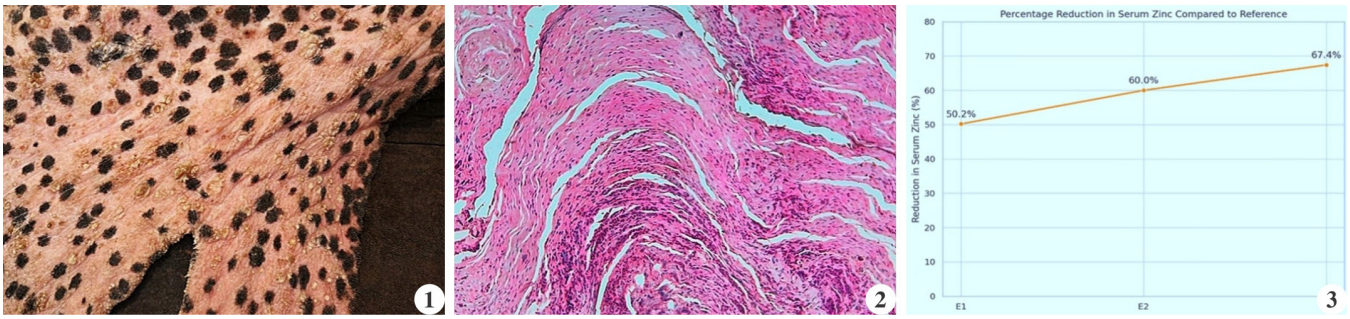

Fig. 1. Rough, thick and scaly skin with prominent crusts and flakes in auricular surface - Hypozincemia - Parakeratosis - Skin - Ear;
Fig. 2. Abnormal keratinization with retention of nuclei in the stratum corneum - Hypozincemia - Parakeratosis - Skin (H&E stain x400);
Fig. 3. Line Plot displaying the percentage reduction in serum zinc levels for each elephant relative to the reference.

surface of hind limbs. Affected areas exhibited thick, rough, scaly skin with crust and exfoliation on the ears and cobblestone-like plaques with deep fissures and cracks on the limbs. Crusty and exfoliated skin lesions were collected and fixed in 10% formalin for histopathological studies. The tissue samples were processed as per standard paraffin embedded technique. The tissue sections of 3-4 μm thickness were processed and stained with haematoxylin and eosin (H&E) stain[9]. Blood samples were collected for haematological analysis, serum biochemistry and mineral profiling. Serum from blood samples were separated for estimation of biochemical parameters such as zinc and cobalt using Atomic Absorption Spectrometry (AAS).

Grossly, cutaneous lesions were characterized as thick, irregular with broad base, crusty and measured 0.7-3.0 cm in width and 3-12 mm in height. Microscopically, cutaneous lesions revealed marked epidermal changes consistent with abnormal keratinization. The stratum corneum appeared thick, compact and irregular with dense eosinophilic keratin layers, hyperkeratosis (Fig. 2). It showed prominent parakeratosis, evidenced by retention of pyknotic nuclei within the stratum corneum. The epidermis beneath the layer of hyperkeratosis showed moderate acanthosis with elongation and thickening of the rete ridges. Serum biochemistry showed decreased zinc concentration ranged from 66.1 to 101.2 μg/dL, indicating a 50-70% reduction compared to the standard reference range of 203-275 μg/dL. Specifically, elephant E1 showed a 50.2% reduction (101.2 μg/dL), E2 showed 60.0% (81.3 μg/dL) and E3 had a 67.4% reduction (66.1 μg/dL) (Fig. 3). Cobalt levels were assessed to rule out any contributory deficiency. While zinc concentrations showed a substantial reduction consistent with clinical manifestations, cobalt levels remained within a normal range.

Zinc plays a crucial role in maintaining the structural integrity and function of epithelial tissues, particularly the skin[6]. Zinc deficiency generally arises, when dietary sources are inadequate due to low zinc content in soil and water which in turn leads to reduced zinc availability in forage crops[10]. In ruminants and other herbivores, it can be exacerbated by high dietary levels of calcium, phytates or iron, which interfere with zinc absorption from the gastrointestinal tract[11]. In the present study, all three elephants demonstrated significantly reduced serum zinc levels, accompanied by gross dermatological changes such as thickened, crusty and exfoliative lesions with fissures. Histopathologically, parakeratosis was evident, characterized by abnormal keratinization with retention of nuclei in the stratum corneum. The pathogenesis of parakeratosis in zinc deficiency involves disruption of normal keratinocyte proliferation and differentiation[12]. Zinc is a cofactor for numerous metalloenzymes and transcription factors (including zinc-finger proteins) that regulate gene expression during epidermal maturation. In the absence of adequate zinc, there is impaired protein synthesis, delayed cell turnover and faulty keratinization resulting in a hyperplastic epidermis with parakeratotic changes. The present observations were in parallel with the findings of earlier documented evidence from elephant of the Zoological Society of London, where an adult female Asian elephant developed parakeratosis, alopecia and dermatitis due to dietary zinc insufficiency following changes in feed quality[13].

Reduced zinc levels were correlated well with the clinical presence of hyperkeratotic skin lesions and the histopathological findings of parakeratosis suggesting that suboptimal zinc status played a contributory role in the skin pathology observed. A strong association between hypozincemia and cutaneous pathology of the present study highlighted the importance of adequate trace mineral nutrition in elephant health management.

The findings of the present study highlight the significance of zinc deficiency as a potential cause of chronic dermatological conditions such as parakeratosis in captive elephants. Based on clinical signs, serum mineral analysis and histopathological findings a targeted therapeutic approach was implemented. Dietary supplementation included zinc sulphate syrup

(200 ml once daily), multivitamin syrup (100 ml once daily) and a region-specific mineral mixture (150 g/day) was followed to address the underlying nutritional deficiencies. Additionally, topical application of zinc oxide ointment combined with antifungal cream and olive oil was performed twice daily to promote skin healing and prevent secondary infections. Dietary care was taken to avoid administering zinc syrup concurrently with mineral supplements, as calcium can interfere with zinc absorption and bioavailability.

ACKNOWLEDGEMENT

We extend our gratitude to the Dean, Veterinary College and Research Institute, Orathanadu - 614625, Thanjavur and Tamil Nadu Veterinary and Animal Sciences University, Chennai for the facilities provided to carry out this study. We wholeheartedly acknowledge the support rendered by the Deputy Director, Mudhumalai Tiger Reserve and the District Forest Officer, Mayiladuthurai Forest Division for assisting in sample collection.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 64-67, 2026: DOI: https://doi.org/10.56093/cf9vtg64

SHORT COMMUNICATION

Diagnosis and management of oral squamous cell carcinoma in an eight-year-old labrador retriever

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Received: 16.7.2025; Accepted: 7.8.2025

ABSTRACT

Squamous cell carcinoma (SCC) is a malignant epithelial tumour originating from squamous cells and is among the most common oral neoplasms in dogs. It typically affects older animals and is known for its locally invasive behaviour, with variable potential for metastasis. This case report describes the clinical presentation, diagnosis and management of oral SCC in an eight-year-old male Labrador Retriever. The dog presented with a proliferative, ulcerated mass involving the gingiva of the right mandible, accompanied by halitosis, difficulty in mastication, drooling and intermittent bleeding. Clinical examination revealed a friable, haemorrhagic oral mass along with mandibular lymphadenopathy. The mass was surgically excised under general anaesthesia with help of electrocautery and submitted at Department of Veterinary Pathology, Bihar Veterinary College, Patna for histopathological examinations. Microscopic examination revealed invasive cords and islands of malignant squamous epithelial cells exhibiting marked nuclear atypia, keratin pearl formation and frequent mitotic figures, confirming a diagnosis of squamous cell carcinoma. Bleeding was ceased after surgical removal of tumour and starts normal feeding from next days. The animal was fully recovered without any complications after 3 days. Upon re-examination after three months, there was no evidence of recurrence. Present case emphasizes the importance of early detection and histopathological confirmation for appropriate therapeutic planning in canine oral tumours.

Keywords: General anaesthesia, nuclear pleomorphism, oral cavity, squamous cell carcinoma, surgical excision

Neoplastic diseases of the oral cavity in dogs represent a significant concern in veterinary oncology due to their clinical impact, potential for local invasiveness and implications for overall prognosis. Among these, squamous cell carcinoma (SCC) is one of the most frequently diagnosed malignant oral tumours in dogs accounting for approximately 17-25% of all canine oral neoplasms, second only to malignant melanoma in prevalence[1]. Oral SCC typically arises from the gingiva, tongue, tonsils or buccal mucosa and is characterized by locally aggressive behaviour with variable metastatic potential depending on tumour location[2]. The pathogenesis of SCC in dogs is multifocal, with chronic exposure to Ultraviolet light, lack of skin pigmentation, chronic inflammation and papillomavirus infection identified as key predisposing factors[3,4]. Breeds such as Labrador Retriever, Golden Retriever and Dalmatians are reportedly more susceptible to SCC particularly when tumours occur in sun exposed lightly pigmented regions[5].

The biological behaviour of SCC varies markedly between non-tonsillar and tonsillar forms. Non-tonsillar SCCs commonly located on the gingiva or hard palate exhibit a relatively low metastatic rate (less than 20%) but are highly invasive to local bone and soft tissue[6]. In contrast, tonsillar and base of tongue SCCs demonstrate a much higher rate of regional and distant metastasis often presenting at an advanced clinical stage[7]. Clinical signs are often insidious and may include halitosis, oral bleeding, drooling, difficulty eating or a visible mass with diagnosis frequently delayed until the lesion is well advanced[8]. Histologically, oral SCCs are composed of invasive islands or cords of squamous epithelial cells with varying degrees of keratinization, nuclear atypia and mitotic activity[9]. Keratin pearl formation is a common feature although poorly differentiated variants may lack overt squamous differentiation[10]. Differentiation from other malignant oral tumours such as fibrosarcoma, melanoma or undifferentiated carcinomas is essential due to differences in biological behaviour, treatment strategies and prognosis[11].

How to cite this article : Sinha, V.K., Kumar, K., Kumar, D., Ali, I. and Kumar, R. 2026. Diagnosis and management of oral squamous cell carcinoma in an eight-year old labrador retriever. Indian J. Vet. Pathol., 50(1) : 64-67.

While oral SCCs are more frequently reported in medium to large-breed, older dogs (typically aged 8 years and above), breed predispositions have been documented in Labrador Retrievers, German Shepherds and Standard Poodles[12]. Treatment typically involves wide surgical excision with or without adjunctive radiotherapy or chemotherapy[13]. The overall prognosis depends on tumour

location, surgical margins, presence of metastasis and histological differentiation[14]. This clinical case report describes the spontaneous occurrence of SCC in the oral cavity of an 8-year-old male Labrador Retriever. The primary aim of the study is to document the clinical presentation, cytological findings and histopathological characteristics of this tumour. The report highlights the significance of early diagnosis and comprehensive histopathological evaluation in facilitating effective management and improving the prognosis in canine patients affected by oral SCC.

The present study was conducted on a clinical case of oral squamous cell carcinoma in an eight-year-old male Labrador Retriever dog reported to the Surgery OPD, Teaching Veterinary Clinical Complex, Bihar Veterinary College, Patna, Bihar, India with a history of halitosis, difficulty in mastication, drooling and intermittent bleeding from the oral cavity. On the day of presentation, bleeding was observed from oral cavity. Clinical examination revealed a tumorous mass at the level of the right maxillary canine tooth, adjacent to mucosa and upper lip. However, physiological parameters like rectal temperature, respiration rate and heart rate were within normal limits. Radiographic examination of chest showed no signs of metastasis. Haematological parameters showed slight variations with neutropenia and anaemia.

Based on clinical examination, the lesion was tentatively diagnosed as a neoplasm and surgical excision was planned to save the life of animal. Surgery was performed under general anaesthesia. The animal was premeditated with atropine sulfate, butorphanol and diazepam followed by induction with propofol. After induction animal was intubated and maintained with isoflurane. The tumorous mass was excised with the help of electrocautery to minimise the bleeding (Fig. 1). The excised tumour mass was submitted for histopathological examination. Postoperatively, the animal was treated with injection Amoxicillin sodium-sulbactam sodium at 12-hour intervals for 7 days and analgesic agent injection Meloxicam 0.2 mg/kg body weight I/M for 3 days.

Bleeding stopped after surgical removal of tumour and the case started normal feeding from next day. The animal was fully recovered without any complications after 3 days. Upon re-examination after three months, there was no evidence of recurrence. Cytological examination of a canine oral tumour showed pleomorphic epithelial cells with eosinophilic cytoplasm, hyperchromatic nuclei, anisocytosis, anisokaryosis and occasional multinucleation (Fig. 2). Histopathological evaluation of the mandibular mass revealed classic features of a well differentiated squamous cell carcinoma. The neoplastic tissue was composed predominantly of irregular islands, cords and nests of atypical squamous epithelial cells invading the underlying submucosa and adjacent connective tissue. These tumour cells exhibited marked nuclear pleomorphism, hyperchromasia and prominent nucleoli with abundant eosinophilic cytoplasm (Fig. 3). Frequent mitotic figures were observed indicating moderate to high proliferative activity. Notably, concentric keratinized structures keratin pearls were visible which is a hallmark of differentiated SCC (Fig. 4). The tumour margins were infiltrative extending beyond the basement membrane and into adjacent muscle bundles

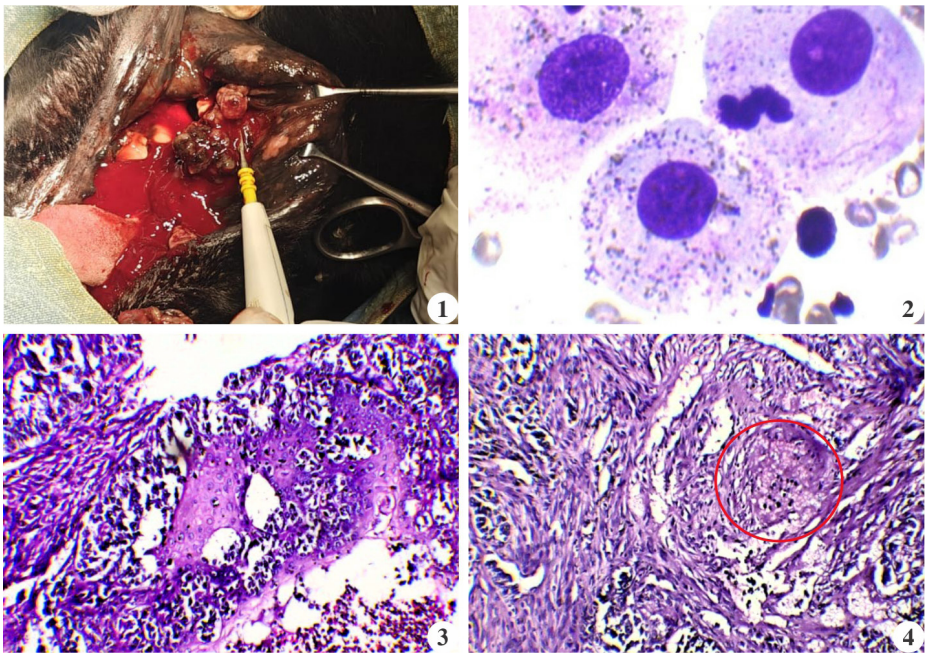

Fig. 1. Intraoperative view showing excision of an ulcerated, proliferative oral mass from the right mandible of dog. Surgical resection was performed using electrocautery under general anesthesia; **Fig. 2.** Cytology of a canine oral tumour (H&E 100x) showing pleomorphic epithelial cells with eosinophilic cytoplasm, hyperchromatic nuclei, anisocytosis, anisokaryosis and occasional multinucleation; **Fig. 3.** Histological section of a canine oral tumour (H&E 10x) revealing invasive nests and islands of neoplastic epithelial cells within a fibrous stroma. The tumour cells exhibit moderate pleomorphism, abundant eosinophilic cytoplasm and central to eccentric hyperchromatic nuclei. Areas of keratinization and stromal invasion are also evident; **Fig. 4.** Histological section of canine oral tumour (H&E 10x) showing well-differentiated squamous cell carcinoma characterized by concentric keratin pearl formation surrounded by malignant epithelial cells.

confirming the malignant and locally invasive nature of the lesion.

Multifocal areas of necrosis and chronic inflammatory infiltrates composed of lymphocytes and macrophages were also observed within and around the tumour tissue. There was no evidence of vascular invasion or metastatic spread in the submitted tissue sections. However, regional lymphadenopathy was clinically noted warranting further staging. SCC is a common malignant epithelial tumour in dogs particularly affecting the oral cavity, where it tends to behave aggressively at the local level with a relatively low rate of distant metastasis especially in non-tonsillar forms[2]. Although SCC is considered to have a low metastatic potential, the risk increases, when the tumour is located in high risk areas such as oral cavity, sublingual regions or tonsils[15]. In contrast, cutaneous SCC particularly those on sun exposed skin rarely metastasize but can cause local tissue destruction if not treated early[5]. Gingival and rostral oral SCCs such as the one in this case are known to be more amenable to surgical resection and have better long-term outcomes compared to caudal or tonsillar lesions[1]. The observed histological features such as keratinization, intercellular bridges and infiltrative growth are consistent with prior descriptions of canine oral SCC[9,10].

The identification of keratin pearls and low to moderate mitotic activity in this case supports classification as a well differentiated variant which may carry a more favourable prognosis compared to poorly differentiated SCCs[16]. Nonetheless, the presence of local tissue invasion and mandibular bone involvement (confirmed radiographically) underscores the importance of early diagnosis and complete surgical excision with wide margins to prevent recurrence[17]. Adjunctive therapies such as radiation or chemotherapy may be considered in cases with incomplete resection, recurrence or confirmed metastasis[13].

Oral SCCs are more commonly diagnosed in older dogs. The present study also reports an oral SCC in an eight-year-old Labrador Retriever, a breed that has been noted in several studies to exhibit a moderate predisposition to this tumour type[5]. Factors such as breed, age and tumour location play critical roles in therapeutic planning and determining the overall prognosis[18]. In the present case, the tumour was localized to the mandibular gingiva. Although it exhibited local invasiveness, there was no evidence of distant metastasis on imaging or histopathological examination. A definitive diagnosis of squamous cell carcinoma was established through histopathological evaluation, which remains the gold standard for differentiating SCC from other oral neoplasms such as fibrosarcoma, malignant melanoma or undifferentiated carcinomas. The identification of organized squamous differentiation, along with the absence of melanocytic or mesenchymal features effectively ruled out these differential diagnoses.

Present case highlights the need for prompt veterinary evaluation of any oral masses in dogs especially given the subtlety of early clinical signs such as halitosis or difficulty chewing. Early diagnosis, staging and surgical management are essential to achieving a favourable outcome in oral SCC. Furthermore, histopathological confirmation not only aids in accurate diagnosis but also guides the selection of appropriate adjunctive therapies and helps predict biological behaviour.

CONCLUSION

The present case report describes a locally invasive squamous cell carcinoma in the oral cavity of an eight-year-old male Labrador Retriever and its successful management. The tumour exhibited infiltrative growth of atypical squamous epithelial cells with keratin pearl formation and moderate mitotic activity, consistent with a diagnosis of well-differentiated squamous cell carcinoma. Clinical and histopathological evaluations confirmed the malignant nature of the lesion and its potential for local tissue destruction. The absence of distant metastasis and the tumour localization to the gingiva indicated a more favourable prognosis compared to caudal or tonsillar variants. Early detection and histopathological confirmation played a pivotal role in accurate diagnosis and formulation of an effective treatment strategy.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 68-70, 2026: DOI: https://doi.org/10.56093/rgm7hq42

SHORT COMMUNICATION

Occurrence of Necrotic Enteritis associated with Coccidiosis in a Desi Chicken (*Gallus gallus domesticus*) flock

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Received: 19.8.25; Accepted: 09.9.25

ABSTRACT

The present investigation describes the occurrence of necrotic enteritis and a concurrent intestinal coccidiosis in a desi chicken (*Gallus gallus domesticus*) flock (Size:-1400). Necrotic enteritis is an acute enterotoxaemia caused by *Clostridium perfringens;* a quite ubiquitous bacterium readily found in soil, dust, faeces, feed and used poultry litter. It is considered as a normal inhabitant of the intestine of healthy chickens. Along with many other factors, the coccidiosis predisposes birds to necrotic enteritis. The 5 carcasses of 137 days old desi chicken were presented to the Department of Veterinary Pathology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara for the necropsy with the history of dullness, inappetence and mortality. Gross examination revealed diffuse congestion of small intestine. On opening severe necrosis of mucosa of small intestine along with foul-smelling reddish-brown contents in the lumen were observed. Mucosa had diphtheritic appearance due to yellowish material covering it. Multifocal haemorrhages were also noted. The wet mount preparation of intestinal content revealed presence of oocysts of *Eimeria spp*. The impression smear from jejunum stained with Grams Stain showed large number of Gram-positive rod-shaped bacteria. Representative samples were collected for histopathological and molecular diagnosis. Histopathological examination showed severe necrosis of the epithelial cells lining the small intestine, fusion of villi and severe infiltration with polymorphonuclear cells, presence of oocysts and other developmental stages of *Eimeria spp.* The PCR amplification of *16s rRNA* gene of *Cl. perfringens* species and subsequent agarose gel electrophoresis of PCR product revealed bands of 481 bp to confirm the infection. The gross and microscopic lesions, microscopic examination of intestinal contents and PCR confirmed the necrotic enteritis in the desi chicken flock which is of economic and public health significance.

Key words: Necrotic enteritis, coccidiosis, gross & histopathological lesions, PCR

Necrotic enteritis has devasting economic effects on production due to high mortality rates and poor feed efficiency. *Clostridium* spp. are considered to be one of the most important agents causing enteric diseases in poultry. Diagnosis of enteric diseases produced by Clostridia is usually challenging mainly because many clostridial species can be normal inhabitant of gut[1], making it difficult to determine their role in virulence. Most common clostridial enteric disease in poultry is necrotic enteritis caused by *Clostridium perfringens* Type A and to lesser extent Type C.[2] *Clostridium* is Gram positive, rod shaped, spore forming and anaerobic bacteria. Necrotic enteritis develops when *Cl perfringens* multiplies anaerobically in the chicken intestinal tract, producing toxins that leads to necrosis. In healthy chicken *Cl. perfringens* is present at level of less than 10^5cfu/g intestinal content.[3] Most important known predisposing factor for necrotic enteritis, is intestinal damage caused by coccidial pathogens.[4] Intestinal damage will result in release of plasma proteins into lumen of intestinal tract. Leaking plasma of intestinal lumen can provide a necessary growth substrate for extensive proliferation of these bacteria to cause necrotic enteritis. The association between *Emeria spp.* and *Cl.perfringens* leads to necrotic enteritis[5] and has been recorded in India also. However, there are very few reports regarding the same in India, hence reported.

The 5 carcasses of 137 days old desi chicken with history of clinical signs *viz.* dullness, inappetence and sudden mortality were presented to the Department of Veterinary Pathology, Krantisinh Nana Patil College of Veterinary Science, Shirwal, Dist. Satara for the necropsy and subsequent diagnosis. Through necropsies were conducted to record gross lesions in various organ systems. The impression smears from affected intestine were prepared and stained with Gram's stain. Wet mount examination of intestinal content was also done. The parts of affected intestine were collected for histopathological examination and fixed in 10% neutral buffered formalin. After fixation, tissues were processed by paraffin embedding technique, blocks

How to cite this article: Prajakta, D., Dhaygude, V.S., Budhe, M.S., Chandan, H.U., Bhandekar, S. and Tumlam, U.M. Occurrence of Necrotic Enteritis associated with Coccidiosis in a Desi Chicken (*Gallus gallus domesticus*) flock. Indian J. Vet. Pathol., 50(1) : 68-70.

were prepared and sections were cut at 5µm thickness with a rotatory microtome and stained with routine Haematoxylin and Eosin staining method. Swabs from intestinal content were collected and inoculated in Robertsons cooked meat broth. It was incubated at 37°C for 24 hrs in an anaerobic condition and subsequently cultured on TSC (Tryptose-Sulfite-Cycloserine) agar.

The DNA was extracted from fresh colonies by snap chill method of DNA extraction. For molecular detection of the organism the PCR targeting *16s rRNA* gene was done with the forward (TAACCTGCCTCATAGAGT) and reverse (TTTCACATCCCACTTAATC) primers. The PCR conditions for 30 amplification cycles included denaturation at 94°C for 1 min, annealing at 55ºC for 30 sec, extension at 72°C for 90 sec and final extension at 72°C for 4 min. PCR product was confirmed by agarose gel electrophoresis.

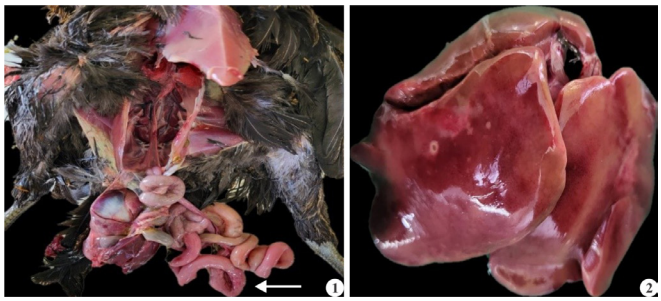

Fig.1. Congested loops of small intestine (arrow); **Fig.2.** Multifocal, pale strips with focal areas of necrosis and haemorrhages on liver

The gross examination revealed diffusely dark red (congested) intestinal loops (Fig.1) along with distended lumen due to accumulation of gas. Cut section of intestinal loop showed presence of foul smelling dark brownish fluid. Mucosal surface of intestine was covered with diphtheritic pseudomembranous made of fibrononecrotic material and mucosa had typical dirty turkish towel like appearance (Fig.3 and 4). Multifocal, pale white strips/ roundish areas of necrosis and haemorrhages were observed on liver (Fig.2). The microscopic examination of section of small intestine showed severe diffusely necrosed mucosa with fusion of villi, loss of epithelial

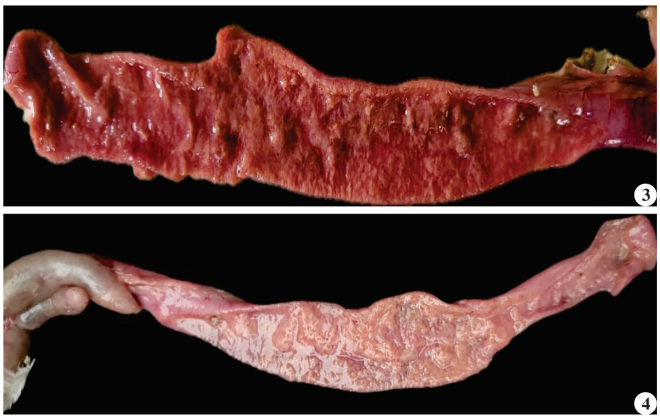

Fig.3. Severe diffuse necrotizing and haemorrhagic enteritis (Small intestine) with focal areas of mucosal sloughing. Mucosa revealed typical dirty turkish towel like appearance; **Fig.4:** Typical dirty turkish towel like appearance of the mucosa of intestine

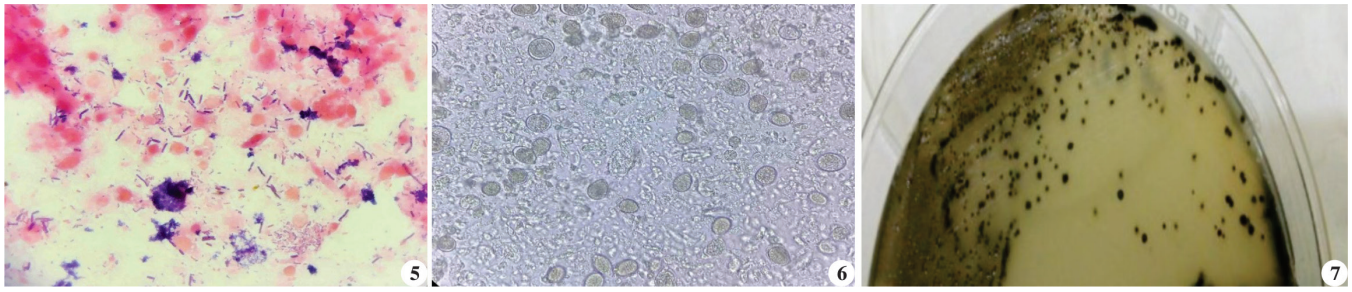

Fig.5. Typical Gram positive rods of *Clostridium spp.*in impression smear from jejunum (Gram stain, 100X); **Fig.6.** Oocysts of *Eimeria* spp. (400X); **Fig.7.** Black colonies of *Cl.perfringens* on clostridial agar with TSC supplement

cells and microvilli. Severe diffuse infiltration of polymorphonuclear cells predominantly heterophils in mucosa and submucosa was also a characteristic finding. There was sloughing of epithelial cells. Focally dark bluish purple colonies of *Cl. perfringens* were also noted. Also, round/oval oocysts and schizonts of *Eimeria* spp. were noted in mucosa (Fig.8 and 9).

The wet mount preparation of intestinal content revealed presence of oocysts of *Eimeria* spp. (Fig.5). Impression smear from jejunum stained with Gram stain revealed large number of Gram-positive rod-shaped bacteria (Fig.6). The bacterial growth on Robertsons Cooked Meat broth was characterized by production of acid and gas (without digestion of meat). On TSC agar typical black colonies were produced due to reduction of sulphite to ferrous sulphate (Fig.7) which indicated successful cultivation of *Cl perfringens*.

The DNA was successfully extracted from the bacteria colonies and the PCR amplification of *16s rRNA* gene of *Cl perfringens* yielded bands *of* expected 481 bp size confirming the involvement of *Cl. Perfingens* in necrotic enteritis (Fig.10).

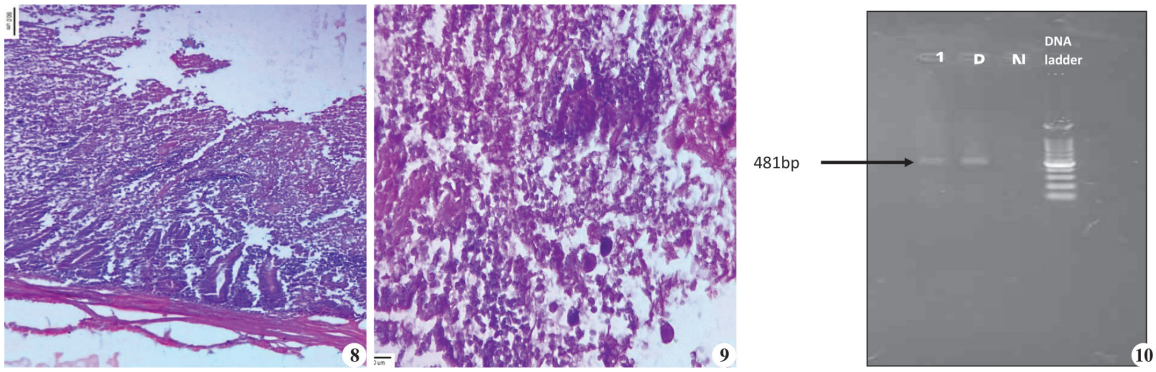

Fig.8. Severe and diffuse necrosed mucosa with fusion of villi, loss of epithelial cells and microvilli, severe infiltration of polymorphonuclear cells and focally dark bluish purple colonies of *Cl.perfringens* in the mucosa (H and E Stain ,100X); **Fig.9.** Section of intestine showing severe necrosis, bacterial colonies, oocysts and other development stages of *Eimeria* spp. (H and E Stain,400X); **Fig.10.** Result of PCR assay showing amplification of 481 bp specific for *Cl perfringenes* (Lane L : 100 bp DNA ladder, 1 : sample, P : Positive control, N : Negative control)

Necrotic enteritis is one of the major enteric diseases caused by *Cl perfringens* mostly Type A and Type C in rare cases. There are many predisposing factors, like damage to intestinal epithelium, disturbance in gut microbial composition, immunosuppression, etc. But main predisposing factor is coccidial infection caused by *Eimeria spp*[6]. Damage to intestine due to coccidia results in inflammatory response and release of plasma proteins into lumen of intestine. These plasma proteins provide necessary growth substrate for proliferation of these bacteria[5]. *Cl perfringens* multiplies anaerobically in intestinal tract and produces toxin. These toxins are proteolytic and collagenolytic enzymes that damage intestinal epithelium and causes necrosis. Gross lesions such as diffusely congested intestinal loop with fibrononecrotic material in lumen was noted and recorded earlier[8-10]. Histopathological changes including diffusely necrosed mucosa, sloughed off epithelial cells, infiltration of inflammatory cells and presence of coccidial oocysts and schizonts were noted and also reported earlier[8-11]. *Cl. perfringens* goes to liver through portal bloodstream and biliary duct and causes damage to liver.[7] Gross lesions such as necrotic foci and heamorrhages were observed and same has been recorded on the present investigations also[9,10].Histopathologically loss of hepatocytes, sinusoidal fibrosis, congestion and infiltration of inflammatory cells has been recorded earlier[7,12]. The association between *Emeria spp.* and *Cl.perfringens* leads to necrotic enteritis[5] and has been recorded in India also. However, the records regarding the same are scant, hence reported.

Based on characteristic gross lesions, wet mount examination and bacterial examination of intestinal content, histopathological findings and PCR detection of 16S rRNA gene; the case was confirmed as necrotic enteritis and concurrent coccidiosis.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI) Assisted technology for manuscript preparation: The authors confirm that there was no use of AI – Assisted Technology for assisting in the writing of the manuscript and no images were manipulated using AI

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Indian J. Vet. Pathol., 50(1) : 71-74, 2026: DOI: https://doi.org/10.56093/xcka6f05

SHORT COMMUNICATION

Clostridium perfringes associated enteritis in a pigeon *(Columbia livia domestica)*: Clinical, gross and histopathological observations

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Received: 25.8.2025; Accepted: 10.9.2025

ABSTRACT

Clostridium infection in pigeons is an emerging concern in avian medicine, characterized by gastrointestinal disturbances and high morbidity. It is primarily caused by *Clostridium perfringens*, an anaerobic, spore-forming bacterium capable of producing potent toxins that lead to necrotizing enteritis. This case report describes the clinical presentation, diagnosis and management of intestinal *Clostridium* infection in an adult domestic pigeon *(Columba livia domestica)*. The bird was presented with symptoms including lethargy, decreased appetite, watery greenish droppings and progressive weight loss. Clinical examination revealed signs of dehydration, ruffled feathers and soiled vent. Post-mortem examination showed severe enteritis with haemorrhagic lesions along the small intestine and fibrinous exudates. Intestinal contents were collected and submitted to the Department of Veterinary Pathology, Bihar Veterinary College, Patna for microbiological and histopathological examination. Gram staining confirmed the presence of large Gram positive rods consistent with *Clostridium perfringens*. Histopathological analysis revealed extensive mucosal necrosis, inflammatory cell infiltration and bacterial colonization in the intestinal lumen confirming clostridial enteritis. The findings highlight the pathogenic role of *Clostridium perfringens* in pigeons and underline the need for timely diagnosis and appropriate antibiotic therapy. The case emphasizes the importance of incorporating routine microbial and histological evaluations in avian gastrointestinal disorders for accurate diagnosis and effective management.

Keywords: *Clostridium perfringens*, histopathology, mucosal necrosis, necrotizing enteritis, Pigeon

How to cite this article : Sinha, V.K., Kumar, S., Kumar, K., Kumar, D. and Ali, I. 2026. Clostri-dium perfringes associated enteritis in a Pigeon (Columbia livia domestica): Clinical, Gross and Histopathological Observations. Indian J. Vet. Pathol., 50(1) : 71-74.

Clostridial enteric infections in birds especially in domestic pigeons *(Columba livia domestica)* represent a growing concern in avian veterinary medicine due to their acute onset, high morbidity and mortality and significant pathological consequences. Among these, *Clostridium perfringens*, a Gram positive, spore-forming, anaerobic bacillus is recognized as the primary causative agent of necrotizing enteritis in avian species[1]. This organism is capable of producing a range of potent exotoxins, including alpha and beta toxins which contribute to severe mucosal damage, necrosis and enteric hemorrhage[2]. The disease predominantly affects young or immunocompromised birds and is often precipitated by stress, poor hygiene, concurrent infections or dietary imbalances[3,4].

Clostridium perfringens associated enteritis in pigeons typically presents with nonspecific clinical signs such as anorexia, lethargy, watery greenish droppings, progressive emaciation and sudden death in some cases[5]. Diagnosis can be challenging and is often confirmed post-mortem through bacteriological culture, toxinotyping and histopathological evaluation which may reveal necrosis of the intestinal epithelium, mucosal sloughing, inflammatory infiltration and the presence of large Gram positive rods[6]. The pathogenesis involves rapid bacterial proliferation in the intestinal lumen, leading to toxin mediated disruption of the intestinal barrier and systemic toxaemia[7]. In pigeons, the condition is frequently under diagnosed due to its overlapping symptoms with other enteropathies and the need for specialized diagnostic approaches.

Despite its clinical relevance, detailed case-based documentation of *Clostridium perfringens* infection in domestic pigeons is relatively scarce in the veterinary literature. Early diagnosis, combined with appropriate antimicrobial therapy and improved husbandry practices is critical for effective disease control and reducing flock level outbreaks[8]. This clinical case report describes the natural occurrence of intestinal *Clostridium perfringens* infection in an adult domestic pigeon. The primary aim of the study is to document the clinical presentation, gross and histopathological findings and microbiological confirmation of the infection. The report under scores the importance of integrated diagnostic methods for accurate identification and timely intervention in managing clostridial infections in avian species.

The present study was conducted on a clinical case of

intestinal *Clostridium perfringens* infection in an adult domestic pigeon *(Columba livia domestica)* submitted to the Department of Veterinary Pathology, Bihar Veterinary College, Patna, Bihar, India for post-mortem examination. A backyard flock of 50 pigeons of which 15 died, had a reported history of progressive weight loss, anorexia, greenish watery droppings, lethargy and sudden death. The affected pigeon had shown signs of illness for approximately three days before being found dead. According to the owner, the bird was being fed a homemade high protein diet primarily consisting of soaked grains and pulses with no recent deworming or anti-microbial treatment.

Out of a backyard flock of 50 pigeons, 15 birds died and were subjected to gross post-mortem examination which revealed consistent findings across all cases including moderate dehydration and emaciation, soiled vent feathers and distended intestinal loops. Fifteen pigeons that died from a backyard flock of 50 were submitted for necropsy. Each bird underwent a thorough gross post-mortem examination following standard protocols. Observations were systematically recorded for external and internal lesions. Particular attention was given to the gastrointestinal tract, liver, spleen, lungs and heart. The small intestines were examined for segmental thickening, congestion and mucosal haemorrhages. The nature of intestinal contents (colour, consistency, presence of mucus or blood) was noted. The liver was assessed for size, colour and texture and the spleen for swelling or discoloration. The lungs and heart were examined for any gross abnormalities.

Tissue samples were collected from the small intestine and fixed in 10% neutral buffered formalin for a minimum of 48 hours. Following fixation, the samples were processed through a routine paraffin embedding technique. Sections of 4-5 µm thickness were cut using a rotary microtome and mounted on glass slides. These sections were stained with Haematoxylin and Eosin (H&E) for microscopic examination. Histopathological evaluation focused on identifying lesions such as inflammation, necrosis, haemorrhage, congestion and other pathological changes.

Post-mortem and laboratory findings confirmed a diagnosis of necrotizing enteritis caused by *Clostridium perfringens* infection. Gross lesions in the small intestine were characterized by showing multiple circulars, raised, dark necrotic foci on the mucosal surface (Fig. 1). The small intestine showed segmental areas of thickening and severe congestion with multiple foci of mucosal haemorrhages. A moderate amount of yellowish, mucoid and occasionally blood stained intestinal content was observed. The liver appeared enlarged and pale and the spleen was mildly swollen. No significant lesions were observed in the lungs/heart. Impression smears revealed large Gram positive bacilli and no other pathogens were observed (Fig. 2). Haematoxylin and eosin stained intestinal sections revealed extensive mucosal necrosis, desquamation of villous epithelium and dense infiltration of heterophils and mononuclear cells in the lamina propria and submucosa. Numerous large Gram positive rods were observed within necrotic debris and among epithelial crypts.

Histopathological examination showed widespread necrosis of the intestinal villi, infiltration of heterophils and mononuclear cells and dense

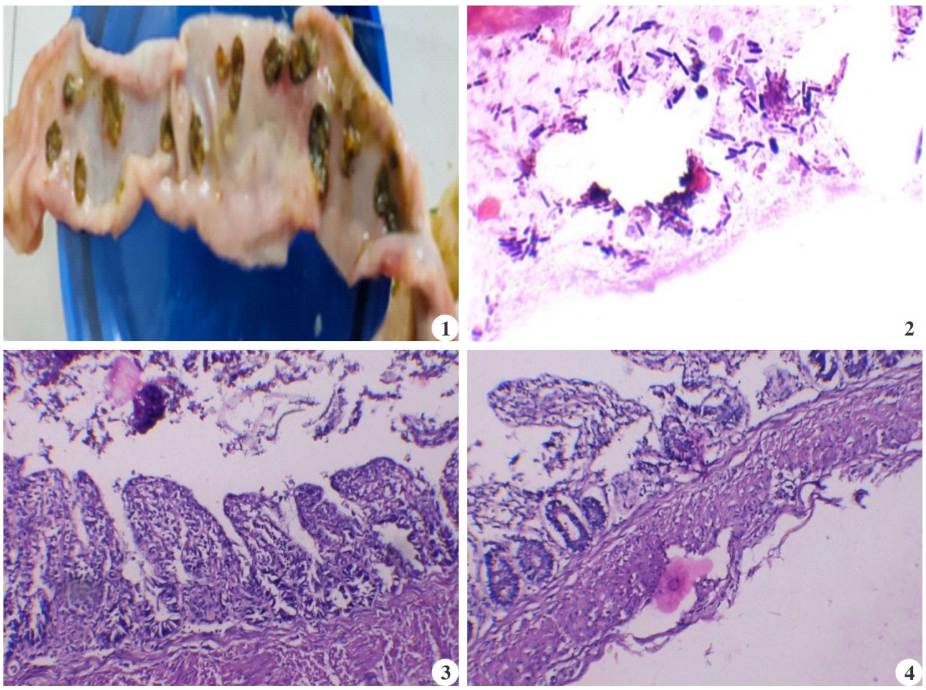

Fig.1. Gross photograph of pigeon intestine showing multiple circular, raised, dark necrotic foci on the mucosal surface characteristics of necrotic enteritis caused by *Clostridium perfringes*; **Fig.2.** Gram stained section of pigeon intestine showing numerous large Gram positive rod shaped bacteria (*Clostridium* spp.) adherent to the necrotic mucosa; **Fig. 3.** Histological section of intestine of a pigeon (H&E,10x) shows severe necrosis and loss of epithelial lining in the intestinal villi with congested lamina propria having inflammatory cells; **Fig.4.** Histological section of intestine of a pigeon (H&E, 10x) shows disrupted, shortened and fused villus architecture. The lamina propria and submucosa show congestion, edema and fibrin deposition. The surface has necrotic debris with bacterial colonies.

colonies of large Gram positive rods in the mucosal layers (Fig. 3). These findings were consistent with necrotizing enteritis due to *Clostridium perfringens*, a condition previously described in poultry and pigeons suffering from acute enteric disease[1,5].

The intestinal architecture was markedly disrupted, with the lamina propria replaced by necrotic debris and inflammatory exudates. The villi appeared shortened, fused or entirely sloughed in certain regions. Bacterial colonies were evident along the luminal surface and crypts, accompanied by desquamated epithelial cells and fibrin (Fig. 4). No parasitic or protozoal organisms were identified. These features were indicative of a severe toxin mediated mucosal injury typical of *C. perfringens* infection[6].

Clostridial enteritis is frequently observed in birds maintained under poor hygienic conditions or fed high protein diets without proper microbial balance[2]. In this case, the affected pigeon was part of a backyard flock fed on high protein soaked grains with no routine probiotic or antimicrobial supplementation. Such nutritional and environmental imbalances may contribute to clostridial overgrowth in the intestine[4]. The sudden death observed was likely the result of acute toxaemia and fluid loss due to mucosal necrosis and haemorrhagic enteritis.

Although clostridial infections have been extensively reported in commercial poultry operations their documentation in domestic pigeons remains limited. However, the pathogenesis, gross lesions and histopathological features in this case were consistent with previous descriptions in chickens, turkeys and pigeons[7]. The presence of Gram positive bacilli in intestinal smears and characteristic necrotic lesions on histopathology confirm the diagnosis and rule out differential diagnoses such as coccidiosis, salmonellosis or trichomoniasis[8].

Effective prevention of *C. perfringens* enteritis relies heavily on improved husbandry practices such as regular cleaning of enclosures, controlled feeding strategies and minimizing stress[9]. Probiotic supplementation and use of toxin binding agents have been shown to reduce intestinal colonization and toxin production in susceptible avian species[10]. In future cases, early antimicrobial intervention with agents such as penicillin, metronidazole or bacitracin may help limit mortality when clinical signs are first observed[11]. Vaccination strategies against *C. perfringens* are being explored in poultry but such approaches remain unavailable for domestic pigeons at present[12].

Based on the clinical history of progressive weight loss, anorexia, greenish watery droppings, lethargy and sudden death along with consistent gross pathological findings (segmental thickening and congestion of the intestines, mucosal haemorrhages, pale enlarged liver and mildly swollen spleen), impression smears revealing large Gram positive bacilli and histopathological evidence of necrotizing enteritis, the condition was diagnosed as *Clostridium perfringens* induced necrotizing enteritis[13,14]. No concurrent parasites or protozoal organisms were detected in intestinal smears ruling out other common enteric pathogens.

The present case underscores the importance of integrating clinical history, postmortem evaluation and histopathological confirmation for definitive diagnosis of clostridial enteritis in pigeons. Early recognition of subtle signs like weight loss and diarrhoea, combined with timely laboratory testing can help mitigate the risk of flock level outbreaks. Proper hygiene, balanced diet and routine health monitoring are essential components of a successful prevention plan.

CONCLUSION

The present case report describes a fatal case of necrotizing enteritis suggestive of being caused by *Clostridium perfringens* infection in an adult domestic pigeon based on the postmortem, cytological and histopathological evaluations. The intestinal lesions exhibited extensive mucosal necrosis, dense inflammatory infiltration and the presence of large Gram positive bacilli consistent with clostridial enteritis. Clinical history and gross findings highlighted the rapid and aggressive nature of the disease especially under suboptimal husbandry conditions. The absence of concurrent parasitic or protozoal infections supported *Clostridium perfringens* as the primary etiological agent. This case emphasizes the critical importance of early detection, proper dietary management and hygiene in the prevention of enteric clostridial infections in pigeons. Histopathological confirmation remains essential for definitive diagnosis and should guide future preventive and therapeutic strategies in avian practice.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 75-79, 2026: DOI: https://doi.org/10.56093/f5pm5282

CASE REPORT

Atypical subtype of sporadic bovine leukosis in an adult crossbred bull: A case report

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Received: 31.10.25; Accepted: 27.11.25

ABSTRACT

In the present study, a bull aged nine years with a history of fever and enlarged lymph nodes (LNs) was presented to the post mortem facility of Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences, University thorough necropsy examination revealed enlargement of prescapular, bronchial and mesenteric lymph nodes with loss of corti-comedullary differentiation and yellow creamish surface with haemorrhages. An interesting solitary hard mass was found near the right kidney. Cytological examination of LNs and hard mass revealed anaplastic changes in lymphoid cells. Histopathological examination of affected LNs and hard mass revealed medium to large sized lymphocytes with few mitotic figures that replaced the follicles and sinuses. Moreover, these neoplastic cells of LNs and hard mass were found to be immunopositive with CD3 antibody only and immunonegative with PAX-5 and BLV p24 (capsid protein) antibodies suggesting a case of sporadic bovine leukosis. However, the intriguing aspect of present case was that, neither the thymus nor the skin was affected along with the presence of atypical hard mass, indicating that it does not fall under either of the categorization of sporadic bovine leucosis. .Hence, the present case based on age, gross, cytological, histological and immunohistochemical findings, was classified as an atypical form of Sporadic Bovine Leukosis.

Keywords: Atypical, bovine leukosis, BLV p24, CD3, Lymphoma, Pax-5, Sporadic

Lymphosarcomais a round cell tumor which has hematolymphoid origin[1]. It is the second most common tumor in cattle after squamous cell carcinoma. Bovine lymphosarcoma has been categorized into two forms: Sporadic Bovine Leukosis (SBL), which has an unknown etiology and Enzootic Bovine Leukosis (EBL), which is caused by infection with BLV (Bovine Leukemia Virus)[2]. Further, SBL is commonly divided into three types: juvenile/ calf, thymic and cutaneous which mainly affects the bovine population under 3 years old while EBL is mostly found in dairy cows and is contagious with a peak incidence in mature animals above 3 years of age[3].

Juvenile form mainly occurs in calves up to 6 months of age and characterized by multicentric lymphadenopathy[4]. The thymic form of SBL mainly affects the animals of 6-24 months and its clinical symptoms vary according to the tumour's location and size which is characterised by thymus involvement, cervical enlargement, bloating, dyspnoea, tachycardia, jugular distention, fever and forequarters edema[5]. In skin or cutaneous form, dermal plaques of diameter 1-5cm can be observed on the neck, rump, back and thigh which can be found in animals up to 30 months of age[3]. The cases which do not fit into these forms are classified as atypical subtype of sporadic bovine leukosis[6].

The present communication case report described the pathomorphological diagnosis of atypical subtype of sporadic bovine leukosis in an adult crossbred bull, which was not fit in any category of sporadic bovine leukosis based on presence of mass, criteria of age, gross, cytological, histopathological and immunohistochemical findings.

Carcass of a crossbred bull aged nine years old was presented to the post mortem facilityof Department of Veterinary Pathology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana with a history of fever and enlarged lymph nodes. A blood sample was collected from the animal at the time of its presentation to the Outpatient Department of the Large Animal Clinics, GADVASU, prior to its death. Total leukocyte count (TLC) and hemoglobin (Hb) weredone by automatic haematology analyser machine (Orphee Mythic 18-VET) and differential leukocyte count was done manually after staining blood smear with Leishman stain[7]. A thorough necropsy was conducted and various

How to cite this article: Syal, P., Singh, N.D., Leishangthemlesha G.D. Atypical subtype of sporadic bovine leukosis in an adult cross-bred bull: A case report. Indian J. Vet. Pathol., 50(1) : 75-79.

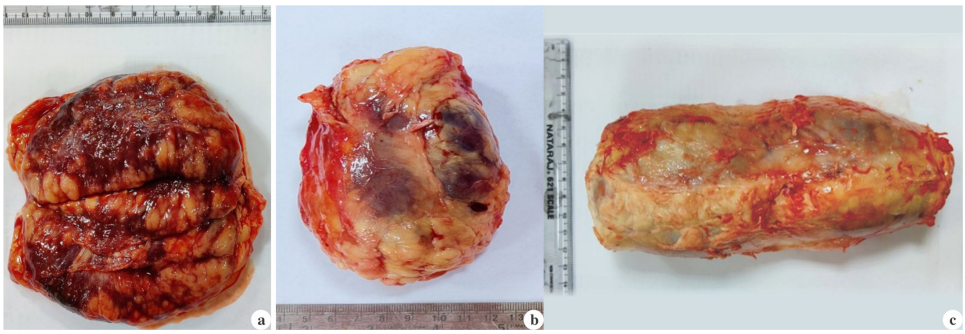

Fig. 1. Enlarged Prescapular (A), Bronchial (B) and Mesenteric (C) Lymph nodes

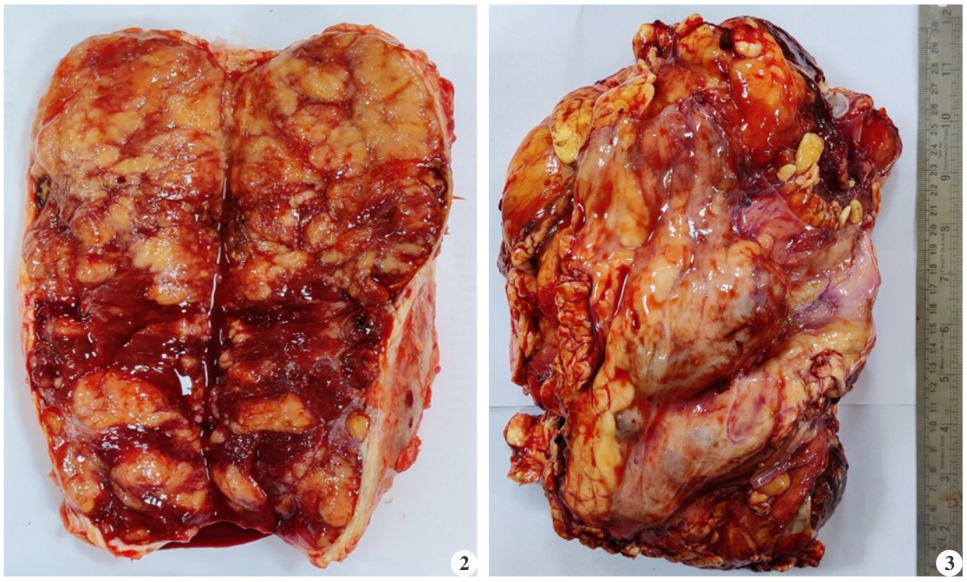

Fig. 2. Cutting of lymph node showed loss of corticomedullary differentiation and pale yellow surface with haemorrhages; **Fig 3.** Solitary hard mass with 27cm length and 17cm width near kidneys.

gross findings were recorded. Impression smears were made by taking cytological samples from enlarged lymph nodes (prescapular, bronchial and mesenteric lymph nodes) and hard mass found in the abdominal cavity near the right kidney on a clean glass slide. All impression smear were stained with Leishman stain. Representative tissue samples were collected *viz.*, lymph nodes, liver, spleen, lungs and hard mass in 10% neutral buffered formalin for histopathological analysis. The tissue sections of 5µm thickness were cut with a rotary microtome (Leica) and stained with the standard Haematoxylin and Eosin method[8]. For confirmatory diagnosis immunohistochemistrywas performed by using CD3 antibody (Pathnsitu, Polyclonal antibody, Ready to use), PAX-5 antibody (Invitrogen, Monoclonal antibody and Dilution 1:250) and monoclonal antibodies against the p24 protein of the bovine leukemia virus capsid with a dilution of 1:500 (VMRD, Pullman, WA, USA)[9]. Antigen labelling was performed by using DAB (3, 3-diaminobenzidine) solution (Vector, ImmPACT peroxidase substrate kit, USA) for visualizing the antigen-antibody-peroxidase reaction. All the images were taken by using an Olympus Microscope (BX51).

On clinical examination, an animal was found to be suffering from fever and enlarged lymph nodes. Haematological examination revealed 9.5 g/dl of Hb and 14900/ µl TLC with a differential count of 68% lymphocytes and 32% neutrophils. Resultant absolute count of lymphocytes and neutrophils were 10132/µl and 4768/ µl respectively. Therefore, hematology revealed leukocytosis with absolute lymphocytosis.

Grossly, prescapular (11cm length and 8.5cm width), bronchial (9cm length and 6.9cm width) and mesenteric (20.1cm length and 7.6cm width) lymph nodes were enlarged (Fig. 1A, 1B, 1C). On cutting the lymph nodes, the gross corticomedullary differentiation was found to be lost with pale yellow to reddish discoloration (Fig. 2). The kidneys were slightly congested and one interesting solitary hard mass of 27cm length and 17cm width was seen near right kidney (Fig. 3). The mass had yellowish discoloration with haemorrhages on their cut surfaces. Liver was enlarged with round edges and multiple sterile cysts were observed on lungs, liver and spleen. Moreover, the spleen revealed petechiae on its surface and lungs showed emphysema as crepitating sounds were evident by tactile sensation.

Cytological examination of the impression smear from lymph node revealed medium to large sized population of lymphocytes (89.29µm to 160.36µm in size) having large nucleus varying from 82.85µm to 157.08µm with prominent nucleoli of varying sizes (15.85µm to 24.26µm), coarse aggregated chromatin and less cytoplasm with basophilic intensity suggesting lymphoma. Mean ± S.E of cell size, nucleus size and nucleolus size were 108.83 ± 7.16, 107.66 ± 5.90 and 19.57 ± 1, respectively. In some areas, two to four

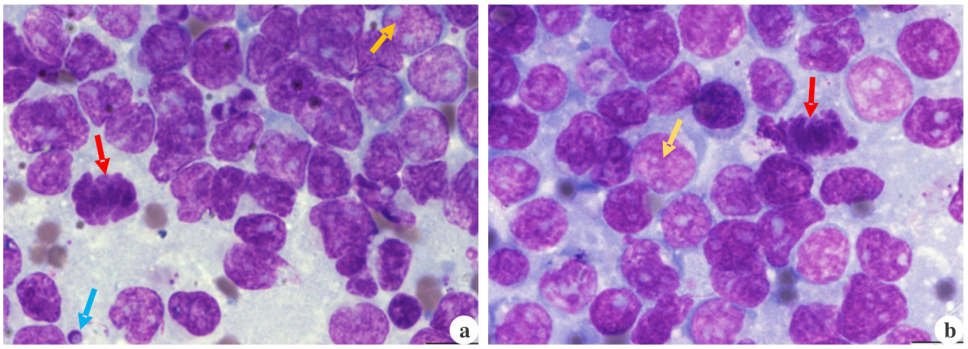

Fig. 4. Cytological examination of impression smear of mesenteric lymph node (A) and solitary mass (B) near kidney revealed uniform population of lymphoblasts having large nucleus with prominent multiple nucleoli (yellow arrow) and small basophilic cytoplasm, mitotic figures (red arrow) and lymphoglandular bodies (blue arrow) suggesting lymphoma (Leishman, Bar=10µm)

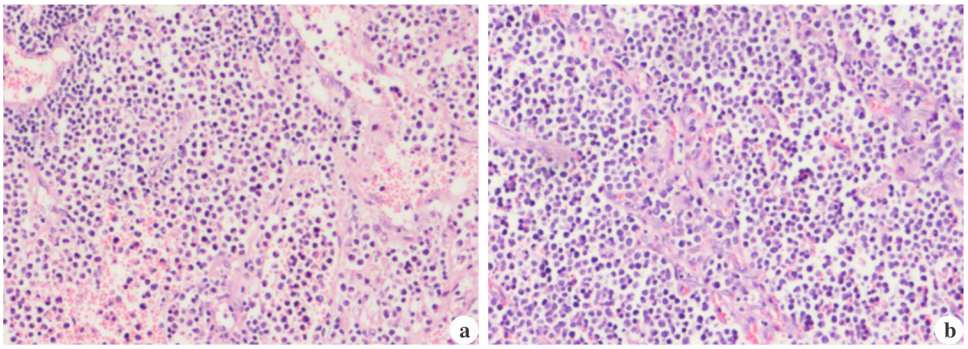

Fig 5. Lymph node (5A) and Solitary mass (5B) revealed small to medium sized neoplastic cells and lymphoblasts (H&E, Bar=100µm).

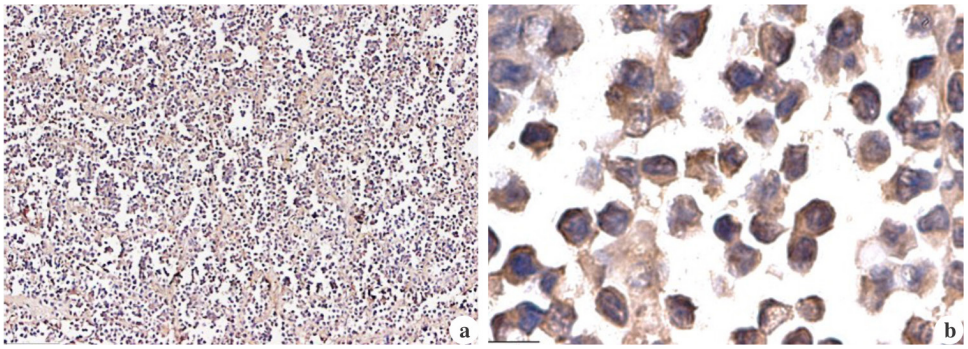

Fig 6. Immunoreactivity with CD3 by neoplastic cells in lymph node (Bar=100µm) (A) and solitary mass (B) (Bar=10µm).

mitotic figures were also observed (Fig. 4A). Further, impression smear from the solitary hard mass revealed similar changes such as medium to large sized lymphoblasts, with large nucleus, prominent nucleoli and coarsely aggregated chromatin (Fig. 4B).

Histopathological examination of enlarged lymph nodes revealed small to medium sized lymphocytes and lymphoblasts with neoplastic characteristics *viz.*, pleomorphic nucleus, karyomegaly, prominent nucleoli and coarse chromatin with few mitotic figures. These cells totally replaced the normal lymphoid follicles and sinuses of the lymph nodes (Fig. 5A). The neoplastic cells were separated by thin connective tissue and nucleus was surrounded by a thin margin of pale eosinophilic cytoplasm. A widespread sclerotic area was detected with marked proliferation of fibrous tissue in a lymph node suggesting desmoplasia. Moreover, the tissue section of solitary mass seen in the abdominal cavity near right kidney also revealed similar neoplastic changes (Fig. 5B), which led to the conclusion that this mass also represented lymphosarcoma. The histology of this mass was fully altered and only neoplastic cells were observed. Liver, spleen and lungs revealed sterile cyst showing a thin, fibrous capsule on the outside and a single layer of cuboidal tissue inside. There was lymphoid depletion and hemosiderosis in spleen. Lungs revealed severe dilatation of alveoli suggesting emphysema. The thymus was not located as due to the animal's age, it might have involuted. Further, Immunohistochemical staining of the lymph nodes and solitary hard mass showed immunopositivity with CD3 antibody only (Fig. 6A, 6B). No immunoreactivity was found withPAX-5 antibody (Fig. 7A, 7B) and BLV viral capsid protein p24 (Fig. 8A, 8B).

In the present case, cytological and histopathological examination of lymph node and hard mass discovered that the animal was suffering from lymphosarcoma or malignant lymphoma[10]. Further, the confirmatory diagnosis was done by immunohistochemical studies that revealed immunoreactivity of neoplastic cells with CD3 only which is a membrane T-cell marker suggesting T-cell origin of neoplastic cells[11] and no immunoreactivity was found with PAX-5 antibodies

which is expressed by B-lymphocytes[12].

Moreover, no viral isolate was found by using BLV capsid protein p24 antibody that revealed lymphoma in aged bull was not induced by bovine leukemia virus *i.e.*, EBL form[13]. Therefore, it was confirmed that the animal was suffering from sporadic bovine leukosis.

According to previous reports, T-cells are primarily involved two forms of sporadic bovine leukosis *viz.*, thymic and cutaneous form which primarily affects young animals i.e., up to 30 months of age[14]. However, the intriguing aspect of present case was that, neither the thymus nor the skin was affected, indicating that it does not fall under either of these forms of sporadic bovine leukosis. The third category of SBL is juvenile form that is seen up to six months of age with multicentric lymphadenopathy and B-cell involvement[3]. Although multicentric lymphadenopathy with T-cell involvement was evident in the present case, but, the curious turning point was the bull's age which was nine years that precluded it from being classified as a juvenile form.

Therefore, the present case deviated from the classification of SBL and classified as atypical subtype of SBL. Moreover, along with the age factor, a solitary hard mass of unknown origin due to its altered histology found in the abdominal cavity showing features of lymphosarcomaalso representing an atypical form of sporadic bovine leukosis. The comparable results of hard mass with the lymph nodes might represent it as an enlarged mesenteric lymph node but it was not confirmed. Additionally, the lymphocytic leucocytosis of present case also corroborated the finding that the cancer was limited to lymph nodes and had not transformed into leukemic form as no bizarre lymphocytes were observed in the blood smear.

Most of the cases of atypical SBL were reported in young animals[15,16,17,18.] Only one case of atypical SBL was reported in an adult cattle[6]. SBL is rarely detected and is estimated to affect one animal out of every 100,000 cattle[19]. It is nontransmissible and noncontagious, and it normally exclusively affects young cattle typically those under a year old in a herd.

Moreover, the fever in bull might be a paraneoplastic syndrome as neoplasia leads to release of various cytokines like IL-1, IL-6, IL-8 and INF-γ which are mainly responsible for affecting the thermoregulatory centre[20] and concomitant secondary infections that was evident from increased total leukocyte count of present case. Bovine tuberculosis was ruled out as a differential diagnosis based on enlarged lymph nodes. However, the pathological examination revealed no evidence of tuberculosis and acid-fast staining came negative on impression smear taken from lymph nodes and their tissue sections. Cysts on liver, lung and spleen of present case was incidental finding that was investigated on histopathological examination for scolex of tapeworm *Echinococcus granulosus* but it was not found anywhere. Therefore, these cysts could be suggested as sterile cysts. Sporadic bovine leukosis remains a clinical and pathologic mystery despite recent attempts to gain a better understanding of the condition. In comparison to enzootic bovine leukosis, it is far less common.

In the present study, an aged adult bull presented with fever and enlarged lymph node was found to be suffering from lymphosarcoma based on cytological and histopathological findings. Further, to confirm the form of lymphoma i.e., sporadic bovine leukosis or enzootic bovine leukosis, immunohistochemical analysis was done that revealed immunoreactivity with T-lymphocytes only describing the present case was of sporadic bovine leukosis. Nevertheless, the criteria of age and atypical hard mass with altered histology deviated the animal to not fall under any described categories of sporadic bovine leukosis. Hence, the present case based on age, gross, cytological, histological and immunohistochemical findings, was classified as an atypical form of Sporadic Bovine Leukosis. This was the first instance of atypical sporadic bovine leukosis in an adult cattle reported in India.

ACKNOWLEDGMENT

Authors are thankful to the Head of Department of Veterinary Pathology, College of Veterinary science, Guru Angad Veterinary and Animal Sciences University, Ludhiana for providing all facilities in the Department.

Financial support & sponsorship: None.

Conflict of Interest: There exists no conflict of interest among authors

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 80-82, 2026: DOI: https://doi.org/10.56093/9d9t6s03

CASE REPORT

Congenital melanoma in a crossbred Jersey calf – A case report

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Received: 18.10.25; Accepted: 04.12.25

ABSTRACT

Congenital melanocytic tumors are rather frequently occurring neoplasms in domestic animals especially in bovines. The present paper document a condition of congenital cutaneous melanoma in a 3 days old crossbred Jersey calf. Clinically, a large, firm, irregularly round growth measuring about 20X17cms was noticed on the right lateral side of the neck. The tumour mass was removed by surgical excision under local anaesthesia. The mass had a dry, dark and blackish cut surface. Cytological evaluation revealed few individual cells with blackish-brown cytoplasmic pigments. Histopathologically, round to fusiform neoplastic melanocytes were noticed proliferating in the dermis. Abundant brownish-black melanin pigments were noticed in the cytoplasm obscuring the nuclear details. Junctional activity and intra-epidermal nesting of neoplastic cells were noticed. Blood vessels and connective tissue stroma were evident amidst the neoplastic melanocytes. Bleached tissue sections revealed numerous spindle and round cells with mild or no cytoplasmic pigmentation. Nucleus was large and round with multiple nucleoli arranged near to nuclear membrane. Based on the gross and histopathological findings, the current case was diagnosed as congential cutaneous malignant melanoma.

Keywords: Congenital, crossbred Jersey calf, cutaneous, melanoma

Melanocytes and melanoblasts are the pigment producing cells, usually seen in the skin and mucous membranes[1]. They are dendritic cells derived from neuroectoderm, which migrate into the dermis and epidermis during embryogenesis. In the skin, they form 'epidermal melanin unit' along with keratinocytes of stratum basale in a highly ordered organisational pattern[1]. Any disruption of such a close association between melanocytes and basal keratinocytes may trigger a continuous proliferation of the melanocytes leading to the development of melanocytic tumors[2].

Melanocytic tumors have been reported earlier in wide range of animal species like fishes, reptiles, birds and mammals including humans[3]. Among domestic animals, they are being reported in bovines, dogs, goats, horses and pigs arising from various body locations[4]. In cattle, melanocytic tumors account for ~6% of all neoplasms, mostly arising from the skin[5], with a higher prevalence in the Aberdeen Angus breed[6] and among cattle with red, grey or black colour coats[7]. On the other hand, congenital tumors are rather rare in cattle with sporadic occurrence[8]. Various congenital tumors like granulosa cell tumor, teratoma, hemangioma, lymphangioma, ameloblastoma and sertoli cell tumors were reported earlier in bovines[9,10,11]. The present report is on a case of congenital cutaneous malignant melanoma in a neonatal calf.

A three days old male, crossbred Jersey cross calf was presented to the Veterinary Clinical Complex, College of Veterinary Science – Garividi, Andhra Pradesh with the history of a growth at the neck region since birth. The growth was surgically resected under local anaesthesia and the tissue sample was submitted for histopathological diagnosis to the Department of Veterinary Pathology, CVSc., Garividi in 10% neutral buffered formalin. The tissue sample was subjected to routine histopathological tissue processing and staining for microscopic evaluation[12]. Few heavily pigmented tissue sections were subjected for bleaching by treating with 0.3% aqueous solution of potassium permanganate for 30 min. to 1 hour followed by 1% oxalic acid solution for 1-2 min. as per the standard protocol[12]. The bleached tissue sections were further subjected to routine Hematoxylin and Eosin staining.

How to cite this article: Hemanth I., Ch. Kumar V. and Kumar K.M. Congenital melanoma in a crossbred Jersey Calf - A case report. Indian J. Vet. Pathol., 50(1) : 80-82.

Clinically, a large, irregularly round and non-ulcerated mass was noticed on the right lateral side of the neck region completely covered with skin (Fig. 1). The resected growth measured about 20X17cm in size and weighed about 750gm. The cut surface of the mass was dry, firm and blackish in appearence. Impression smear cytological evaluation from the cut surface revealed a few individual cells laden with blackish cytoplasmic pigments.

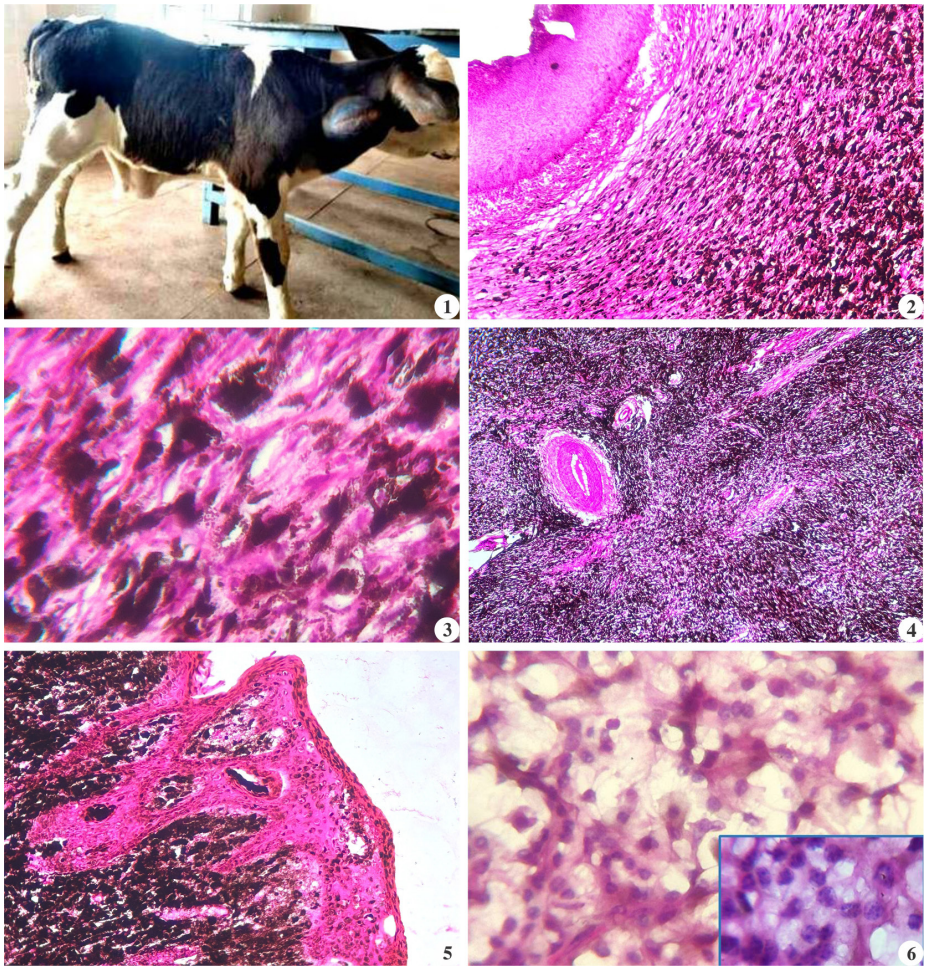

Fig. 1. Large and irregularly round growth on right lateral side of the neck in a Crossbred Jersey calf; **Fig. 2.** Diffuse proliferation of spindle shaped melanocytes below the epidermis with abundant brownish-black melanin pigment. H&E x100; **Fig. 3.** Fine brownish granules of melanin pigment within the melanocytes and stroma. H&E x400; **Fig. 4.** Proliferating melanocytes streaming around blood vessels and capillaries interspersed by islands of connective tissue. H&E x100; **Fig. 5.** Dermal-epidermal proliferation (Junctional activity) and intra-epidermal nesting of malignant melanocytes. H&E x400; **Fig. 6.** Bleached tissue sections revealing malignant melanocytes with large and round nucleus. H&E x100. Inset: Nucleus with multiple prominent nucleoli. H&E x1000

Histopathologically, diffuse proliferation of neoplastic melanocytes was noticed all over the dermis. The neoplastic cells were spindle or fusiform shaped near to epidermis (Fig. 2), while polygonal or round shaped in the deeper region suggesting a combined variant of melanoma as reported earlier[13]. Cytoplasm revealed an abundant dark brown to blackish coloured melanin pigment completely obscuring the nuclear details. At certain areas, the pigment appeared as fine brownish individual granules both within the melanocytes and in the adjacent stroma (Fig.3). Neoplastic cells were also seen streaming around the blood vessels and capillaries of the dermis. Fine strands and islands of connective tissue were evident amidst the proliferating melanocytes (Fig.4). Overlying epidermis appeared thin and undifferentiated at certain areas, whereas it was completely differentiated with infiltration of neoplastic cells underneath at other regions. Brownish granules of melanin pigment were also evident within the cytoplasm of epidermal cells. Junctional activity of melanocytes characterised by proliferation of neoplastic cells at dermo-epidermal junctions was noticed at certain areas. In addition, intra-epithelial nests of neoplastic cells were also evident within the epidermis suggestive of tumor malignancy (Fig.5). No evidence on the involvement of skin appendages was noticed. Examination of bleached tissue sections revealed numerous spindle and round shaped melanocytes within the dermis characterised by a large round nucleus with multiple prominent nucleoli that were mostly arranged at the nuclear periphery (Fig.6). Cytoplasm appeared non-pigmented with fine cytoplasmic processes.

Based on above gross, cytological and histopathological findings, the present case was diagnosed as congenital cutaneous malignant melanoma. Further, this condition can be categorised as dermal variant due to the infiltration of neoplastic cells into the dermis as detailed earlier[14]. Although, malignant melanomas were reported to have significant metastatic activity into regional lymph nodes and other organs[15], it was not noticed in the present case, since the calf recovered eventually with no further reoccurrence.

Financial support & sponsorship: None.

Conflict of Interest: There exists no conflict of interest among authors

Use of Artificial Intelligence (AI)-Assisted Technology for manuscript preparation: The authors confirm that there was no use of AI-assisted technology for assisting in the writing of the manuscript and no images were manipulated using AI.

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Indian J. Vet. Pathol., 50(1) : 83-85, 2026: DOI: https://doi.org/10.56093/xp0cmf41

CASE REPORT

Case of hepatocellular carcinoma in horse-report

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Received: 26.8.25, Accepted: 10.9.25

ABSTRACT

Hepatocellular carcinoma (HCC) in horses is an uncommon hepatic neoplasm often presenting with vague clinical signs and diagnosed postmortem. This case report describes the clinical presentation, pathological findings and histopathological confirmation of hepatocellular carcinoma with concurrent pericardial haemorrhage in an adult horse. The animal exhibited nonspecific symptoms including lethargy, anorexia and respiratory distress prior to death. Clinical deterioration was rapid and the horse succumbed despite supportive treatment. A complete postmortem examination was performed revealing a markedly enlarged and nodular liver with pale to tan patches on surface, unclotted blood within the pericardial sac and heavy edematous lungs with frothy exudate. Microscopic examination of hepatic tissue revealed neoplastic hepatocytes arranged in trabecular and pseudoglandular patterns with marked cellular atypia and areas of necrosis consistent with hepatocellular carcinoma. The pericardium showed extensive haemorrhage and fibrin deposition while pulmonary sections exhibited alveolar congestion and emphysema. The histopathological findings confirmed a diagnosis of hepatocellular carcinoma with associated pericardial haemorrhage. This report highlights a rare multisystemic manifestation of hepatic neoplasia in equines and underscores the importance of thorough postmortem and histopathological investigations in diagnosing complex systemic conditions.

Keywords: Equine, Hepatocellular carcinoma, Histopathology and Pericardial haemorrhage.

INTRODUCTION

Hepatic neoplasms in horses are infrequently diagnosed and often remain clinically silent until they reach an advanced stage making them a diagnostic challenge in equine practice. Among these, hepatocellular carcinoma (HCC) is a rare but aggressive malignant tumour originating from hepatocytes with limited documentation in veterinary literature[1]. Hepatocellular carcinoma in horses may present with vague, nonspecific signs such as weight loss, lethargy, jaundice or may even remain asymptomatic until sudden death occurs[2]. In horses, hepatic neoplasms are infrequently diagnosed often due to their deep anatomical location, the liver's significant functional reserve and the non-specific clinical signs that can accompany hepatic disease[3]. Diagnosis is typically achieved post-mortem supported by gross pathological findings and histopathological examination of hepatic tissue[4].

Equine HCC is known for its potential to metastasize and invade surrounding tissues but concurrent involvement of other vital organ systems such as the cardiovascular and respiratory systems is exceedingly rare[5].

Comprehensive diagnostic efforts including detailed necropsy and histopathological evaluation are crucial in elucidating such conditions and expanding the current understanding of equine neoplastic pathology[7].

Despite the severity and clinical significance of these findings, there is a paucity of case-based reports describing hepatocellular carcinoma with associated cardiorespiratory pathology in horses. The rarity and vague clinical presentation make antemortem diagnosis of HCC particularly challenging and definitive diagnosis is frequently established post-mortem through gross pathological and histological examination[8]. This case report presents a rare instance of hepatocellular carcinoma in a horse with concurrent pericardial haemorrhage. The report aims to detail the clinical signs, gross pathology and microscopic lesions highlighting the importance of thorough post-mortem evaluation in cases of unexplained equine mortality.

How to cite this article : Kumar, D., Kumar, S., Kumar, K., Ali, I. and Sinha, V.K. 2026. Case of hepatocellular carcinoma in Horse-report Indian J. Vet. Pathol., 50(1) : 83-85.

CASE PRESENTATION

The present case study involves an adult male horse of mix breed of age 8 years 7 months submitted to the Department of Veterinary Pathology, Bihar Veterinary College, Patna (Bihar, India) for post-mortem examination (Fig.1). The animal had a reported history of progressive weight loss, reduced appetite, intermittent lethargy and mild respiratory distress. The animal had shown signs of clinical deterioration over the preceding week and died suddenly despite of symptomatic treatment. No history of trauma or previous hepatic disease was noted.

For histopathological analysis, representative tissue samples from the liver and heart

were fixed in 10% neutral buffered formalin and processed using standard paraffin embedding techniques.

RESULTS AND DISCUSSION

Postmortem and histopathological findings confirmed a diagnosis of hepatocellular carcinoma (HCC) with concurrent pericardial haemorrhage. Gross examination of the liver revealed marked enlargement with multiple pales to tan, firm nodular masses of varying sizes scattered throughout the hepatic parenchyma (Fig. 2). The pericardial sac was distended with dark, unclotted blood. Lungs appeared edematous, heavy and failed to collapse with frothy, reddish fluid evident in the trachea and primary bronchi (Fig. 4).

Microscopic examination of liver sections revealing neoplastic hepatocytes arranged in irregular trabecular patterns with anisokaryosis, hyperchromatic nuclei, prominent nucleoli (Fig. 5). It also reveals clusters of atypical hepatocytes with pleomorphic, hyperchromatic nuclei, disorganized architecture and intracellular pigment along with prominent nucleoli (Fig. 6). Histological section of heart of Horse reveals cardiac muscle fibres with numerous red blood cells extravasated in between (Fig. 7). Histological section of lungs of Horse shows congestion and emphysema of alveoli along with inflammatory cells infiltration (Fig. 8).

Haematoxylin and eosin-stained liver sections revealed disorganized hepatic architecture with neoplastic hepatocytes arranged in trabecular and pseudoglandular patterns. The neoplastic cells exhibited marked nuclear pleomorphism, hyperchromasia, prominent nucleoli and frequent mitotic figures consistent with hepatocellular carcinoma. Pericardial sections showed diffuse haemorrhage with fibrinous exudate and scattered inflammatory cells.

These findings were consistent with primary hepatocellular carcinoma, a rare hepatic malignancy in equines that has been previously described in individual case reports and retrospective studies[2,5]. The nodular hepatic lesions and disrupted histoarchitecture, along with typical cytological features of malignant hepatocytes confirm the diagnosis[1].

Although direct neoplastic invasion of the pericardium was not evident histologically, similar

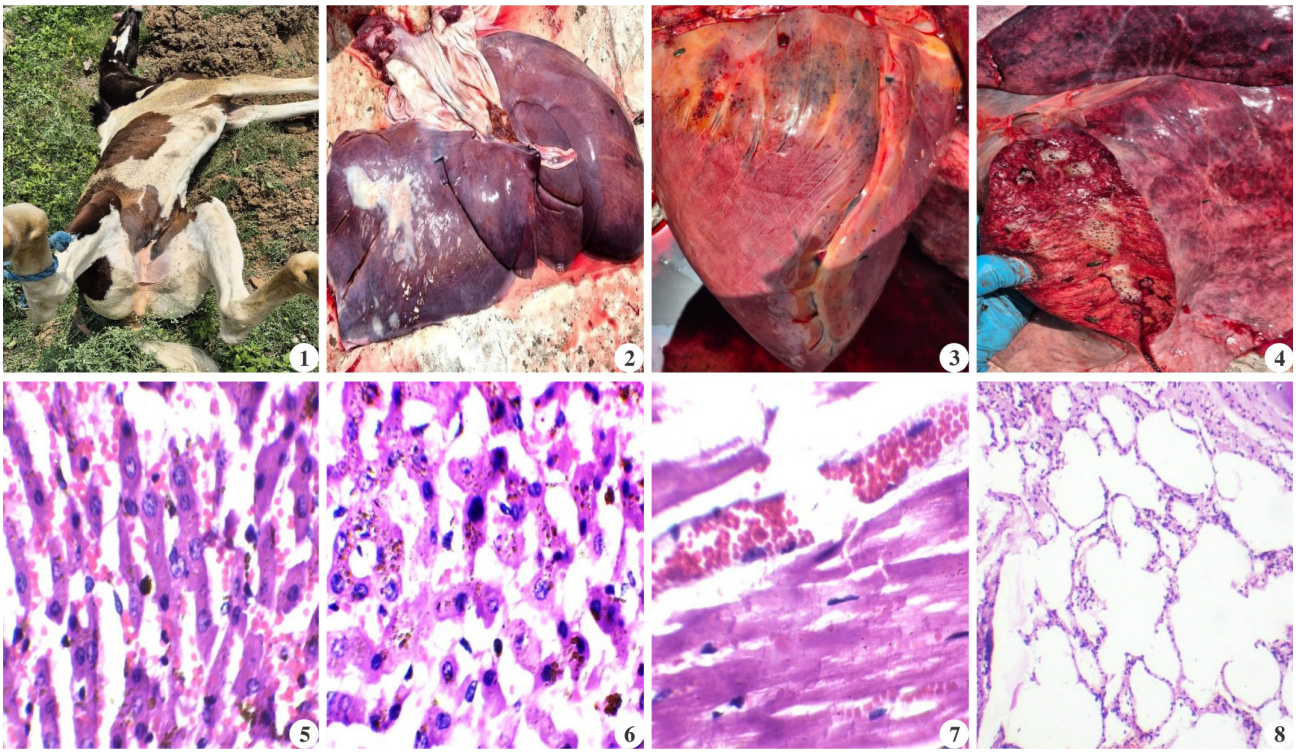

Fig.1. Gross photograph of horse with poor body condition and mild abdominal distension; **Fig.2.** Markedly enlarged liver with multiple pales to tan nodules of varying sizes; **Fig. 3.** Heart showing petechial haemorrhages on the epicardium; **Fig.4.** Lungs showing severe congestion with dark red, firm and non-collapsing parenchyma and frothy exudates., **Fig.5.** Histological section of liver of horse (H&E,400x) revealing neoplastic hepatocytes arranged in irregular trabecular patterns with anisokaryosis, hyperchromatic nuclei, prominent nucleoli; **Fig.6.** Histological section of liver of horse (H&E,400x) reveals clusters of atypical hepatocytes with pleomorphic, hyperchromatic nuclei, disorganized architecture and intracellular pigment along with prominent nucleoli; **Fig.7.** Histological section of heart of horse (H&E,400x) revealing cardiac muscle fibres with numerous red blood cells extravasated in between; **Fig. 8.** Histological section of lungs of horse (H&E,100x) showing congestion and emphysema of alveoli along with inflammatory cells infiltration.

presentations have been documented in horses in association with terminal neoplastic conditions[7].

Previous studies have indicated that hepatocellular carcinoma in horses may be clinically silent until the disease is advanced, with nonspecific signs such as weight loss, lethargy and respiratory distress often being the only indicators[9]. The rapid clinical decline and sudden death observed in this case reflect the decompensated systemic response to multisystemic compromise induced by hepatic neoplasia. The absence of gross or microscopic gastrointestinal lesions ruled out enteric diseases or parasitic causes of systemic illness.

Although hepatocellular carcinoma is well documented in small animals and humans, its occurrence and associated complications in horses remain underreported. The combined presentation of hepatic neoplasia with concurrent pericardial and pulmonary pathology as seen in this case represents a rare and diagnostically challenging condition that necessitates comprehensive postmortem evaluation and histological analysis[10].

In equine practice, early diagnosis of hepatic tumours is hindered by the deep anatomical location of the liver and the lack of specific clinical signs[11]. Ultrasonography and liver biopsy may aid antemortem detection in suspected cases, but histopathology remains the gold standard for definitive diagnosis[12]. While therapeutic options for equine hepatic neoplasms are limited, understanding their pathogenesis and systemic impact is essential for accurate prognosis and case management.

The present case underscores the importance of integrating clinical history, gross pathology and microscopic evaluation in diagnosing rare multisystemic manifestations of neoplastic disease in horses. Routine postmortem analysis remains a critical tool in uncovering underlying causes of unexpected equine mortality and contributes valuable insights to veterinary oncology.

CONCLUSION

This case highlights the importance of thorough postmortem examination in unexplained equine deaths and emphasizes histopathological analysis as a definitive diagnostic tool. Early recognition of hepatic disease, even in the absence of specific signs along with comprehensive diagnostic work-up is essential for timely intervention and understanding of rare neoplastic conditions in equine practice.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI) Assisted technology for manuscript preparation: The authors confirm that there was no use of AI – Assisted Technology for assisting in the writing of the manuscript and no images were manipulated using AI

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Indian J. Vet. Pathol., 50(1) : 86-89, 2026: DOI: https://doi.org/10.56093/q4k9t243

CASE REPORT

A rare case of Triorchidism in indigenous Fowl

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Received: 21.9.25; Accepted: 14.11.25

ABSTRACT

A case of triorchidism in an indigenous fowl was recorded in Ranchi, Jharkhand. Triorchidism is an exceptionally rare congenital abnormality, which could be ascribed to faulty embryological development with developmental cause that are distinct from testicular asymmetry. During postmortem examination of this fowl, three testes were observed. The third testis which was smaller as compared to the right and left testes was attached to the proximal end of the left testes with which it shared the same epididymis. The left, right and third testes weighed 4.2983 gm, 2.2193 gm and 0.8475 gm, respectively, whereas the length and width of the left, right and third testes were 1.2x2.9cm, 1.2x2.0cm and 1.0x1.2cm, respectively. The testes revealed, white fluid on cutting. This appears to be the first case of triorchidism in poultry from Jharkhand, India.

Keywords: Congenital, first, indigenous fowl, triorchidism

INTRODUCTION

Triorchidism is a condition described to the incidence of having three testes in an individual and has been reported earlier in poultry. Supernumerary right[2] and left[3] testes have been reported in domestic chicken earlier. These authors also reported that the small round supernumerary testis was softer in consistency, and normal in color and showed spermatogenic activity on histological examination. Hocking systematically examined the testes of 378 male domestic chickens and found three cases of triorchidism, each of which comprised of two left testes[4]. Both right and left testis could be affected by this phenomenon. Triorchidism is different from testicular asymmetry, which is widespread in birds and has been found to correlate with age and secondary sexual characteristics[5]. The phylogenetic distribution of triorchidism in vertebrates is poorly known due to dearth of information.

METHODOLOGY

An adult male indigenous fowl was brought for postmortem examination to the Department of Veterinary Pathology, College of Veterinary Science & A.H, Ranchi, Jharkhand. Critical postmortem examination revealed presence of three testes (Fig 1) in this bird. It was noted that the third testis was smaller than the right and left testes and was attached to the proximal end of the left testis and shared the epididymis with the latter. The weight and length of all right, left and the third testes were determined using Metler's analytical balance and meter rule respectively. The left, right and third testes weighed 4.2983gm, 2.2193gm and 0.8475gm, respectively while the length and width of the left, right and third testes were 1.2x2.9 cm, 1.2x2.0 cm and 1.0x1.2 cm, respectively. When the third testis was cut open, a milky white fluid oozed out similar was the observation when the right and left testes were cut open. The right and left testes were bean-shaped whereas the third testis was slightly elongated in shape. The three testes were nearly identical in color and firmness (Fig 1, 2). The testicular tissues were then fixed in 10% formalin for histopathology. The tissue was routinely processed, sectioned and stained with Hematoxylin and Eosin (H&E) stain[6].

How to cite this article : Sharma S., Lakra P.P., Gupta M.K., and Mehta A.K. 2026. A rare case of Triorchidism in indigenous Fowl. Indian J. Vet. Pathol., 50(1) : 86-89.

RESULT

Histopathological examination (Fig. 2) of right testis (T1) revealed presence of seminiferous tubules of variable sizes, which showed intact germinal layer, however in most of the tubules, there was detachment of spermatid and other developing stage of spermatogonia from germinal epithelium. In few places, the developing stages of spermatozoa showed continuity from germinal epithelium. In most of the seminiferous tubules, degenerative changes were observed. Spermatozoa were mostly in degenerative stages characterized by hypocellularity. Lumen of seminiferous tubules showed significant number of spermatozoa. No infiltrative changes in parenchyma or interstitium was seen. Calcification was also not observed.

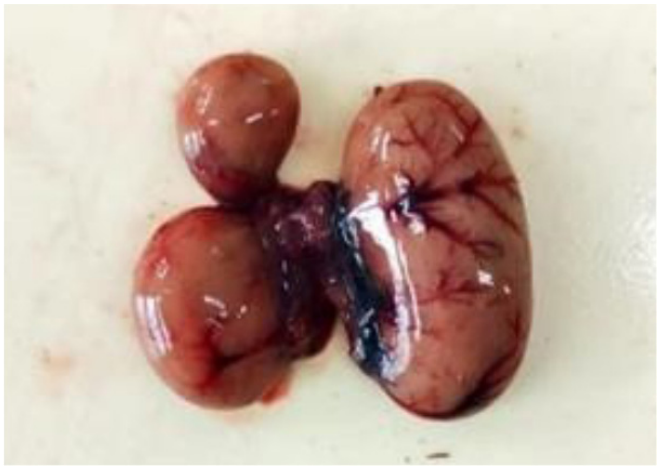


Fig. 2. Gross: Triorchid testis found in desi fowl

In the left testis (T2), the epididymal lumen showed necrosis and calcification. Loss of columnar lining epithelial cells of duct was observed with lesser number of spermatogonia.

Hypocellularity was also marked. Seminiferous tubules were lesser in diameter and loss of germinal epithelial cells was evident along with calcified zone and necrosis.

In the smaller left testis (attached to proximal end of left testis, T3) there was loss of germinal cells. In most of the places, the seminiferous tubules revealed loss of germinal cells with only thin basement membrane or sparely distributed germinal cells. In few seminiferous tubules, there was presence of spermatogonia but in most of the area spermatids were seen without formation of spermatogonia. Hypocellularity was significant.

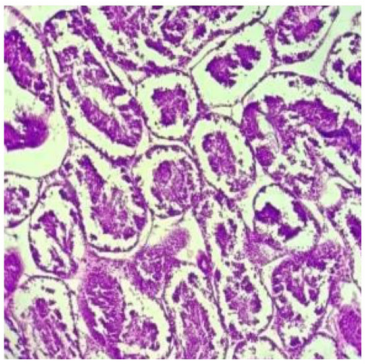
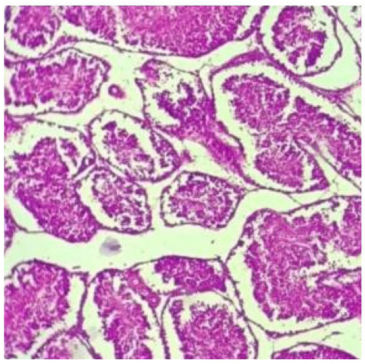
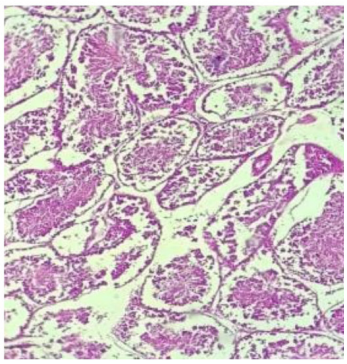
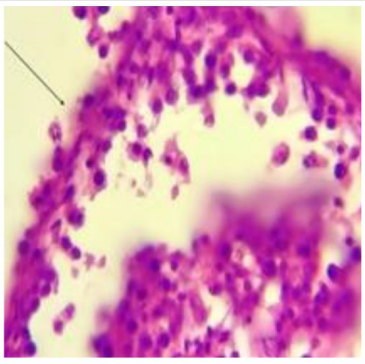
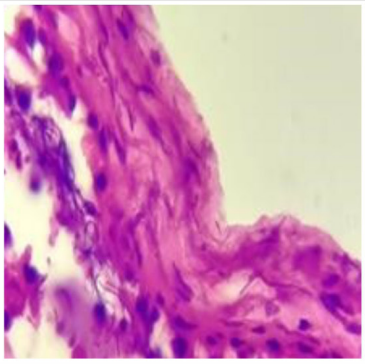
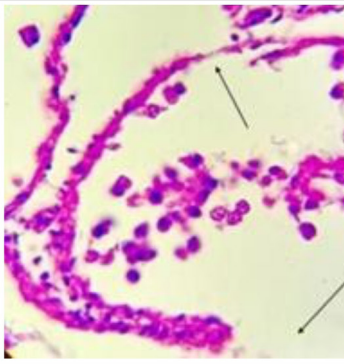
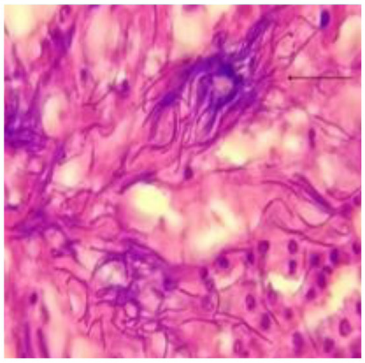
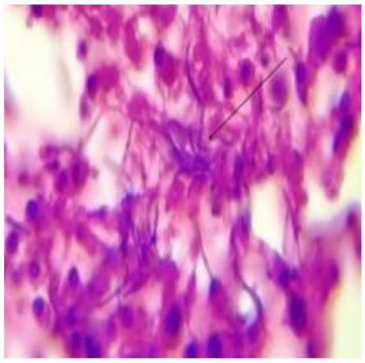
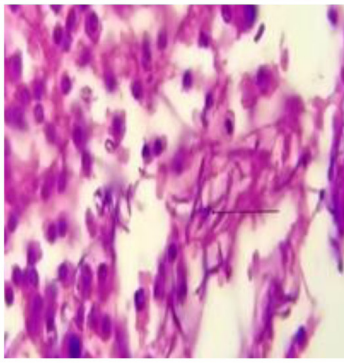
Testis	T1	T2	T3
Seminiferous tubule size Hypocellularity in T3 H&E x400			
Germinal Layer 1. Intact germinal layer in T1, T2 H&E x1000 2. Loss of germinal layer in T3 H&E x1000			
Spermatozoa Lumen showed high Presence of spermatozoa in T1&T2 compared to T3. H&E x1000			

Fig.2. Histopathology

McFarland[1], who examined 2,000 male Japanese Quails and found one case of triorchidism in which the right testis was divided into two nearly co-equal sections, both of which were undergoing normal spermatogenesis.

DISCUSSION

In chicken, testis development is governed by the Z-chromosome-linked DMRT1 gene, which directly or indirectly activates the male factors, HEMGN, SOX9 and AMH. Recent single cell RNA-seq has defined cell lineage specification during chicken testis development, while comparative studies point to deep conservation of avian testis formation[7].

Normally, the reproductive tract of poultry consists of a testis, epididymis and highly convoluted ductus deferens running alongside ureter. Poultry testes are paired organ, bean-shaped, light-yellow structures located within the abdominal cavity; along the backbone; near the cranial pole of the kidneys. Each testis is connected to a vas deferens, which transports sperm to the cloaca. Each testis is an aggregate of anastomosing seminiferous tubules, with associated interstitial space enveloped by connective tissue capsule. Testicular capsule is an important component of three layers: tunica mucosa, tunica albuginea and tunica vascularis. Tunica albuginea represents main tissue layer and comprises cellular elements that alternate with thick bundles of collagen fiber. Capsule is thinner in birds. Normally, the testis weighs from 0.4 - 28g. After 28 weeks, weight of testis significantly decreases, reaching 15g by week 42[8]. Gonadal differentiation in chick embryo occurs at 6.5 to 7 days of incubation. Embryonic testis is characterized by a germinal epithelium that recedes with time, a thicker capsule, absence of secondary or cortical sex cord as well as the presence of primary sex cord surrounded by stroma. Biochemically, gonadal differentiation is evident in terms of increased cyclic nucleotide concentration, increased protein synthesis and the pattern of sex steroid synthesis[9]. Once the testis has formed, the Mullerian duct ceases to develop and undergoes regression under the effect of AMH (*Anti Mullerian Hormone*). The gene encoding AMH is expressed in both male and female but higher in male during sexual differentiation[10].

There are various factors which can lead to pathology/ supernumerary testes during developmental stages. Any disruption in mesodermal cells during gonadal development could lead to formation of accessory gonadal structure[11].Gonadal development is regulated by FSH (follicle stimulating hormone) & Testosterone. Genes like DMRT1 (*Doublesex and mab-3 Related Transcription factor 1*), SOX9 (*Sex-determining region Y-box9*) and PITX2 (*Paired haemodomain transcription Factor 2*) play crucial role in testicular differentiation along with regulating the left-right symmetry of the gonads during development[12]. The mutations in FGF9 (*Fibroblast growth factor 9*) and SF1 (*Steroidogenic factor 1*) lead to atypical gonadal development which can result in extra testicular lobules. Sertoli cells express SOX9 and DMRT1 as markers which are involved in spermatogenesis whose improper differentiation can result in development of additional testicular lobule[13].Anomalies in basement membrane and tubular structure due to dysregulation in extracellular matrix protein facilitate atypical testicular formation in birds.

DMRT1 plays a central role in testicular development. DMRT1 is present on both ZZ chromosome in males but only on Z of ZW chromosome in female[14]. It is confirmed that DMRT1 is master Z-linked genetic trigger for testis formation in chicken[15,16]. It encodes for zinc finger like transcription factor with DNA binding DM domain[15,17]. Through the analysis of 5′ regulatory region, it is proposed that DMRT1 first plays role in germ cell development and then is recruited to somatic cell of gonads, where it plays role in Sertoli cell specifications[18]. DMRT1 is required for SOX9, HEMGN (Hemogen) and AMH expression, while inhibiting the FOXL2/ Aromatase female pathway.

Testis formation in poultry is also attributed to activation of SOX9 by DMRT1. SOX9 is central hub gene required for initiation of pre-Sertoli cell development in gonadal medulla. In chicken, SOX9 in male is upregulated during testis formations[17,19,20]. Downregulated SOX9 expression following DMRT1 knockdown in male gonads (ZZ) is ectopically activated when DMRT1 is mis expressed in ZW[15,17,18].

Another target FGF9, is required for proper Sertoli cell development and testis formation. DMRT1 protein is therefore likely to act as transcriptional activator and transcriptional repressor in embryonic avian gonads. Misexpression of the gene in the gonad induces SOX9, HEMGN, AMH and represses aromatase expression. HEMGN plays role in chicken testis development. Signals from the Sertoli cell lineage must drive interstitial cell development, and formation of the squamous surface epithelium, and induction of germ cell mitotic arrest though the exact nature of those signals are unknown. The resulting organ is a structurally and functionally integrated unit, supporting gain to genesis and male sex hormone production.

A similar case of triorchidism was reported in an indigenous breed of fowl in India[21].

CONCLUSION

Avian testis development represents an ideal model for understanding the molecular genetics of vertebrate gonadal sex differentiation. Much of our knowledge in this area has come from studies on the chicken embryo. Thus, the cause of triorchidism affiliates to

all those factors or genes which play significant roles in the development of testes during embryonic stages. Congenital anomalydue to abnormal development might be the possible cause for triorchidism in this case.

Financial support & sponsorship: None

Conflicts of Interest: None

Use of Artificial Intelligence (AI) Assisted technology for manuscript preparation: The authors confirm that there was no use of AI – Assisted Technology for assisting in the writing of the manuscript and no images were manipulated using AI

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Title of Thesis : Incidence, Pathomorphological and Molecular Detection of Peste des petits ruminants in Goats of Malwa Region in Madhya Pradesh

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Name of the Advisor : Dr Rashmi Choudhary

Degree/Year : MVSc/2024

Name of the University : College of Veterinary Science & AH, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur, MP

The study was carried out to determine the incidence and pathology of PPR in goats of Malwa region of Madhya Pradesh followed by molecular confirmation of virus through RTPCR. During six months duration, 500 goats were screened irrespective of age, sex and breed with 100 goats showing clinical symptoms similar to PPR were taken for study.

Haematobiochemical analysis revealed significantly decreased haematological parameters, leukocytosis with significant neutrophilia and lymphopenia. On biochemical analysis, significant changes were observed in total protein and ALT values. Gross and microscopic lesions suggestive of PPR were observed in respiratory, digestive and lymphoid system in lungs, abomasum and mesenteric lymph nodes, respectively. Positive lung tissues and their nasal swabs were amplified for N gene based RT-PCR. Out of total 66 samples, 14 samples (21.21%) were successfully amplified for N gene of expected size. Incidence rate of PPR was recorded as 1.6% (8/500), on the basis of RT PCR.

Title of Thesis : Pathology of experimentally induced subacute and subchronic ziram toxicity in kadaknath chickens

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Name of the Advisor : Dr Supriya Shukla

Degree/Year : MVSc/2025

Name of the University : College of Veterinary Science & AH, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur, MP

Dithiocarbamate fungicides are widely used across the globe for various crops, primarily because of their effectiveness in managing plant fungal diseases and their relatively low acute toxicity to mammals. Ziram, a specific dithiocarbamate is utilized to combat various fungal diseases affecting potatoes, nuts, certain fruits and grains.

The current study was conducted from May to October 2024 at the college poultry farm unit and Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Dr Ambedkar Nagar (MHOW), aimed to study the pathomorphological and haemato-biochemical alterations in the ziram induced subacute and subchronic toxicities in the Kadaknath chickens.

The clinical signs of toxicity included lethargy, loss of appetite, huddling, ruffled plumage, muscle weakness, uncoordinated gait and oedema in the crop. Weight gain and overall live weight were significantly lower in the experimental groups compared to the control group. Haematological changes included decreased haemoglobin, TEC, PCV and lymphocytes, with increased leucocyte count and heterophils. Biochemical analysis showed increased AST, ALT, creatinine and globulin levels, while total protein and globulin decreased. Zinc levels were significantly elevated, while copper showed no significant change. Gross pathology revealed liver congestion with necrotic foci, dark red spleen, hyperaemic intestines and atrophied bursa of Fabricius and thyroid. Histopa-thological findings included liver oedema, necrosis, spleen haemorrhages, intestinal necrosis and kidney degeneration. The study highlights the toxic effects of ziram on various organs in Kadaknath chickens.

Title of Thesis : Pathohaematobiochemical studies of amphistomosis in goats *(Capra hircus)*

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The study was carried out to determine the incidence, haematobiochemical and histopathological changes of amphistomosis in goats of Malwa region of Madhya Pradesh. During the six-month duration, 200 goats were examined at slaughter house, Mhow. Gross examination of the rumen and faecal examination by sedimentation method revealed an incidence rate of 20.5% and 17.5% amphistomosis, respectively. Females showed greater susceptibility to amphistomosis than males. Age wise higher incidence was observed in goats above 1 year age group.

The values of haemoglobin, packed cell volume and total erythrocyte count were found to be decreased highly significantly ($p \leq 0.01$) and values of total leucocyte count, neutrophil count and eosinophil count were increased significantly ($p \leq 0.05$) whereas non-significant changes were observed in lymphocyte, monocyte, basophil and platelet count in amphistomosis infected goats as compared to non - infected ones. Biochemical changes in the serum of infected goats revealed significantly decreased ($p \leq 0.01$) total protein and albumin values and significantly increased ($p \leq 0.05$) ALT level whereas non-significant changes were observed in globulin, A:G ratio and ALP levels. Positive faecal samples revealed mean egg per gram was 220.58 ± 21.84, indicating moderate infection of amphistomosis in goats. Grossly, the rumen of infected goats showed numerous pink-coloured conical flukes attached on or in between papillae. Flukes were processed and identified as *Paramphistomum cervi* based on their morphological characters. The mucosal surface was found severely inflammed and congested. The liver of affected goats were observed as soft, pale and friable in consistency. The duodenum appeared congested and oedematous, with mucoid exudate. No significant gross changes were observed in the reticulum of infected goats. Histopathologically, the parasites were lying freely as well as partly attached in the villi of rumen. The lining mucosal epithelium of rumen revealed degenerative changes, coagulative necrosis, sloughing and hyperplasia of villi mucosa and infiltration of lymphocyte and plasma cells. Microscopically, duodenal mucosa showed destruction of glandular epithelial cells, glandular dilatation and diffused infiltration of lymphocytes and eosinophils. Degenerative changes, coagulative necrosis, areas of haemorrhages and infiltration of mononuclear cells in the liver parenchyma and surrounding bile ducts were observed.

Title of Thesis : Pathological studies of induced subacute ochratoxicosis in Kadaknath chickens and amelioration with *Saccharomyces boulardii*

Name of the Student : **Ritesh Verma**

Name of the Advisor : Dr Supriya Shukla

Degree/Year : MVSc/2025

Name of the University : College of Veterinary Science & AH, Mhow, Nanaji Deshmukh Veterinary Science University, Jabalpur, MP

The poultry industry in India has seen remarkable progress over the years, with indigenous breeds like Kadaknath gaining recognition for their disease resistance and black meat with medicinal value. However, OchratoxinA (OTA), amycotoxin produced by *Aspergillus* and *Penicillium* species, poses a severe threat to poultry production causing nephrotoxicity, hepatotoxicity, immunosuppression and reduced growth performance. This study explored the pathological effects of OTA on Kadaknath chickens and evaluated the efficacy of *Saccharomyces boulardii* in mitigating its toxicity. The experiment, conducted from May to October 2024 at the College of Veterinary Science and Animal Husbandry, Mhow, utilized 40 day old Kadaknath chicks divided into four groups: Group A (control), Group B (OTA-exposed), Group C *(S. boulardii)* and Group D (OTA + *S. boulardii*). Parameters assessed included productive performance (body weight, weight gain, feed consumption, feed conversion ratio), haematobiochemical markers (Hb, TLC, ALT, AST, Cr and BUN) and pathological alterations in the liver, kidney, spleen, bursa of Fabricius, thymus and small intestine.

Results revealed that OTA exposure significantly reduced growth performance, impaired feed efficiency and caused severe biochemical and pathological changes including elevated ALT, AST, Cr and BUN levels. Gross and histopathological examination showed hepatic and renal damage, lymphoid depletion in the spleen, thymus and bursa of Fabricius and congestion and fatty changes in the small intestine. Supplementation with *S. boulardii* improved growth performance, preserved haematobiochemical parameters and mitigated organ damage with the probiotic-only group (Group C) exhibiting the best outcomes. In Group D, the probiotic partially alleviated OTA-induced toxicity but did not completely restore normal parameters. This study establishes *Saccharomyces boulardii* as an effective strategy to mitigate ochratoxicosis in poultry, improving growth performance, physiological health, organ integrity and enhancing productive performance. These findings highlight the potential of probiotics as a practical and effective solution to combat mycotoxin challenges and promote sustainable poultry production.

Title of Thesis	:	Pathomorphological Studies on Bovine Pneumonia with special reference to *Mannheimia haemolytica*
Name of the Student	:	**Shazaan Murtaza**
Name of the Advisor	:	T. Rajendra Kumar
Degree/Year	:	MVSc/2025
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Livestock forms a cornerstone of the Indian agricultural economy, contributing 6.8% of the GDP, with its share steadily rising over the past decades. India possesses the largest global bovine population *i.e.*, 193.46 million cattle and 109.85 million buffaloes, yet full utilization of animal resources remains hindered by persistent animal diseases, notably affecting food security. Among these, bovine respiratory disease complex (BRDC) stands out for its multifactorial etiology, encompassing a diverse array of pathogens, stress factors and management issues. *Mannheimia haemolytica*, a Gram-negative coccobacillus naturally inhabiting the upper respiratory tract, emerges as a principal agent of pneumonic pasteurellosis and BRDC. Its pathogenicity intensifies under stress or comorbid conditions, driven by virulence factors such as capsule, lipopolysaccharides and leukotoxin, resulting in rapid lung damage and significant economic losses in cattle and buffalo farming. The present study investigates the prevalence of pneumonia in bovines, focusing on the pathomorphology of the disease and the detection of *Mannheimia haemolytica* through isolation and polymerase chain reaction (PCR) in pneumonic cases. The research was conducted between December 2023 and October 2024 on 315 samples out of 2015 lung samples from cattle and buffaloes obtained from abattoirs, post-mortem facilities and veterinary dispensaries in and around border regions of Bidar, Karnataka. The study observed an overall pneu-monia prevalence rate of 15.63%. The findings indicated that the highest prevalence of pneumonia (59.68 percent) was in bovines aged 4-7 years, with female bovines (71.4%) being more affected than males. Weak and Emaciated animals were more prone to pneumonia than healthy, thin and fat animals. The types of pneumonia identified grossly and histopathologically were broncho-interstitial pneumonia (37.46%), interstitial pneumonia (28.57%), bronchopneumonia (18.09%), fibrinous broncho-pneumonia (3.80%) and other less common conditions like granulomatous pneumonia (0.63%) and pulmonary adenocarcinoma (0.31%). Notably, broncho-interstitial pneumonia was the most prevalent form of pneumonia, characterized by consolidation, thickening of interlobular septa and capillary congestion. In contrast, pulmonary adenocarcinoma was the least common condition, affecting only 0.31% of the cases. Bacteriological analysis of the samples identified *Mannheimia haemolytica* in 42 (13.33%) pneumonic cases. These isolates underwent biochemical tests, including catalase, oxidase and urease tests and were further screened for the virulence-associated gene *Hp* and the *16S* gene using PCR. The results confirmed the presence of these genes in all isolates, highlighting the bacterial origin of most pneumonia cases. However, pulmonary echinococcosis, accounting for 4.76% of cases, was parasitic in nature. The study concludes that the high prevalence of pneumonia in the region indicates favourable environmental conditions for the disease. The predominant involvement of lungs in poorly conditioned animals emphasizes the economic importance of pneumonia in the bovine industry. These findings are critical for guiding the development of preventive and therapeutic strategies to control bovine pneumonia in the region.

Title of Thesis	:	Transcriptional Dynamics of Pro-inflammatory and Immune Markers in the Endometrium of Bitches affected with CEH-Pyometra Complex
Name of the Student	:	**Ajeet Kumar**
Name of the Advisor	:	Dr Deepak Kumar
Degree/Year	:	MVSc/2024
Name of the University	:	Bihar Veterinary College, Bihar Animal Sciences University, Patna

Canine cystic endometrial hyperplasia (CEH)-pyo-metra complex is a significant reproductive disorder in bitches, characterized by chronic or subacute metritis or endometritis. The condition involves abnormal thickening of the endometrium due to hypertrophy and hyperplasia of the uterine lining. It is often a sequel to repeated estrous cycles without conception, leading to persistent hormonal stimulation, particularly by progesterone and predisposing the uterus to secondary bacterial infections. Pyometra is considered a life-threatening condition, with systemic effects resulting from the absorption of bacterial toxins and inflammatory mediators into circulation.

The present study aimed to investigate both the patho-morphological changes and transcriptional dynamics of key pro-inflammatory and immune markers, namely tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta 1 (TGF-β1), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β), in the context of CEH-pyometra. A total of 40 bitches, divided equally into affected and healthy control groups, were included in the study. These dogs were from various breeds, aged 2-6 years and weighed between 25-45 kg. Each animal underwent a thorough clinical evaluation, ultrasonographic examination and vaginal cytology to confirm diagnosis. Blood samples were collected for serum and hematological analysis, while uterine tissue samples were obtained for both histopathological evaluation and gene expression studies.

Endometrial samples were carefully collected by incising both uterine horns and scraping the inner lining with forceps. These were subjected to RNA extraction for transcriptional profiling of the target cytokines. Additional tissue samples of approximately 1 cm^2 full-thickness were collected for histopathological processing and stained with hematoxylin and eosin (H&E) to visualize morphological alterations.

Haematological analysis revealed significant differences in parameters such as haemoglobin, packed cell volume (PCV), red blood cell (RBC) count, total leukocyte count (TLC), platelet count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and differential leukocyte counts between affected and control animals. These findings indicated systemic inflammatory and immune responses associated with pyometra. Histopathology of the affected uterus showed marked cystic dilation of endometrial glands, infiltration of inflammatory cells and thickening of the endometrial lining, in contrast to the normal histoarchitecture of healthy controls.

Gene expression profiling demonstrated a significant ($P < 0.05$) downregulation of IL-1β mRNA in pyometra-affected uteri compared to controls. TGF-β1 expression showed a non-significant decrease, suggesting a potential dampening of certain immune-regulatory pathways. Conversely, IL-6 and TNF-α transcripts were significantly upregulated ($P < 0.05$) in pyometra cases, highlighting their role as key mediators of inflammation in the disease process.

Overall, the findings indicate that CEH-pyometra is not merely a localized uterine disease but has systemic implications. The altered hematological profile, combined with elevated IL-6 and TNF-α expression, points toward an intense pro-inflammatory environment, contributing to disease severity and progression. These cytokines may serve as potential biomarkers for early diagnosis and therapeutic monitoring in canine pyometra.

Title of Thesis	:	Pathological and Molecular Characterization of Porcine Circovirus Associated Diseases in Pigs
Name of the Student	:	**Alok Kumar Chaurasiya**
Name of the Advisor	:	Dr Chandrakanta Jana
Degree/Year	:	MVSc/2025
Name of the University	:	ICAR-Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh

Porcine circovirus associated diseases (PCVAD) are serious health problem of pigs due to mortality losses, immunosuppression and coinfections. The main etiological agent is porcine circovirus 2 (PCV2) under the family *Circoviridae*. The present study investigated the occurrence, pathology and molecular characterization of PCV2 in pigs. Both clinical and necropsy samples (n = 219) from Bareilly, Uttar Pradesh were screened by PCR. PCV2 was confirmed in 59 samples (26.94%), of which 27 were mono-infections and 32 were co-infections with porcine parvovirus (PPV1), *Streptococcus suis* and *Haemophilus parasuis*. All samples were found to be negative for PCV3. Highest percent positivity was noticed in grower pigs (33.92%), followed by suckling piglets (25%), weaners (24.35%) and adults (23.07%). Pure breeds exhibited 27.69% positivity, crossbreds 26.80%, while no infection was recorded in desi pigs. Lungs, lymph nodes, spleen, tonsils, intestines, kidneys and liver were major organs positive for PCV2. Clinically, infected pigs displayed progressive wasting, poor body condition, respiratory distress, anorexia and sudden death in some cases. Hematobiochemical study revealed anemia, neutrophilia, lymphopenia and elevated liver enzymes (ALT, AST), indicating systemic immunosuppression and organ dysfunction, respectively. Cytokine profiling demonstrated stage-dependent immune responses: early infection marked by strong IFN-γ activity, while late stages showed elevated IL-10, suggesting immunoregulation favoring viral persistence. Pathological investigations revealed both PCV2-systemic disease (PCV2-SD) and PCV2-enteric forms. Gross lesions included hydrothorax, hydropericardium, pulmonary edema, hemorrhage and enteritis. Microscopically, lymphoid depletion, bronchointerstitial pneumonia, interstitial pneumonia, myocarditis, hepatic necrosis, nephritis and enteritis were observed. In PCV2-affected lungs, Shorr's staining revealed large, spherical, magenta intracytoplasmic inclusion bodies located within the cytoplasm of alveolar epithelial cells, indicating viral presence. Masson's trichrome staining of the intestine showed proliferation of fibroblasts, which stained blue, in the submucosa surrounded by the smooth muscle layer stained red, reflecting fibrotic changes. Brown and Brenn staining of lung tissue highlighted multiple discrete patches of blue-stained bacterial colonies predominantly found in the peribronchial and interstitial regions, consistent with bacterial co-infections. Immuno-histochemistry detected PCV2 antigen widely in lymphoid and bronchial epithelial cells across multiple organs except brain. Apoptosis in lymphoid tissues and lungs was confirmed by TUNEL assay, supporting the role of PCV2 in apoptosis and immunosuppression. Molecular characterization of the ORF2 gene from two isolates showed high genetic similarity (97.8-99.0%) with Asian PCV2 strains, clustering within the PCV2d genotype. In conclusion, PCVAD is highly prevalent in the area, frequently co-infected with PPV1 and the circulating porcine circovirus is of the PCV2d genotype, which causes PCV2-SD and PCV2 enteric forms.

Proceedings of Executive Committee (EC) / General Body (GB) Meeting of XLII Annual Conference of Indian Association of Veterinary Pathologists and XVI Annual Meeting of Indian College of Veterinary Pathologists and National Symposium on "Bridging Conventional and Artificial Intelligence Based Digital Pathology for Maximum Outreach – A Futuristic Approach to Veterinary Disease Diagnosis" held during 04-06 December, 2025 at Ranchi Veterinary College and Animal Husbandry, Birsa Agricultural University, Kanke, Ranchi, Jharkhand, India

The Executive Committee / General Body Meetings were held under the Chairmanship of Dr B.N. Tripathi, President, IAVP on 03rd and 04th December, 2025, respectively at Ranchi Veterinary College and Animal Husbandry, Birsa Agricultural University, Kanke, Ranchi, Jharkhand. The meeting was attended by Vice President, the Secretary General, Joint Secretary, Web Manager, Zonal Secretaries, EC Members, and Life Members. Dr G.A. Balasubramaniam, Secretary General, IAVP welcomed all office bearers and EC/GB members. Dr B.N. Tripathi, President, IAVP & Vice Chancellor, Sher-e-Kashmir University of Agricultural Sciences & Technology, Jammu, Union Territory of Jammu & Kashmir, India gave opening remarks and appreciated the efforts of office bearers for advancement of IAVP/ICVP.

1. He emphasized that all the Life Members should made sincere efforts to improve the NAAS rating of the Indian Journal of Veterinary Pathology.
2. Members should be encouraged to submit a greater number of quality research papers to improve the standard of the Indian Journal of Veterinary Pathology.
3. He reiterated that the Heads of the Department of Veterinary Pathology from various states should encourage their students to submit their MVSc and PhD thesis abstracts to the journal for publication without fail.
4. He also emphasized that a greater number of Veterinary Pathologists should be encouraged for appearing in the ICVP Diplomate examination.

Agenda No. 1: Approval of the minutes of the Proceedings of last Executive Committee / General Body meeting held at Sher-e-Kashmir University of Agricultural Sciences & Technology, Jammu, Union Territory of Jammu & Kashmir, India

1. The Secretary General informed the members about the action taken report (ATR) based on the Proceedings of 41st IAVP Conference, 2024 held at SKUAST-Jammu.
2. The Proceedings have been circulated among the EC members and all the members agreed with the same.
3. The approval of minutes of the 41st Proceedings of IAVP held at SKUAST-Jammu was proposed by Dr Pankaj Goswami, Executive Committee Member and seconded by Dr Saminathan M., Joint Secretary, IAVP. The minutes were approved by EC/GB and Life members.

Agenda No. 2: Report by the Secretary General

Letter to all the Zonal Secretaries to collect all the HoDs addresses from their respective zones: The communication was sent to all the six Zonal Secretaries to collect the addresses of all the Heads of the Department of Veterinary Pathology who come under their respective zones in order to request them to submit the MVSc and PhD thesis abstracts to Indian Journal of Veterinary Pathology (IJVP) for publication and to enrol the PG students as Life members of the society. As per the request, all the Zonal Secretaries had responded promptly and the HoDs in concerned zones were addressed accordingly for the above actions and they all took initiative promptly. As a result, total of 29 abstracts were submitted to IJVP for publication.

New IAVP Life Members: A total of 54 new Veterinary Pathologists had been enrolled as Life Member of IAVP in 2024-25. As on date, there are around 1651 life members in the society. All the HoDs were requested to take this process of enrolment seriously to strengthen the society.

Zonal Activities: Letter to all the Zonal Secretaries to organise an event in their respective zones: The communication to all the Zonal Secretaries requesting to organise Zonal level symposium/ workshop was sent on dated 20.03.2025 individually. Among the all, the South Zone took initiative and organised a South Zonal IAVP Conference-2025 and the National Symposium on "Synergising Academia, Industry and Clinical Practice: The Role of Pathology" in the Department of Veterinary Pathology, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry, India on 12th-13th September, 2025. A total of 150 delegates including faculty, scientists, veterinarians, and postgraduate students from various institutions and contract research organizations participated. While appreciating the organisers of the South Zonal Conference, the President, IAVP insisted all the Zonal Secretaries should come forward to organize at least one Conference/ Symposium/ Workshop event each year in their respective zone in order to motivate the youngsters as well as to strengthen the society. Further, it was decided that each Zonal Secretary should organize two webinars for each year.

Database Updating: Updating of the addresses of Life Members of the IAVP was discussed and the Zonal Secretaries were requested to expedite the process and complete the work at the earliest.

Agenda No. 3: Report of the Chief Editor, IJVP

Dr A. Anand Kumar, Chief Editor, IJVP, could not turn up for the EC/GB meeting due to personal reasons and expressed inability to attend the same in advance. On his behalf, Secretary General briefed the house about the IJVP report and informed that:

1. A total of 96 research articles and 29 thesis abstracts were received for publication in the IJVP.
2. The chief editor report as follows:

S. No	Activity	No.
1.	Total no. of research articles received from Dec., 24 to 25th Nov., 25	96
2.	Total no. of thesis abstracts received	21
3.	Total no. of manuscripts published in Vol. 49(1-4) includes the manuscripts received in 2024-25 (89) [Review articles-4, Research articles-21, Short communications and Case reports-43, and Thesis abstracts-21]	89
4.	No. of manuscripts rejected this year a. No. of manuscripts rejected after review-5	5
5.	Per cent of rejection for this year (Jan., 25 - 25th Nov., 25) a. Manuscripts received –87 b. Manuscripts rejected –12 x 100/87 =13.79%	13.79%
6.	No. of manuscripts rejected over all a. No reply after review besides several reminders- 2 b. No. of manuscripts rejected after review- 9 c. No. of manuscripts rejected without review- 1	12
7.	a. Manuscripts received – 9 (last year) + 87 (this year)- 96 b. Manuscripts rejected - 9x100/96 = 9.38% c. Manuscripts rejected including no response from authors 12x100/96 =12.5%	12.5%

3. All four issues of IJVP of 2025 were published well in time and uploaded in ICAR and IAVP websites.

4. The acceptance and rejection rate of the articles were 86.21% and 13.79%, respectively.

5. Provision for issue of Reviewers certificate: After extensive discussion, it was decided to issue the soft copy (e-copy) of a Reviewer certificate to all the reviewers.

6. After extensive discussion, it was decided to host the Indian Journal of Veterinary Pathology in both Indian Journals.com and Indian Council of Agricultural Research-Directorate of Knowledge Management in Agriculture (ICAR-DKMA) websites. Discussion need to be initiated with Indian Journals.com to finalize the guidelines and publication cost for open access. Further, it was decided that the newly inducted Life Members must create an account in Indian Journals.com to access and download the articles.

Reducing the hard copies of the IJVP: The Managing Editor and Editor were proposed to reduce the hard copies of the IJVP journal from 500 to 300. After extensive discussion, it was decided to reduce the IJVP journal to 300. Further, the Editorial Team should send one hard copy of the journal to all the Heads of the Department of Veterinary Pathology and Libraries in Veterinary Colleges/Universities.

Agenda No. 4: Non-return of the advance/seed amount (Rs. 50,000/-) received by Dr M. Lakshman, Organizing Secretary of XXXIX Annual Conference of IAVP during 17-20th November, 2022 held at Department of Veterinary Pathology, College of Veterinary and Animal Science, Rajendranagar, PVNRTVU, Hyderabad, Telangana.

The Secretary General explained in detail about the action taken so far in this regard. The first letter was sent on 28.08.2024 to Dr M. Lakshman requesting to refund the long pending issue of seed money (Rs. 50,000/-) and the 20% of the registration fee of the Conference he organised in PVNRTVU, Hyderabad in 2022. He requested for exemption from the refund of the said amount by citing various reasons. But the association did not accept his request. Subsequently, based on the proceedings of the IAVPCON2024 that held in Jammu, Dr. M. Lakshman was again requested through many reminder (Dt:25.02.2025, 08.07.2025 &14.11.2025) to refund the seed money (Rs.50,000) alone and after the receipt of the seed money, the possibility for exemption for the 20% of the registration fee may be considered. However, there was no reply for that also. After a long deliberation on the issue, the EC/GB members were in the view that Secretary General should send a letter to Dr M. Lakshman to refund the seed money alone (i.e. Rs.50,000/-) to the society. If he fails to do so, the General Body of the society will be free to take any decision including the suspension of Dr. M. Lakshman from the association.

Agenda No. 5. Treasurer, IAVP Report

Dr Pawan Kumar, Treasurer presented the Audit Report of the income and expenses of the IAVP for the year 2024-25. He informed the house:

1.	Opening Balance for the year 2024-25	Rs. 2,97,961.00
2.	Income during the year (Subscription fee, membership fee, publication charges, financial assistance from ICAR, interest earned from FDR, Award processing fee etc.)	Rs. 12,23,516.00
3.	Expenditure during the year (Printing IJVP charges, remunerations and salary, award mementoes purchase, website maintenance, postal stamps, zonal conferences, audit fee, etc.)	Rs. 12,96,043.00
4.	Excess Expenditure	Rs. 72,527.00
5.	Total Balance	Rs. 55,16,820.00
6.	Cash in hand	Rs. 1,893.00
7.	Fixed deposit in SBI	Rs. 46,05,288.00

Treasurer's Report was accepted by EC/GB members.

Concerned IAVP office bearers should make attempt to enhance income of by making new LMs, sale of journal and other measures.

Agenda No. 6: Web Manager Report

- Dr R. Somvanshi, Web Manager informed that IAVP website (https://www.iavp.org/) is regularly updated and functioning smoothly.
- He also informed that The Lesion, 2025 was published and circulated to life members at Ranchi and also uploaded in IAVP website.

Agenda No. 7: Others

- **Nominating Returning Officer to conduct the Election for selecting the next Executive Committee for 2026-28:**
- In the EC meeting, for conducting the election for the new Executive Committee Members for 2026-28 was discussed. In the GB meeting, after the detailed discussion, Dr Desh Deepak Singh, DUVASU, Mathura, UP was confirmed to act as the Returning Officer to conduct the election for the new EC members. Further, it was decided to complete all the election processes within 6 months by the Returning Officer.
- Starting the online and offline Continued Professional Development (CPD) in Veterinary Pathology:
- It was decided that no separate programme is required. The training programmes organized by Veterinary Colleges may be utilized by the faculties for this purpose.

Agenda No. 8: Venue for the IAVP Conference 2026

- The venue for the IAVP Conference 2026 was discussed in detail. Department of Veterinary Pathology, College of Veterinary Sciences & Animal Husbandry, DUVASU, Mathura, Uttar Pradesh was proposed as the venue for the Veterinary Pathology Conference- 2026. Dr Desh Deepak Singh, Professor and Head was proposed to be the Organizing Secretary of the Conference. Further, it was decided to organize the conference in the month of November, 2026.

Agenda No. 9: Miscellaneous

- **Increasing the Life Membership Fee of IAVP and Article Processing Charges (APC) of IJVP:**
- After extensive discussion in the GB meeting, it was decided to increase the Life Membership Fee of IAVP from Rs. 3,000/- to Rs. 4,000/- to enhance the financial status of the society. The Life Membership Fee of IAVP for the non-Indian citizens was also modified for the pathologists of the SAARC countries and other than North America, Europe and Australia from $600 to $200 considering their low income status. Further, it was also decided after extensive discussion in the GB meeting, to increase the Article Processing Charges (APC) of IJVP from Rs. 2,000/- to Rs. 3,000/- for Life Members of IAVP to enhance the financial status of the journal and to cover the charges of open access to have wider reach among the academicians, scientists and research scholars. Both the change viz. Life Membership Fee of IAVP and Article Processing Charges (APC) of IJVP will be effective from 1st January, 2026 onwards.
- **Renaming of the award:** Dr M.K. Gupta, University Professor & Chairman, Department of Veterinary Pathology, Ranchi College of Veterinary Science & Animal Husbandry, BAU, Ranchi donated Rs 1.00 lakh to IAVP to initiate a new award in the honor of Dr H.V.S. Chauhan. Henceforth, the existing IAVP-Best Poultry Pathologist Award may be named after Dr Chauhan viz. IAVP-Dr H.V.S. Chauhan-Best Poultry Pathologist Award w.e.f. 2025.
- **IAVP Achievement Awards in Specialty Subjects:** To promote and recognize pathologists working in specialty fields that are not currently mentioned in the IAVP Constitution or approved by the IAVP General Body (GB) meeting, such as the Best Aquatic Animal Pathologist Award, it was decided to constitute a committee. The committee will formulate appropriate criteria to either incorporate these awards into the existing award structure or to create new biannual awards, thereby avoiding confusion among pathologists.

Agenda No. 10: Condolence for Deceased IAVP Members

- The house was informed that 7 eminent Veterinary Pathologists viz. Dr M.C. Prasad (05.12.2024); Dr Gopal Yadgirkar (23.12.2024), Prof. C.R. Lalitha Kunjamma (11.02.2025), Dr Anil Purthi (16.02.2025), Dr Lakshmi Narayan Acharjyo (30.04.2025), Dr J.L. Vegad (29.08.2025), and Dr M. Jeevana Latha (20.10.2025) have passed away. The house appreciated the significant contributions and services of the above individuals to the profession and IAVP and a silence condolence was observed for peace of departed souls.

Agenda No. 11: Vote of Thanks

- Dr G.A. Balasubramaniam, Secretary General proposed the Vote of Thanks to all the members of the EC/GB for their contributions in fruitful discussion in the meeting.

RESULTS OF IAVP AWARDS-2025

The Indian Association of Veterinary Pathologists has instituted a number of awards. Awards are distributed to Veterinary Pathologists, who have excelled in professional competence as assessed by appropriate criteria and independent evaluation committee. The IAVP administers more than 6 groups of awards where an application is required from the participants, the last date for receiving award application is one month before the opening day of Annual Conference. The awards results for IAVP, 2025 are listed below:

I. IAVP-Young Scientists Awards

1. IAVP-Dr Balwant Singh Memorial Young Scientist Award for Best Oral Presentation

Title: Pathological and immunological evaluation of vaccine candidate against infectious bronchitis in specific pathogen free (SPF) chicken

Authors: Suraj Dhankar, Shyma K. Latheef, Ajay Kumar, Vikramaditya Upmanyu, Rahul Singh, Divya Yadagiri, Deva Ramu, Megha Sharma, Umesh Kumar Singh, Pawaiya, R.V.S. and Asok Kumar, M.

Affiliation: ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh

2. IAVP-Dr S.K. Nigam Memorial Young Scientist Award for Second Best Oral Presentation

Title: Pathology of porcine parvo virus 1 infection in domestic pigs and optimization of recombinase polymerase amplification assay for its diagnosis

Authors: Sangvi K.R., Deepti Singh, Alok Kumar Chaurasiya, Ankit Prasad Kelwan, Richa Gupta, Karikalan, M., Asok Kumar, M., Jana, C. and Pawaiya, R.V.S.

Affiliation: ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh

3. IAVP-Prof. S. Ramachandran Memorial Best Molecular Oncologist Presentation Award

Title: Integrative analysis of the expression of p53 associated biomarkers and exosomal microRNAs in canine mammary tumors

Authors: Pranathi, J., Nakul, P., Vinay Kumar, S.D., Sree Lakshmi, P., Veena R., Sutar, Manohar, S., Tanaya, Swati Singh, Kumar Sugyan, Karuna Irungbam, Vidya Singh, Pawaiya, R.V.S., Pawde, A.M. and Pawan Kumar

Affiliation: ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh

4. a. IAVP-Prof. C. Balachandran Molecular Pathology Award

Title: Comparative assessment of biological fluids for miRNA-based detection of canine lymphoma

Authors: Mohanapriya Thangaraj, Vishal Mahajan, Geeta Devi Leishangthem, Ashwani Kumar and Banga, H.S.

Affiliation: College of Veterinary Science, GADVASU, Ludhiana, Punjab

b. Title: Role of exosomes in canine mammary tumor and evaluation of GW4869 as inhibitor of cancer cell progression

Authors: Manohar, S., Vinay Kumar, S.D., Neha, Sree Lakshmi, P., Nakul, P., Deva, R., Karuna Irungbam, Pawde, A.M., Vidya Singh, Pawan Kumar and Pawaiya, R.V.S.

Affiliation: ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh

II. A. IAVP-Poster Presentation Awards

1. IAVP-Best Poster Presentation Award

Title: Pathomorphology of granulomatous pneumonia in a marsh crocodile (Crocodylus palustris)

Authors: Jayanthi, N., Saahithya, R., Rajan, T. and Senthil Kumar, T.M.A.

Affiliation: Veterinary College and Research Institute, Udumalpet, TANUVAS, Tamil Nadu

2. IAVP-Organizing Secretary Second Best Poster Presentation Award

Title: Foot rot: Pathology and molecular detection in migratory Gaddi sheep and goats in Himachal Pradesh

Authors: Bruce Richard Barreto, Rinku Sharma, Surender Kumar, Payal Bhatia, Gorakh Mal, R.D. Patil, Ajayta Rialch, Birbal Singh, Devi Gopinath, Mohammad Altaf Bhat, and R.V.S. Pawaiya

Affiliation: Disease Investigation Laboratory, ICAR-Indian Veterinary Research Institute, Regional Station, Palampur, Himachal Pradesh

II. B. Sessional IAVP Poster Presentation Awards (Two per session)

Session: AI-Driven Pathology, Molecular Pathology and Oncology

1. **Title:** Canine distemper virus in Northeast India: Genetic diversity and spillover potential in the Indo-Burma biodiversity hotspot.

   Authors: Kiran Jayappa, T.K. Rajkhowa, and F.A. Choudhury

   Affiliation: College of Veterinary Sciences & Animal Husbandry, CAU, Aizawl, Mizoram

2. **Title:** Histopathological diagnoses of malignant splenic neoplasm in dogs

   Authors: Abhilash Jadhao, Rajat Sood, Reanne Carvalho, Vijay Kumar, Thomas Heathcote, Rohit Prajapati, Abhisaar Kumar, Pramod Batra, and N.K. Sood

   Affiliation: College of Veterinary Science, GADVASU, Ludhiana, Punjab

Session: Farm Animal, Pet/Companion Animal Pathology

1. a. **Title:** Acute Orbivirus infection in sheep: Pathology of pulmo-vascular alteration in disease outbreaks in Tirunelveli, Tamil Nadu

   Authors: Kumar, V., Babu Prasath, N., Thangathurai, R., Mahaprabhu, R. and Rajalakshmi, S.

   Affiliation: Veterinary College and Research Institute, Tirunelveli, TANUVAS, Tamil Nadu

1. b. **Title:** Haemato-cytological and molecular diagnosis of Theileria orientalis in cattle

   Authors: Mohanapriya, T., Thangapandiyan, M., Sasi Kumar, S., Sivaraman, S., Bharathi, R. and Theophilus Anand Kumar, C.

   Affiliation: Veterinary College and Research Institute, Salem, TANUVAS, Tamil Nadu

2. a. **Title:** Detection of African swine fever virus in Haematopinus suis: Evidence of a potential vector in domestic pigs

   Authors: Kiran Jayappa, T.K. Rajkhowa and Laldinliana Khiangte

   Affiliation: College of Veterinary Sciences & Animal Husbandry, CAU, Aizawl, Mizoram

2. b. **Title:** Histopathological diagnosis from biopsies from the oral cavity in 88 dogs in Punjab

   Authors: Sood Rajat, Abishek, Omer K. Baba, Kuldip Gupta, N.K. Sood, L. Geeta Devi, Nitin Dev Singh, Vishal Mahajan, Nischal Dutta, Jagmeet Kaur and Navrose Sangha

   Affiliation: College of Veterinary Science, GADVASU, Ludhiana, Punjab

Session: Toxicopathology, Immunopathology and Forensic Pathology

1. **Title:** Evaluation of copper oxide nanoparticle-induced hepatic alterations in male Wistar rats

Authors: Barbaile A., Singh R., Lendewad, R.J., Yadav, A., Gangwar, K., Tiwari, S., Prabhu, S.N., Gangwar, N.K. and Singh, D.D.

Affiliation: College of Veterinary Science & Animal Husbandry, DUVASU, Mathura, Uttar Pradesh

2. **Title:** Clinico-pathological diagnosis of paraquat poisoning in a non-descript dog

   Authors: Mohanapriya, T., Enbavelan, P.A., Bharathi, R., Subapriya, S., Venkatesa- kumar, E. and Theophilus Anand Kumar, C.

   Affiliation: Veterinary College and Research Institute, Salem, TANUVAS, Tamil Nadu

Session: Avian Pathology and Clinical Pathology

1. **Title:** Diagnostic evaluation of canine cutaneous Langerhans cell histiocytosis: Cytological, histological, and immunohistochemical perspectives

   Authors: Venkatesh Yadav, J., Mohitha Sree, D., Sravanthi, M. and Nagaraj, P.

   Affiliation: College of Veterinary Science, Korutla, PVNRTUV, Hyderabad, Telangana

2. a. **Title:** An integrated pathological and molecular approach to fowl adenovirus outbreak (FAV) causing inclusion body hepatitis in commercial poultry

   Authors: Devaraj, C.K., Anjan Kumar, K.R. and Tajunnisa, M.

   Affiliation: Veterinary College, Hebbal, Bengaluru, Karnataka

2. b. **Title:** Emergence of methicillin-resistant Staphylococcus aureus in bumblefoot lesions of white Pekin ducks: Evidence and alternative therapeutic approaches

   Authors: Mini Kumari, Monika Kumar, Adithyan K.M., Soumajit Sarkar, Nikhil K.C., Ganesh N. Aderao, Kanaka K.K. and Soumen Naska

   Affiliation: ICAR–Indian Institute of Agricultural Biotechnology, Ranchi, Jharkhand

Session: Laboratory, Aquatic and Wild animal Pathology

1. a. **Title:** A case of soft tissue fibroma in wistar rat (Rattus norvegicus)

   Authors: Deepak Kumar, Kaushal Kumar, Vishal K. Sinha and Rajesh Kumar

   Affiliation: Bihar Veterinary College, BASU, Patna, Bihar

1. b. **Title:** Clinicopathological study of Theileriosis in captive Indian gaur (Bos gaurus)

   Authors: Vishal K. Sinha, Kaushal Kumar, Deepak Kumar, Sanjiv Kumar and Imran Ali

   Affiliation: Bihar Veterinary College, BASU, Patna, Bihar

2. **Title:** Uterine leiomyoma in a captive Asian elephant (Elephas maximus)

   Authors: Gadhave, P.D., Bhuyan, S., Lasya, T., Thorat, Y., Tripathi, S.A., Raul, K. and Valsarajan, D. and Kadam, D.P.

   Affiliation: Mumbai Veterinary College, Parel, Mumbai, Maharashtra

III. Journal Awards, 2024

1. IAVP-Dr C.M. Singh Award for Best Full Research Article (Non-Pack Animals)

Title: Concurrent occurrence of wet form of feline infectious peritonitis (FIP) and feline parvovirus (FPV) infection in a cat

Authors: Reshma, K.A., Devi, S.S., Divya, C., Anoopraj, R., Sankar Surya, Prasanna, K.S., Sajitha, I.S., Bharathi, R. and Udhayakumar, C.

Issue: Indian J. Vet. Pathol., 48(2): 123-131, 2024: DOI: 10.5958/0973-970X.2024. 00022.8

Affiliation: College of Veterinary and Animal Sciences, Mannuthy, KVASU, Pookode, Wayanad, Kerala

2. IAVP-Dr S. Damodaran Award for Best Oncology Paper/Case Report

Title: Immunopathological characterization of pulmonary lesions of ovine pulmonary adenocarcinoma in small ruminants

Authors: Krishnachari Kumar, Pawan Kumar, Shweta Valecha, Sonali Mishra, Monika Bhardwaj, Bhupesh Kamdi, Vidya Singh and Rajendra Singh

Issue: Indian J. Vet. Pathol., 48(4): 320-326, 2024: DOI: 10.5958/0973-970X.2024. 00055.1

Affiliation: ICAR-Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh

3. IAVP-Dr B.S. Rajya Award for Best Non-Oncology Short/Rapid Communication

Title: Chronic hyperplastic ingluvitis: Synergistic effect of invasive Candida and Trichomonas in a desi chicken

Authors: Babu Prasath, N., Selvaraj, J. and Velusamy, R.

Issue: Indian J. Vet. Pathol., 48(1): 67-70, 2024: DOI: 10.5958/0973-970X.2024.00010.5

Affiliation: VCRI, TANUVAS, Orathanadu, Thanjavur, Tamil Nadu

IV. IAVP-Best Post Graduate Thesis Awards

IV. a. IAVP-Best MVSc Thesis Awards

1. IAVP-Prof. P.K.R. Iyer Memorial Best MVSc Thesis Award

Thesis Title: Pathology and molecular characterization of canine distemper virus in Indian wild carnivores

Name of Student: Dr V. Deekshita

Major Adviser: Dr M. Karikalan, Centre for Wildlife, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh

2. IAVP- Second Best MVSc Thesis

Thesis Title: Pathology of porcine circovirus associated with respiratory disease complex in pig

Name of Student: Dr Thesni Mariam Thomas

Major Adviser: Dr C. Jana, Division of Pathology, ICAR-IVRI, Izatnagar, Uttar Pradesh

IV. b. IAVP-Best PhD Thesis Awards

1. IAVP-Best PhD Thesis Award

Thesis Title: Molecular and pathological studies on chicken infectious anaemia and its concurrent infections in chicken

Name of Student: Dr Sedeneinuo Suohu

Major Adviser: Dr G.A. Balasubramaniam, VCRI, Namakkal, TANUVAS, Tamil Nadu

2. IAVP-Dr Patri Rama Rao Memorial Second Best PhD Thesis Award Thesis

Thesis Title: Immunophenotyping and microRNA expression as biomarkers in canine lymphoma

Name of Student: Dr Mohanapriya, T.

Major Adviser: Dr Vishal Mahajan, GADVASU, Ludhiana, Punjab

V. IAVP-Achievement Awards in Specialty Subjects

1. IAVP-Best Farm Animals Pathologist Award

Dr Rajukumar, K, ICAR-National Institute of High Security Animal Diseases (NIHSAD), Bhopal, Madhya Pradesh

2. IAVP–Dr H.V.S. Chauhan Best Poultry Pathologist Award

Dr Dharanesha, N.K., Institute of Animal Health & Veterinary Biologicals (IAH&VB), KVAFSU, Hebbal, Bengaluru, Karnataka

3. IAVP-Dr B.L. Purohit Memorial Best Toxicologist- Pathologist Award

Dr Rinku Sharma, ICAR-IVRI, Regional Research Station, Palampur, Himachal Pradesh

4. IAVP-Wild Life Pathologist Award (Bi-Annual Award)

Dr Karikalan, M., Centre for Wildlife, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh

VI. IAVP-Special Encouragement Awards

1. IAVP-Best Veterinary Pathology Teacher Award

Dr Chandrakanta Jana, Division of Pathology, ICAR-IVRI, Izatnagar, Bareilly, Uttar Pradesh

2. IAVP-Best Women Pathologist Award (Bi-Annual Award)

Dr P. Ezhil Praveena, ICAR-Central Institute of Brackish Water Aquaculture, Chennai, Tamil Nadu

VII. Fellowship of Indian Association of Veterinary Pathologists, 2025

1. Dr G. Saikumar, Division of Pathology, ICAR-IVRI, Izatnagar, Uttar Pradesh
2. Dr Madhu Swamy, Department of Veterinary Pathology, Nanaji Deshmukh Veterinary Science University, Jabalpur, Madhya Pradesh
3. Dr Rajukumar, K., ICAR-National Institute of High Security Animal Diseases, Bhopal, Madhya Pradesh

VIII. IAVP Appreciations/Activities/Recognitions

1. IAVP-Dr P.P. Gupta Oration

Title: Beyond the microscope: AI in veterinary pathology

Speaker: Dr G. Saikumar

Affiliation: Division of Pathology, ICAR-IVRI, Izatnagar, Uttar Pradesh

2. IAVP-Veterinary Pathology Congress-Thematic Lecture

Title: Advances in digital pathology and AI in research and diagnostics

Speaker: Dr Manu M. Sebastian

Affiliation: Professor, Department of Veterinary Medicine and Surgery, The University of Texas, MD Anderson Cancer Center, Houston, Texas-77030, USA

3. **IAVP-Veterinary Pathology Congress-Continuing Veterinary Pathology Education Lecture**

Title: A diagnostic neuropathology: Delving into clinical cases and digital pathology

Speaker: Dr Valerie McElliott

Affiliation: Clinical Assistant Professor/ Residency Training Co-Ordinator, Department of Anatomic Pathology, College of Veterinary Medicine, Oklahoma State University, Stillwater, Oklahoma, USA

4. **IAVP-Appreciation to Organizing Secretary and Team**

Dr M.K. Gupta, Dean & Organizing Secretary, Department of Veterinary Pathology, Ranchi College of Veterinary Science & Animal Husbandry, Birsa Agricultural University, Kanke, Ranchi, Jharkhand

5. **IAVP-President Appreciation Certificate for Best EC Worker/ Zonal/Chapter**

Dr R. Kumar, Organizing Secretary, IAVP-South Zone Conference-2025, Rajiv Gandhi Institute of Veterinary Education and Research (RIVER), Puducherry

SUPERANNUATION

Professor (Dr.) T. V. Anilkumar

PhD (London), FIAVP, Dip ICVP, FRC Path (England), FNASc., FNAAS, Cert LAM (Guelph)

Dr. T. V. Anilkumar's association with IAVP has been marked by vision, integrity, and tireless commitment. As Secretary-General of IAVP (2008-2011), he strengthened the organizational framework, fostered academic cohesion, and elevated the scientific discourse within the veterinary pathology fraternity. He was instrumental coining the term and initiating 'The Lesion', as a IAVP Newsletter. His administrative acumen combined with scholarly excellence set exemplary standards for professional leadership. A Fellow of IAVP (2007), he has been a pillar of the Association, inspiring generations of Veterinary Pathologists through his academic rigor and ethical grounding. His leadership extended beyond national boundaries when he became the President of the Asian Society of Veterinary Pathology (2016–17), reflecting the international recognition of his contributions to the discipline. He played a pivotal role in establishing the Indian College of Veterinary Pathologists and its Board examination system (first Chair of the Examinations). His valuable suggestions as a Member of the Editorial Board of the IJVP is also appreciable.

Dr Anilkumar was trained under Prof. (Dr.) M. Krishnan Nair and Prof. (Dr.) A Rajan, two doyen Veterinary Pathologists who served the IAVP (1987-1993) as President/Secretary General, at the Manuthy Veterinary College (Kerala). He opted for a career in Translational Medicine, after specialization in Experimental and Toxicologic Pathology at the Imperial College School of Medicine at Hammersmith (Royal Postgraduate Medical School, London), that bridges the gap between fundamental research and patient care. He made seminal contributions in Tissue Engineering and Regenerative Medicine and pioneered the concept of using extracellular matrix recovered from cholecyst (gall bladder) of farm animals for fabricating tissue-graft scaffolds. He fabricated Cholederm, through an innovative indigenous technology (Make in India or Atmanirbhar Bharat), an 'advanced wound care product' that fosters faster healing of skin wounds with minimal scarring. This is the first animal-derived (Class-D, high risk/sophistication) Medical Device that satisfied all regulatory requirement of the Central Drug Standards Control Organization for commercial production/sale in India, as per the Medical Device Rules (2017).

Dr Anilkumar, is the first Veterinarian practicing Pathology in India who qualified for the prestigious FRCPath title of the Royal College of Pathologists (England). He also holds Fellowships of the National Academy of Sciences (India), the National Academy of Agricultural Sciences (India) and the National Academy of Veterinary Sciences (India). He is also a visiting Professor in Laboratory Animal Science at the School of Biology of the Indian Institute of Science Education and Research - Thiruvananthapuram (Ministry of Human Resources Development, Government of India). Beyond positions and accolades, he will be remembered for his mentorship, humility, and unwavering dedication for advancing Veterinary Pathology and translational biomedical research. Indeed, he leaves an enduring legacy of professionalism while retiring from formal service in February 2026 at the age of 65 years as Head, Department of Applied Biology, from Sree Chitra Tirunal Institute for Medical Sciences and Technology - Trivandrum (an institution of national importance under the Ministry of Science and Technology, Government of India). The Indian Association of Veterinary Pathologists wishes him a healthy, peaceful, and fulfilling post-retirement life.

OBITUARY

Dr. J.R. Sadana

Dr. J.R. Sadana, born on 9th September 1942, has been a distinguished figure in the field of Veterinary Pathology. He completed his schooling at Government High School, Hisar, followed by his senior secondary education at Government College, Hisar. Driven by a passion for animal health and scientific inquiry, he pursued his professional education at the College of Veterinary Sciences, Hisar and earned B.V.Sc. & A.H. degree. He further advanced his academic credentials by completing his M.V.Sc. degree in 1968 and Ph.D. in July 1973.

Dr. Sadana began his professional career in May 1965 as an Assistant Veterinary Surgeon. His dedication to academics and research soon led him to serve as a Teaching Assistant and Lecturer, where he contributed significantly to veterinary education. In 1974, he joined the Department of Veterinary Pathology as Assistant Disease Investigation Officer (Poultry), marking the beginning of his specialized and impactful work in poultry disease diagnosis and histopathology.

Over an illustrious service span of 28 years, 8 months, and 21 days, Dr. Sadana served the institution and the veterinary profession with exceptional commitment and integrity. His expertise in poultry pathology, particularly in disease diagnostics, toxicity studies (e.g., sodium chloride toxicity in chicks), and immunological studies, earned him wide recognition. He was also honoured by the Dr C.M. Singh-Salihotra Samman instituted by Dr. C.M. Singh Endowment Trust owing to his significant contributions for advancement of veterinary and animal science. He published his research work related to diagnostic assay comparisons and poultry pathology in various national and international journals like Avian Pathology. His administrative acumen and leadership qualities were reflected in the prestigious positions he held, including Head of the Department, Veterinary Pathology, Controller of Examinations (II), and Additional Director Research at CCS Haryana Agricultural University, Hisar from 2000 to 2002, until his retirement.

A dedicated pathologist, academician, and administrator, Dr. J. R. Sadana's contributions have left an enduring legacy in veterinary education, research, and poultry disease diagnostics. His professional journey stands as an inspiration to generations of Veterinary scientists and students. IAVP family extends their condolences to the departed soul.

Prof. Rafeeq Ahmed

MVSc, Ph.D (Mc Gill Univ., Canada)

Dr S. Rafeeq Ahmed started his career from College of Veterinary Science, Tirupati and then served at College of Veterinary Science, Rajendranagar, Hyderabad as Head Department of Veterinary Pathology till his superannuation.

He was very good in teaching, good at performing Postmortem diagnosis of poultry. He was a simple, sobber, friendly and helpful to all.

On February 6th 2026, Dr S. Rafeeq Ahmed departed. IAVP family extends their condolences to the departed soul.