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Official organ of Progressive Veterinary Doctors' Association
37 Belgachia Road, Kolkata-700037, West Bengal, India

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The aim of a biological research journal is to publish original, high-quality scientific work across a wide range of biological and Animal fields to advance understanding and disseminate new knowledge. Its scope includes experimental research, Molecular Genetics, molecular biology, cell biology, biotechnology, and environmental and agricultural sciences, focusing on significant advances, new methods, or crucial topics of special interest.

AIMS

- Advance knowledge: To publish original research that contributes to the body of knowledge in veterinary medicine and animal science.
- Improve animal health and welfare: To provide research that enhances the well-being and healthcare of animals.
- Disseminate high-quality research: To serve as a platform for sharing soundly designed, reproducible, and clinically relevant contributions with a global audience.

SCOPE

Disease-focused research: Studies on the etiology, diagnosis, prevention and treatment of specific diseases in various animal and human diseases.

Experimental biology: Biochemistry, bioinformatics, biotechnology, cell biology, cancer biology, chemical biology, developmental biology, genetics, genomics, and systems biology.

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Dr. Tirtha Kumar Datta
Vice Chancellor

No. : VCS/WBUAFS/ M-5/30

Date : 29.01.2026

MESSAGE

I am extremely happy to learn that the 12th Annual General Meeting of the Progressive Veterinary Doctors' Association will be held on 22nd February, 2026 at West Bengal Veterinary Council Hall, Belgachia, Kolkata. It is also learnt that a large number of Veterinary Doctors at different fields of Animal Husbandry along with the teaching personnel of West Bengal University of Animal & Fishery Sciences are going to take part in this meeting.

I hope that this occasion will throw up opportunities for greater interaction between the practicing Veterinarians and teachers will pave way for tackling the problems faced by the Veterinary practitioners during their field work.

I am also happy to learn that to commemorate the occasion, the Association is going to bring out the First Edition of Journal entitled " Translational Veterinary and Biological Research" which is a commendable event. I hope that the First Edition of the Journal will be informative and helpful for the Veterinary community.

I wish all the best for the success of the 12th Annual General Meeting of the Progressive Veterinary Doctors' Association .

(Tirtha Kumar Datta)

To
Dr. Chayan Bhattacharya
General Secretary
Progressive Veterinary Doctors' Association
37, Belgachia Road, Kolkata- 700037

Message from the Desk of Chief Editor on the Launch of Translational Veterinary & Biological Research (TVBR)

Dear Colleagues and Members of the Scientific Community,

It is a matter of great pride and professional satisfaction to announce the launch of a new peer-reviewed scientific journal, *Translational Veterinary & Biological Research (TVBR),* an official publication of PVDA, West Bengal. The inaugural issue of the journal is scheduled for publication in February 2026.

TVBR has been conceived with the objective of providing a robust and credible platform for the dissemination of high-quality research that bridges fundamental science with its translational applications in the veterinary and biological sciences. The journal will publish Original Research Articles, Review Papers, Short Communications, and Clinical Case Reports that address contemporary challenges and innovations relevant to researchers, academicians, clinicians, and professionals across veterinary, biological, and allied disciplines.

The journal aims to serve a wide and diverse readership, including veterinary scientists, biological researchers, clinicians, postgraduate and doctoral scholars, policymakers, and industry professionals. Through rigorous peer review and strict editorial standards, we are committed to maintaining the highest level of scientific integrity, originality, and academic excellence in all published articles.

On the occasion of the launch of the first issue, we warmly invite researchers, academicians, and clinicians to submit their original and unpublished research work for consideration. Contributions based on both ongoing and completed research are welcome. Special emphasis will be placed on studies with translational relevance and interdisciplinary significance.

The editorial board assures contributors and readers alike that all manuscripts will undergo a transparent and unbiased peer-review process. The journal is committed to steadily advancing toward recognized quality benchmarks, including NAAS rating and other national and international indexing and accreditation systems, in the near future.

We earnestly seek your support in establishing *TVBR* as a trusted and impactful scientific journal. We also request you to encourage your colleagues and research scholars to participate in this academic initiative, either as contributors, reviewers, or readers.

We look forward to your valuable contributions and continued cooperation in this scholarly endeavour.

With warm regards,

Prof. Nilotpal Ghosh
Chief Editor
Translational Veterinary & Biological Research (TVBR)
Kolkata, West Bengal

Message from the Desk of General Secretary on the Launch of Translational Veterinary & Biological Research (TVBR)

Dear Colleagues and Members

Warm greetings from the Progressive Veterinary Doctors' Association (PVDA), West Bengal.

It gives us immense pleasure to inform you that our association is launching a new peer-reviewed scientific journal titled ***"Translational Veterinary & Biological Research"*** (TVBR), the publication of PVDA, West Bengal. The journal aims to provide a vibrant scientific platform for dissemination of high-quality research outcomes, critical reviews, and case studies that bridge the gap between fundamental science and its translational applications in the veterinary and biological sciences. The inaugural issue of the journal is scheduled for publication on 22nd February 2026 on the eve of AGM, PVDA.

Your insightful contribution, based on your ongoing or completed research, will greatly enrich the journal and help establish it as a credible and impactful scientific resource for the veterinary and allied scientific community.

On this momentous occasion, we strongly believe that our journal is a good platform for exchange of scientific thoughts on Veterinary and Biological Research, Training and Development and extension activities.

I would like to thank our scientific committee who has taken such good effort to publish inaugural issue of the journal. We hope that authors, colleagues, researchers, and readers could appreciate all the time and effort expended in preparing this issue.

With warm regards,

Dr. Chayan Bhattacharya
General Secretary
Progressive Veterinary Doctors' Association

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III. All figures should be separately sent in JPEG or TIFF format at 600 dpi.

IV. Article should be written in concise manner and use own language.

V. Plagiarism should be avoided (maximum 5-10% permitted)

VI. Article should contain title, author's name, address, abstract, keywords, introduction, materials & methods, results, discussion, conclusion and acknowledgement and references.

VII. Original research article (2500-5000 words, Clinical case study/ Short communication (1500-2000 words) and review article (6000 words).

General Guideline for author

I. Translational Veterinary & Biological Research will publish articles under the publisher's name of Progressive Veterinary Doctors' Association, Kolkata, West Bengal.

II. Manuscripts for publication will be considered on their merits. All articles will be double-blind peer-reviewed where identity of the reviewer will not be disclosed.

III. The comments from the reviewers along with the editorial board's decision will be communicated to the authors for further course of action.

IV. Editorial board deserves the right to cancel any article if any sorts of violation of ethics noticed.

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III. All the financial and organizational related assistance during the study of experiment should be properly acknowledged.

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Title: The manuscript of the paper begins with the title which should be short, specific, informative and include the species involved in the research when applicable. The title should be such as to be useful in indexing and information retrieval. Abbreviations are not permitted in the title. In addition, a short subtitle not exceeding 50 letters should be provided separately for running headlines.

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The following table clearly portray how the references should be cited for the journal TVBR:

Source	Format
Published Articles	Tran KQ, Nguyen TTD, Pham VH, Pham QM, Tran HD. Pathogenic role and antibiotic resistance of methicillin-resistant Staphylococcus aureus (MRSA) strains causing severe community-acquired pneumonia in vietnamese children. Adv Respir Med. 2023;91(2):135-45. Hansen LF, Nielsen NSK, Christoffersen LC, Kruuse C. Translational challenges of remote ischemic conditioning in ischemic stroke-a systematic review. Ann Clin Transl Neurol. 2021;8(8):1720-1729. Nguyen LTT, Nguyen KNT, Le PNTA, Cafini F, Pascoe B, Sheppard SK, et al. The emergence of plasmid-borne cfr-mediated linezolid resistant-staphylococci in Vietnam. J Glob Antimicrob Resist. 2020;22:462-5
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Translational Veterinary & Biological Research
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Review Article

PHYTOTHERAPEUTICS IN ANIMAL HEALTH: A MECHANISTIC REVIEW OF AYURVEDIC MEDICINAL PLANTS

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ABSTRACT

Ayurvedic medicinal plants have long contributed to animal healthcare, and growing scientific evidence supports their relevance in modern veterinary practice. This review examines ten widely used botanicals-*Withaniasomnifera*, *Azadirachta indica*, *Curcuma longa*, *Ocimum sanctum*, *Zingiber officinale*, *Tinospora cordifolia*, *Picrorhizakurroa*, *Asparagus racemosus*, *Glycyrrhiza glabra*, and *Aloe vera*, highlighting their key phytochemicals, mechanisms, and therapeutic applications. Major constituents such as withanolides, azadirachtin, curcuminoids, eugenol, gingerols, berberine, picrosides, shatavarins, glycyrrhizin, and acemannan act on pathways including NF-κB, Nrf2–ARE, JAK–STAT, AMPK, and HPA-axis signaling. Across species, these plants demonstrate anti-inflammatory, immunomodulatory, antimicrobial, hepatoprotective, antioxidant, adaptogenic, and wound-healing effects. Evidence from controlled studies supports their use in stress modulation, ectoparasite control, metabolic regulation, lactation enhancement, gastrointestinal protection, and dermatologic care. Wider adoption requires standardized extracts, species-specific dosing, pharmacokinetics, and safety data. Overall, these ayurvedic botanicals offer a scientifically grounded, multi-targeted approach to integrative veterinary health.

Keywords: Medicinal plants, Veterinary pharmacology, Phytochemistry, Ayurveda, Mechanisms of action, Animal health

INTRODUCTION

Ethnoveterinary medicine, the traditional use of plants and natural products for animal health, has supported livestock and companion-animal care for centuries across diverse cultures [41]. In the past few decades, however, the landscape of veterinary therapeutics has changed significantly. Widespread and often indiscriminate use of antibiotics in animal husbandry has accelerated the development of multidrug-resistant pathogens, now recognized as a critical threat to animal and public health worldwide [3]. Parallel concerns regarding antimicrobial residues in animal-derived foods and the rising consumer demand for organic production systems have intensified the search for safe, natural, and sustainable therapeutic alternatives [10].

India, designated as one of the planet's major megadiverse regions, possesses an exceptionally rich flora, with many species deeply embedded in Ayurvedic medicinal systems [47]. While this heritage provides a valuable repository of knowledge, contemporary veterinary science requires systematic validation of herbal interventions. Rigorous investigation is needed to confirm efficacy, establish safety profiles, and determine species-appropriate dosing [50]. Such efforts involve isolating bioactive constituents, characterizing their molecular targets, and mapping the biochemical pathways through which they exert pharmacological effects [67]. Mechanistic clarity is essential not only for standardizing botanical preparations but also for anticipating potential herb-

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drug interactions and integrating these remedies into evidence-based veterinary practice [70].

This review aims to bridge traditional Ayurvedic wisdom with modern scientific evidence by examining ten key medicinal plants for which substantial experimental and clinical data support relevance in animal health. By consolidating phytochemistry, mechanisms, molecular targets, and therapeutic implications, the review highlights their potential role in advancing integrative and sustainable veterinary care.

MATERIALS AND METHODS

A comprehensive literature review was conducted using PubMed, Scopus, and Google Scholar. Search terms included combinations of each plant's botanical name with medical subject headings (MeSH) terms "veterinary phytotheraphy", "animal health", "veterinary", "mechanism", "pathway", "pharmacology", "*in vivo*", and "*in vitro*". Priority was given to peer-reviewed studies examining cellular targets, molecular pathways, pharmacological endpoints, and validated therapeutic effects.

Major Ayurvedic Botanicals in Animal Health

Ashwagandha *(Withania somnifera)*

Ashwagandha is widely recognized for its adaptogenic, immunomodulatory, and anti-inflammatory activity. These effects are attributed primarily to its withanolide content, particularly withaferin A and sitoindosides, which contribute to its diverse biological actions [14].

Major constituents- Withanolides, including withaferin A and sitoindosides [14].

Mechanism of action- Withanolides are steroidal lactones that exert neuroprotective and anxiolytic effects by modulating GABAergic neurotransmission, acting in part by enhancing GABA-A receptor activity [14]. Ashwagandha also influences the hypothalamic-pituitary-adrenal (HPA) axis, resulting in reduced serum cortisol concentrations and improved stress resilience [9]. Withaferin A is a strong inhibitor of the NF-κB signalling pathway, a key regulator of inflammatory gene expression [61]. Through these actions GABA-A receptor modulation, attenuation of HPA-axis activity, and suppression of NF-κBAshwagandha demonstrates consistent anxiolytic, adaptogenic, and anti-inflammatory properties [9,14,61,65]. Recent randomized controlled trials in dogs further support its stress-reducing benefits and good tolerability, indicating its potential for use in behavioural and stress-related disorders in veterinary settings [31].

Targets- GABA-A receptors; glucocorticoid receptors; NF-κB transcription factor; cyclooxygenase-2 (COX-2) [9,14,61].

Pathways- Regulation of the hypothalamic-pituitary-adrenal (HPA) axis; inhibition of NF-κB signalling; downregulation of mitogen-activated protein kinase (MAPK) pathways [9,61].

Therapeutic relevance in veterinary medicine- Ashwagandha offers anxiolytic, stress-modulating, anti-inflammatory, and analgesic benefits in domestic animals. To ensure predictable outcomes, standardized extracts with defined withanolide content are essential, as commercial preparations vary widely in potency. Careful dosing is recommended, and potential interactions should be monitored when used alongside sedatives or medications that influence the HPA axis [9,65].

Neem *(Azadirachta indica)*

Neem, traditionally referred to as *Sarva Roga Nivarini* ("the curer of all ailments"), is widely recognized for its antimicrobial, antiseptic, antiparasitic, and anti-inflammatory properties. Its therapeutic breadth is attributed to a diverse array of bioactive phytochemicals [45].

Major constituents- Azadirachtin, nimbin, nimbidin, and quercetin [45].

Mechanism of action- Azadirachtin is a potent limonoid that disrupts arthropod development by antagonizing the ecdysone receptor, resulting in impaired molting and eventual death [45]. Nimbidin inhibits prostaglandin synthesis, contributing to its anti-inflammatory and anti-ulcer effects [48]. It also interferes with microbial membrane integrity and suppresses fungal ergosterol synthesis, thereby exerting broad-spectrum antimicrobial and antifungal actions [6]. Together, azadirachtin and nimbidin provide the pharmacological basis for Neem's traditional and modern use as an ectoparasiticide, antifungal agent, and wound-healing botanical [6,45,48].Recent controlled *in vitro* and *in vivo* studies

have demonstrated significant acaricidal activity of Neem seed extracts and oil preparations. Field evaluations further validate the effectiveness of topical Neem formulations for tick control in cattle, highlighting its utility as a low-toxicity, plant-based option within integrated pest management programs for livestock [6,40].

Pathways- Ecdysone receptor signalling; arachidonic acid and prostaglandin synthesis pathways; oxidative stress pathways [40,45,48].

Therapeutic relevance in veterinary medicine- Neem is an effective natural ectoparasiticide for managing ticks, fleas, and mites [1]. It is also used to treat dermatophytosis (ringworm), support wound healing, and manage infectious dermatoses in cattle and companion animals [4,6,40]. Additionally, it offers gastroprotective benefits due to its anti-inflammatory phytochemicals [6]. For systemic administration or prolonged topical use, careful attention to formulation purity is essential, particularly the removal of potentially toxic anthranilic derivatives. Unstandardized concentrated preparations should be avoided in young or debilitated animals [4].

Haridra *(Curcuma longa)*

Turmeric, often referred to as the "golden spice of India", is rich in curcumin, one of the most extensively researched natural anti-inflammatory compounds. Curcumin is a pleiotropic molecule with potent anti-inflammatory, antioxidant, and cytoprotective properties [2].

Major constituents- Curcuminoids, primarily curcumin (≈77%), along with demethoxycurcumin and bisdemethoxycurcumin[2].

Mechanism of action- Curcumin interacts with numerous molecular targets involved in inflammation, immune regulation, and oxidative stress. It strongly suppresses NF-κB activity, reducing downstream expression of COX-2, LOX, iNOS, and proinflammatory cytokines such as TNF-α and IL-6 [43]. It also activates the Nrf2-ARE antioxidant pathway, enhancing cellular resilience to oxidative damage [58]. Curcumin inhibits the JAK-STAT signalling cascade, a key pathway in many immune-mediated inflammatory conditions [11], and promotes autophagy through modulation of mTOR and associated regulatory proteins such as LC3 and Beclin-1 [7]. Collectively, these mechanisms support curcumin's recognized anti-inflammatory, hepatoprotective, antioxidant, and wound-healing activities across animal species [7,11,43,58].Despite its broad pharmacological potential, curcumin exhibits poor oral bioavailability. Co-administration with piperine, or the use of enhanced formulations (phytosome/lecithin complexes, solid dispersions, or nanoparticles), substantially improves systemic absorption. Both human and animal studies demonstrate several-fold increases in curcumin plasma levels with such formulations, making delivery strategy a key determinant of therapeutic success in veterinary applications [7,43,59].

Targets- NF-κB; COX-2; LOX; iNOS; TNF-α; IL-6; Nrf2; JAK; STAT3; mTOR; autophagy-related proteins LC3 and Beclin-1 [7,11,43,58].

Pathways- NF-κBsignaling; Nrf2-ARE antioxidant pathway; JAK-STAT pathway; MAPK cascade; mTOR/autophagy pathways [7,11,28,43,49,58].

Therapeutic relevance in veterinary medicine-

Curcumin has shown benefits in the management of canine osteoarthritis [28], equine laminitis [18], and inflammatory bowel disease [38]. It also offers hepatoprotective effects against toxic, drug-induced, and metabolic liver injury [52] and contributes to enhanced wound healing and oxidative stress reduction [35,43]. However, its low oral bioavailability remains a practical limitation; incorporation of piperine or phospholipid complexes can markedly improve absorption [69]. Care is advised when combining curcumin with medications metabolized by CYP enzymes [69], and caution is warranted in animals with bleeding tendencies or those receiving anticoagulants.

Tulsi *(Ocimum sanctum)*

Tulsi, or Holy Basil, is revered in India for its broad therapeutic potential and is well recognized for its adaptogenic, antidiabetic, and immunomodulatory activities [17]. Its diverse pharmacological effects are attributed to a rich profile of phenolic and terpenoid compounds.

Major constituents- Eugenol, ursolic acid, and rosmarinic acid [17].

Mechanism of action- Eugenol acts as a natural cyclooxygenase (COX) inhibitor and modulates the

TRPV1 receptor, contributing to Tulsi's analgesic and anti-inflammatory actions [64]. Ursolic acid and rosmarinic acid are potent antioxidants that activate the Nrf2-ARE pathway, enhancing cellular protection against oxidative damage [33]. Tulsi also influences endocrine and metabolic regulation by modulating cortisol levels through HPA axis activity and by exerting insulinotropic effects- enhancing insulin secretion, improving insulin sensitivity, and supporting glucose homeostasis [8,27]. These combined actions position Tulsi as a versatile botanical with benefits in respiratory, metabolic and immunological health.

Targets- COX enzymes; TRPV1 receptors; Nrf2 transcription factor; glucocorticoid receptors; insulin receptors; and K^+-ATP channels in pancreatic β-cells [8,27,33,64].

Pathways- Nrf2-ARE antioxidant pathway; hypothalamic-pituitary-adrenal (HPA) axis; and insulin signalling cascades [8,27,33].

Therapeutic relevance in veterinary medicine- Tulsi supports respiratory health, mitigates metabolic stress, and enhances immune function in both livestock and companion animals. It is generally well tolerated, and the use of standardized extracts, particularly those with quantified phenolic contentcan improve consistency and reproducibility across veterinary applications [8,27,33,64].

Adraka *(Zingiber officinale)*

Ginger is widely used as both a culinary spice and a medicinal plant, with well-documented effects on gastrointestinal function, inflammation, and immune regulation. It has been traditionally employed to manage rheumatism, muscular discomfort, and abdominal cramps [5].

Major constituents- Gingerols (particularly 6-gingerol), shogaols, and zingerone [5].

Mechanism of action- Gingerols and shogaols act as antagonists at the 5-HT_3 receptors, thereby inhibiting the vomiting reflex and reducing nausea [39]. These compounds also suppress inflammatory processes by inhibiting COX and LOX enzymes and by downregulating NF-κB activation [25]. In addition, gingerols exhibit pro-apoptotic activity in certain cancer cell models and demonstrate gastroprotective effects in animal studies through modulation of mitochondrial pathways and oxidative stress responses [71].

Targets- 5-HT_3 receptors; COX and LOX enzymes; NF-κB transcription factor; Bcl-2/Bax regulatory proteins; and the mitochondrial voltage-dependent anion channel [25,39,71].

Pathways- Serotonergic signalling; arachidonic acid metabolism; NF-κB inflammatory pathway; and mitochondrial apoptosis pathway [25,39,71].

Therapeutic relevance in veterinary medicine-

Ginger helps reduce nausea, including postoperative nausea [73], and is beneficial in motility disorders, pain, inflammatory conditions, and oxidative stress-related disorders [5,25,39,71]. It also provides gastroprotective and mild antitumor effects [25]. While generally well tolerated, high doses should be avoided in animals with bleeding risks due to its mild antiplatelet properties [5].

Guduchi *(Tinospora cordifolia)*

Guduchi is widely recognized in traditional and contemporary veterinary practice for its immunomodulatory and metabolic regulatory effects [46]. Its diverse pharmacological activities arise from a range of alkaloids, diterpenoids, and glycosides present in various parts of the plant [46].

Major constituents- Berberine, tinosporin, and cordioside [46].

Mechanism of action- Berberine enhances macrophage proliferation and improves phagocytic activity, contributing to strengthened innate immune responses [63]. Guduchi also modulates the Th1/Th2 cytokine balance and increases immunoglobulin levels, thereby supporting adaptive immunity [63]. Its metabolic effects include activation of AMPK and inhibition of mitochondrial complex I; mechanisms associated with improved glucose regulation and antidiabetic activity [42,60]. Collectively, these actions underpin its role in immunomodulation, metabolic balance, and host defence. Recent reviews further highlight its promise as an adjunct therapy in infectious diseases and immune-compromised states [62].

Targets- Macrophages; B cells and T cells; key cytokines including IL-1, IL-2 and IFN-γ; AMPK; and mitochondrial complex I [42,60,63].

Pathways- Innate and adaptive immune signalling; NF-κB and JAK-STAT pathways involved in immune regulation; and AMPK-mediated metabolic pathways [42,60,63].

Therapeutic relevance in veterinary medicine- Guduchi is useful in immune-suppressed or chronically infected animals, helps enhance immunity in young or geriatric animals, and supports recovery from stress or illness. It also acts as a hepatoprotective and antidiabetic agent, helping regulate blood glucose levels and metabolic function [46]. While Guduchi is generally well tolerated, standardized extracts and species-specific dosing studies are needed to ensure consistent therapeutic outcomes in Veterinary practice.

Katuki (*Picrorhizakurroa*)

Katuki is a bitter Himalayan herb traditionally regarded as one of Ayurveda's foremost hepatoprotective plants. Beyond its liver-protective properties, it has been used for conditions such as splenic disorders, fever, and asthma [57,21].

Major constituents- Kutkin (a mixture of picroside I and picroside II) and apocynin [21].

Mechanism of action- Picrosides exhibit strong antioxidant activity, scavenging free radicals and enhancing intracellular glutathione levels. Apocynin, a well-characterized phytochemical in Katuki, acts as a selective inhibitor of NADPH oxidase, thereby reducing oxidative stress mediated tissue damage [21]. Katuki also exerts choleretic effects by increasing bile synthesis and flow, and demonstrates immunomodulatory activity through modulation of cytokine responses and xenobiotic metabolizing enzymes [29,66]. These combined actions Nrf2 activation, NADPH oxidase inhibition, enhanced bile flow, and regulation of hepatic CYP enzymes, account for the robust hepatoprotective effects observed in animal toxicology models. Accordingly, *Picrorhizakurroa* is considered a rational therapeutic candidate for managing drug-induced or toxin-mediated liver injury in livestock and companion animals [21,29,66].

Targets- NADPH oxidase; cytochrome P450 enzymes; bile salt export pump; Nrf2 transcription factor; and TNF-α, along with broader hepatic antioxidant and detoxification systems [21,29,66].

Pathways- Nrf2-ARE antioxidant pathway; bile acid synthesis and secretion pathways; and NF-κB-associated inflammatory signalling [21,29,66].

Therapeutic relevance in veterinary medicine- Katuki is beneficial in the management of toxin- or drug-induced hepatotoxicity, metabolic liver dysfunction, reduced appetite associated with hepatic disease, and bile flow disorders [21,29]. Its antioxidant and choleretic actions make it particularly valuable in hepatic support protocols where enhanced detoxification and improved bile dynamics are desired [29]. However, attention should be given to potential interactions with drugs metabolized by hepatic CYP enzymes [66], and standardized extracts are recommended to ensure consistent potency.

Shatavari (*Asparagus racemosus*)

Shatavari is regarded in Ayurveda as one of the most important female reproductive tonics and galactagogues. It is also known for its adaptogenic, gastroprotective, and anti-ulcer properties, making it valuable across multiple physiological systems [24].

Major constituents- Shatavarins (including shatavarin IV, a steroidal saponin) and isoflavones [24].

Mechanism of action- Shatavarin IV enhances the synthesis and secretion of prolactin, supporting lactation in mammals [54]. The steroidal saponins found in Shatavari exhibit phytoestrogenic activity, contributing to reproductive support and hormonal modulation. These saponins also increase mucin production in the gastrointestinal tract, providing protection against ulcer formation and improving mucosal integrity [12]. Beyond these effects, Shatavari demonstrates antioxidant and immunomodulatory actions that contribute to its adaptogenic profile. Controlled studies in dairy cows, particularly Karan Fries crossbreds, have reported increased milk yield, improved milk composition, and higher immunoglobulin levels following supplementation, reinforcing its role as a Veterinary galactagogue and nutritional tonic [36,13].

Targets- Prolactin receptors; estrogen receptors; gastric mucosal cells [12,13,36,54].

Pathways- Prolactin signaling pathway; estrogen signaling cascade; mucin synthesis and gastric cytoprotection pathways [12,13,36,54].

Therapeutic relevance in veterinary medicine- Shatavari significantly enhances milk production in lactating dairy animals and improves milk quality parameters [36]. It also supports urinary tract health

through anti-lithiasis activity [16], promotes gastric healing, and enhances stress tolerance and vitality in animals under chronic strain [22,51]. Its broad physiological benefits make it valuable in dairy and general livestock management. However, due to its phytoestrogenic effects, caution is advised when using Shatavari in breeding animals, and reproductive outcomes should be monitored when used across large herds.

Yastimadhu (*Glycyrrhiza glabra*)

Yastimadhu, commonly known as Licorice or Mulethi, is a sweet-tasting demulcent widely valued for its anti-inflammatory, antiviral, expectorant, and mucoprotective properties. It has long been used in traditional medicine for respiratory, gastrointestinal, and dermatologic conditions [34].

Major constituents- Glycyrrhizin and glabridin[34].

Mechanism of action- Glycyrrhizin inhibits 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), thereby increasing local glucocorticoid activity and contributing to its anti-inflammatory effects [34]. It also suppresses prostaglandin synthesis and demonstrates direct antiviral activity through interactions with viral envelope proteins [68]. Glabridin exhibits potent antioxidant and anti-atherosclerotic effects, further expanding the therapeutic potential of Licorice[53]. Additionally, glycyrrhizin binds to high mobility group box 1 (HMGB1), a key pro-inflammatory mediator, thereby exerting immunomodulatory and antiviral effects [44]. Collectively, these actions- 11β-HSD2 inhibition, HMGB1 binding, antioxidant activity, and modulation of inflammatory pathways, explain Licorice's broad therapeutic activity across organ systems.

Targets- 11β-HSD2 enzyme; prostaglandin E2 synthesis pathways; viral envelope proteins; HMGB1; and broader glucocorticoid metabolism and epithelial protection mechanisms [34,44,53,68].

Pathways- Corticosteroid metabolism; arachidonic acid/prostaglandin pathways; and HMGB1-associated inflammatory signalling [34,44,53,68].

Therapeutic relevance in Veterinary medicine- Yastimadhu is an effective antitussive and expectorant for managing cough and respiratory irritation [32]. It provides mucosal protection in gastric ulceration and gastritis, exhibits antiviral properties, and is used topically to manage eczema, dermatitis, pruritus, and other inflammatory skin eruptions [55]. It is valuable across respiratory, gastrointestinal, dermatologic, and inflammatory conditions in Veterinary care. However, chronic or high-dose systemic use can mimic mineralocorticoid excess due to prolonged 11β-HSD2 inhibition, potentially causing hypertension and hypokalaemia in susceptible animals [32,34,44,53]. Prolonged administration should therefore be avoided, and electrolyte balance monitored when necessary.

Aloe vera (*Aloe barbadensis Miller*)

Aloe vera is a succulent plant whose inner leaf gel is widely recognized for its demulcent, wound-healing, moisturizing, antifungal, antidiabetic, anticancer, immunomodulatory, and gastroprotective properties [26]. Its therapeutic actions are largely attributed to its rich polysaccharide and anthraquinone content.

Major constituents-Acemannan (a bioactive polysaccharide) and anthraquinones such as aloin and emodin [26,72].

Mechanism of action-Acemannan enhances innate and adaptive immunity by stimulating macrophage activity [72], leading to increased release of cytokines including TNF-α, IL-1, and IL-6, as well as nitric oxide [30]. Aloe gel also promotes rapid wound healing by increasing fibroblast proliferation, collagen synthesis, and collagen cross-linking, contributing to accelerated tissue repair [15,20]. In addition, Aloe vera exhibits antimicrobial effects against several bacterial and fungal pathogens [37]. Through macrophage activation, fibroblast stimulation, collagen enhancement, and cytokine modulation, Aloe vera supports robust wound healing and exerts topical anti-inflammatory actions. Systematic reviews in mammals substantiate its efficacy in managing burns, excisional wounds and other dermatologic injuries [26].

Targets- Macrophages; dermal fibroblasts; growth factors; inflammatory mediators and microbial cell walls [15,20,26,30,37,72].

Pathways- Macrophage-mediated immune activation; collagen synthesis and tissue repair pathways and antimicrobial response pathways [15,19,20,30,37,72].

Table 1. Ayurvedic medicinal plants in animal health, the targets, pathways and therapeutic Effects

Herbal Name	Primary Compound	Mechanism of Actions	Molecular Targets	Key Therapeutic Effects
Aswagandha *(Withania somnifera*	Withanolides (e.g. Withaferin A, Withanoloide D), Sitoindoside	-GABA receptor modulation -HPA axis -NF-kB pathway inhibition	GABA-A receptor, Glucocorticoid receptor, NF-kB, COX-2	-Reduce stress and anxiety -Anti-inflammatory & analgesic (osteoarthritis) -Neuroprotective
Neem *(Azadirichta indica)*	Azadirachtin, Nimbin, Nimbidin,	-Ecdyson receptor antagonism -Prostaglandin synthesis inhibition -Microbial membrane disruption	Ecdysone receptor, COX enzyme, Fungal/Bacterial cell membrane	-Natural ectoparasiticide (ticks, fleas, mites) -Anti-fungal (ringworm) -Wound healing & gastroprotective
Haridra *(Curcuma longa)*	Curcuminoids	-NF-kB & JAK-STAT pathway inhibition -Nrf2_AER pathway activation -Induction of autophagy	NF-kB, COX-2, LOX, Nrf2, JAK/STAT3, mTOR	-Potent anti-inflammatory (arthritis, IBS) -Hepatoprotective and Antioxidant -Supporting wound Healing
Tulsi *(Ocimum sanctum)*	Eugenol, Ursolic acid, Rosmarinic acid	-COX enzyme -TRPV1 receptor modulation -Nrf2 pathway activation -HPA axis modulation	COX enzyme, TRPV1, Nrf2,Glucocorticoid & insulin receptor	-Support respiratory health -Immunomodulator -Anti-diabetic
Adraka *(Zingiber officinale)*	Gingerols (6-gingerol), Shogaols	-5-HT3 receptor -COX/LOX enzyme inhibition -Induction of apoptosis	5-HT3 receptor, COX/LOX enzymes,NF-kB, Bcl-2/Box	-Anti-emetic (motion sickness & postoperative nausea) -Antiinflammatory & analgesic -Gastroprotective
Guduchi *(Tinospora cordifolia)*	Berberine, Tinosporin	-Macrophage activation - Immunomodulation -AMPK activation -Mitochondrial complex I inhibition	Macrophage, B/T cells, AMPK, Mitochondrial complex I	-Immunomodulator (boost defense) -Hepatoprotective -Antipyretic -Antidiabetic
Katuki *(Picrorhiza kurroa)*	Kutkin (Picroside I and II), Apocynin	-NADPH oxidase inhibition -Antioxidant -Choleretic effect	NADPH oxidase, Cytochrome, P450, Bile salt export pump(BSEP), Nrf2	-Hepatoprotective (against toxins/drugs) -Choleretic (Improve bile flow)
Shatavari *(Asparagus racemosus)*	Shatavarins (Shatavarin IV), Isoflavones	-Prolactin secretion/ stimulation -Mucoprotective -Phytoestrogenic activity	Prolactin receptor, Estrogen receptor, Gastric mucosal cell proliferation	-Galactagogue (increase milk production) -Anti-ulcer -Adaptogen -Anti-lithiasis -Support urinary health
Yastimadhu *(Glycyrrhiza glabra)*	Glycyrrhizin, Glabridin	-11β-HSDH2 enzyme activation -Antiviral replication inhibition -HMGB1 protein inhibition	11β-HSDH2 enzyme, Prostaglandin E2, HMGB1	-Antitussive & expectorant -Soothes gastric ulcer -Antiviral -Anti-inflammatory (topical)
Aloe Vera *(Aloe barbadensis miller)*	Acemannan, Anthraquinones	-Macrophage stimulation -Immunomodulation -Fibroblast & collagen synthesis -Anti-microbial	Macrophages, Fibroblasts, Growth factor (FGF/VEGF), Microbial walls	-Tropical & burn healing -Immunostimulant -Soothing for skin condition

Therapeutic relevance in Veterinary medicine- Aloe vera gel is effective for topical management of burns, wounds, abrasions, "hot spots," and allergic or inflammatory skin conditions in animals [19]. It is also beneficial in managing ulcers and surgical wounds due to its epithelializing and anti-inflammatory effects. For Veterinary use, purified and stabilized gel preparations are recommended. Raw leaf latex, which contains high levels of anthraquinones may cause irritation or act as a laxative if ingested and should therefore be avoided.

The targets, pathways, and therapeutic effects of the Ayurvedic medicinal plants in animal health reviewed are summarized in Table 1. and their integrated mechanistic convergence in Fig 1.

CROSS-CUTTING TRANSLATIONAL CONSIDERATIONS

Standardization and formulation

Efficacy and safety are highly formulation-dependent. For compounds with low oral bioavailability (e.g., curcumin), co-formulants (piperine, phospholipid complexes, nanoparticles) or parenteral delivery (where appropriate and safe) markedly change pharmacokinetics and therefore clinical effect. When translating human literature to animals, dose-scaling must account for species differences in absorption, metabolism and target exposure. Evidence supports use of piperine to enhance curcumin absorption; this approach has been replicated in preclinical and clinical pharmacokinetic studies [56,59].

Safety, toxicology and interactions

Botanicals can interact with Veterinary pharmaceuticals (e.g., CYP-mediated interactions, effects on steroid metabolism, additive sedative effects) [29]. Licorice exemplifies mineralocorticoid-like adverse effects at high exposures [34]. Neem topical preparations are generally safe, but ingestion of concentrated neem oils can be toxic in young animals [6]. Rigorous safety testing (acute, subacute, chronic toxicity) in the intended target species is essential prior to wide adoption.

Evidence strength and clinical trial gaps

Many *in vivo* studies are mechanistic or small-scale; high-quality, randomized controlled trials in target animal species remain relatively few. Notable exceptions include controlled trials of *W. somnifera* in companion animals for stress/anxiety and field trials of neem for ectoparasite control. Continued well-designed clinical trials (adequate sample size, standardized extracts, clear end points) are required to build a stronger regulatory case for Veterinary indications [23,31].

Regulatory and residue considerations

Use of botanicals in food-producing animals requires evaluation of residues in meat, milk and eggs. Some botanicals produce metabolites that are inert at low levels, but regulatory frameworks differ by jurisdiction; producers must document withdrawal periods and residue studies where required.

Practical recommendations for veterinarians and researchers

1. Prefer standardized extracts with assayable active markers (e.g., withanolide percentage, curcuminoid content, glycyrrhizin fraction).
2. Select formulation to match indication: topical gels for wounds (aloe, neem), liposomal or piperine-enhanced curcumin for systemic anti-inflammatory uses, standardized ashwagandha for behavioural stress [31,59].
3. Start with adjunctive use alongside established therapies, using close clinical monitoring and objective outcome measures (e.g., parasite counts, lameness scores, milk yield).
4. Document safety and efficacy in target species; even widely used human formulations require species-specific pharmacokinetic and toxicology data.
5. Report negative as well as positive outcomes to reduce publication bias and guide evidence synthesis.

DISCUSSION

The Ayurvedic medicinal plants reviewed in this article represent some of the most versatile and scientifically validated botanicals used in contemporary Veterinary care. Although these herbs originate from long-standing ethnoveterinary and Ayurvedic traditions, modern research has increasingly clarified their molecular targets, signalling pathways, and therapeutic relevance in domestic animals. Their pharmacological actions are broad and often multitargeted, reflecting the inherent complexity of phytochemicals compared with single-molecule synthetic drugs.

Several plants demonstrate potent anti-inflammatory and antioxidant activity, mediated through suppression of NF-κB, COX-2, iNOS, and proinflammatory cytokines, as seen in *Withaniasomnifera*, *Curcuma longa*, and *Zingiber officinale* [7,9,11,14,17,18,28,33,35,38,43, 49,52,58,59,61,64,65,69]. Others such as *Azadirachta indica* and *Tinospora cordifolia* offer antimicrobial and immunomodulatory benefits through modulation of macrophage activity, T-cell responses, and oxidative stress pathways [1,2,4,6,40,42,45,48,60,63,73]. These multimodal effects are particularly valuable in chronic, multifactorial veterinary conditions where inflammation, oxidative damage, and immune dysregulation often coexist.

Importantly, a subset of these botanicals is supported by controlled animal trials, strengthening their translational relevance. Neem solutions have demonstrated consistent acaricidal efficacy in cattle [6]. standardized Ashwagandha extracts have shown reductions in anxiety and stress behaviours in dogs [31], Shatavari supplementation has improved milk yield and composition in dairy cows [13], and Aloe vera gels have accelerated wound healing across species [15,19,20, 26,30,37,56,72]. Such outcomes provide proof-of-concept validation and highlight the potential for integrating botanicals into evidence-based veterinary protocols.

Despite these encouraging findings, several limitations warrant attention. Many studies lack standardized extract characterization, making reproducibility difficult. Variability in phytochemical concentration due to differences in plant chemotype, geography, harvest timing, and extraction techniques can markedly alter pharmacological outcomes. For example, curcumin bioavailability is inherently low but improves significantly with enhanced formulations such as nanoparticles, phospholipid complexes, or piperine co-administration [56]. Without standardized products, establishing dosage accuracy and safety becomes challenging.

Species-specific pharmacokinetic data remain limited for most herbs. Extrapolating doses from rodent or human studies is inappropriate, as absorption, metabolism, and elimination vary significantly across Veterinary species. Moreover, potential herb-drug interactions, particularly

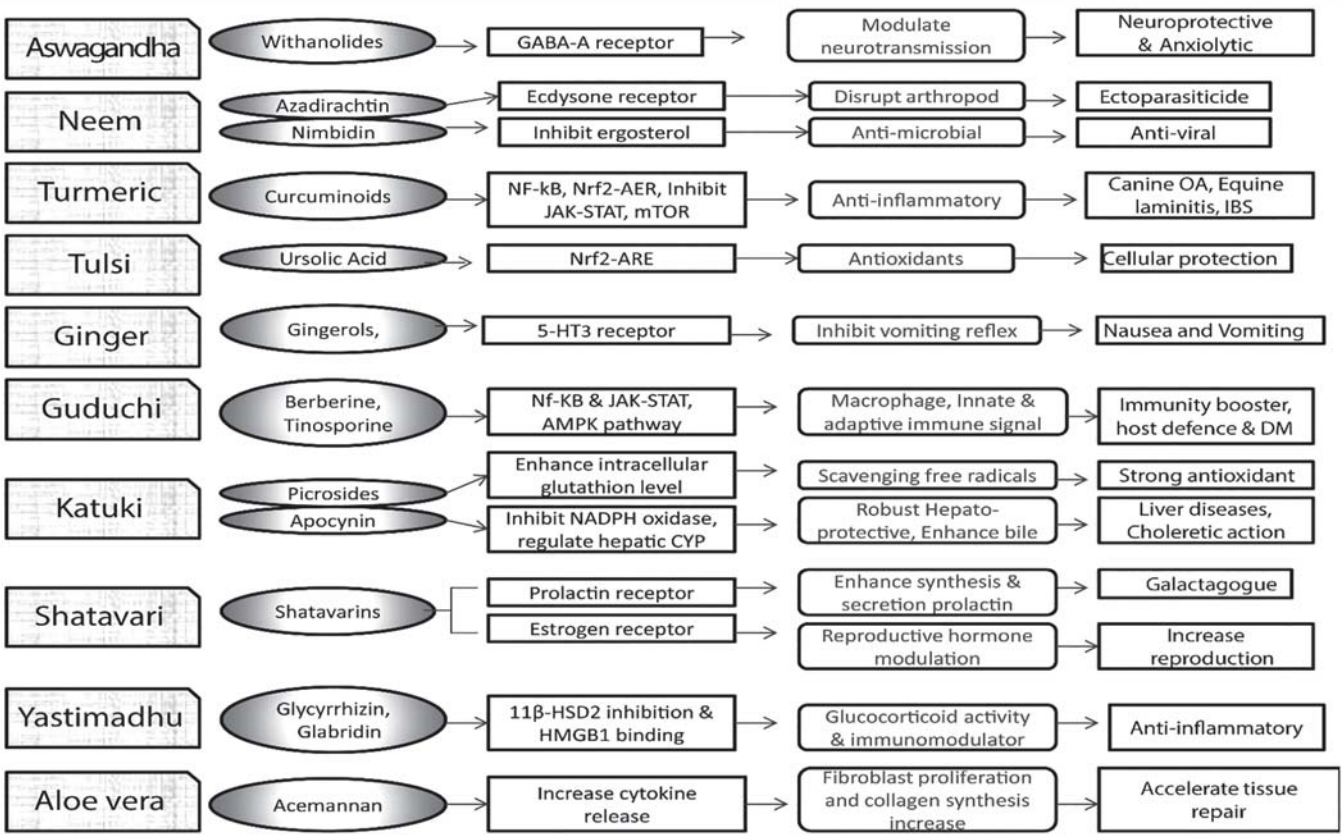

Fig 1. Integrated mechanistic convergence of Ayurvedic medicinal plants in Veterinary health.

with CYP-metabolized pharmaceuticals, must be examined systematically. These considerations are especially critical in food-producing animals, where toxicity, residue profiles, and withdrawal periods must meet regulatory standards.

To fully integrate these botanicals into mainstream veterinary practice, a robust translational research agenda is essential. This includes:

- species-specific pharmacokinetic and pharmaco-dynamic studies;
- well-designed randomized controlled trials;
- long-term safety and residue studies;
- evaluation of herb-drug interactions; and
- large-scale field trials under real-world farm and household conditions.

The broader implications of this work extend to antimicrobial stewardship and sustainable livestock management. Botanical therapeutics offer a promising route to reduce reliance on synthetic antimicrobials and anti-inflammatory drugs, addressing global concerns regarding antimicrobial resistance and drug residues in animal-derived food.

CONCLUSION

Overall, the evidence suggests that Ayurvedic medicinal plants, including Ashwagandha, Neem, Tulsi, Ginger, Guduchi, Shatavari, Licorice, and Aloe veracan serve as effective alternatives or adjuncts for managing stress disorders, ectoparasite burden, inflammatory conditions, metabolic dysfunction, reproductive challenges, and wound healing. Their mechanisms of action align with many unmet needs in veterinary practice, and their generally favourable safety profiles support further development.

In conclusion, medicinal plants offer a scientifically grounded, biologically plausible and clinically meaningful resource for integrative veterinary medicine. With continued advancements in standardization, pharmacokinetics, and controlled clinical research, these botanicals can evolve from complementary remedies into reliable, evidence-based tools for improving the health and productivity of livestock and companion animals.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Review Article

ELECTRO CARDIOGRAPHY BEYOND THE HEART: DIAGNOSTIC INSIGHTS INTO NON-CARDIAC ILLNESSES IN DOGS

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ABSTRACT

Electrocardiography (ECG) remains a fundamental, non-invasive tool for evaluating cardiac function in dogs and is increasingly recognized for its diagnostic value in systemic diseases. While canine cardiovascular disorders affect approximately 10% of the population, ECG demonstrates high specificity (99.5%) for detecting cardiac abnormalities and arrhythmias. Breed-related variations in ECG parameters have been documented, particularly in P-wave duration and mean electrical axis, underscoring the importance of breed-specific interpretation. Beyond cardiac disorders, ECG alterations are closely linked with hepatic, renal, electrolyte, and endocrine disturbances. Hepatic diseases such as cirrhosis and congestive hepatopathy commonly present with low-voltage complexes, axis deviations, and correlations with elevated liver enzymes. In renal disorders, both acute and chronic conditions contribute to arrhythmias driven by metabolic derangements, with significant associations between QT interval, creatinine, and BUN levels. Electrolyte imbalances, especially potassium abnormalities, produce characteristic ECG changes including altered T-wave morphology, PR prolongation, and predisposition to life-threatening arrhythmias. Endocrine disorders such as hypothyroidism and hyperthyroidism also manifest distinct ECG alterations involving heart rate, wave amplitudes, and MEA shifts. Marked alterations in the ECG parameters were also found in hemoprotozoan infection. Collectively, these findings highlight ECG as a sensitive, integrative diagnostic modality capable of reflecting multisystemic physiological changes and enhancing clinical decision-making in canine practice

Keywords: Electrocardiography, Dog, Arrhythmias, Hepatic Disorders, Renal Diseases

INTRODUCTION

The global prevalence of cardiovascular diseases in dogs is estimated to be around 10%, with chronic valvular heart disease (CVHD) constituting nearly 75% of these cases [4]. Over the years, several diagnostic modalities have been developed to identify and characterize canine cardiac disorders, including radiography, electrocardiography (ECG), echocardiography, and more recently, advanced techniques such as right heart catheterization and angiocardiography [6]. Among these, electrocardiography continues to hold a pivotal position as one of the most dependable, non-invasive diagnostic tools for evaluating cardiac function in dogs. Studies have reported an overall diagnostic specificity of 99.5% for detecting cardiac abnormalities, with an even higher specificity of 99.7% for identifying arrhythmias [16]. Beyond its conventional role in cardiology, ECG has emerged as a valuable tool for assessing various systemic and metabolic disorders. Abnormal ECG patterns may reflect secondary effects of hepatic dysfunction, renal impairment, endocrine imbalances, or disturbances in electrolyte homeostasis [7]. This expanding diagnostic relevance indicates that ECG is not merely limited to cardiac disease detection but can serve as a sensitive indicator of multi-systemic physiological alterations. Consequently, it holds significant promise as a preliminary screening and prognostic tool prior to specific clinical and laboratory investigations for non-cardiac conditions [1]. By integrating ECG findings with biochemical and imaging data, clinicians can gain a more holistic understanding of the animal's systemic health status, thereby improving diagnostic accuracy and therapeutic decision-making.

Reference values of ECG parameters in dogs: Variations among breeds, sex, and age groups Breed-specific reference values for various electrocardiographic (ECG)

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reference values for various electrocardiographic (ECG) parameters have been documented in several canine breeds, including Beagles [12], Labradors [18] and German Shepherds [37]. In a subsequent investigation, we conducted a study to establish baseline reference data for ECG parameters in a larger canine population comprising 239 dogs representing 11 different breeds [28]. The findings revealed that most ECG waves and complexes showed no significant variation across breeds, ages, or sexes, except for the P-wave duration, which was significantly ($p < 0.05$) higher in Golden Retrievers and Dobermans (Table 1).Additionally, notable interbreed differences were observed in the mean electrical axis (MEA) values, which were recorded as 75.30° ± 6.80 for Labradors, 60.33° ± 7.84 for Golden Retrievers, and 82.00° ± 19.43 for German Shepherds [27]. These findings highlight the existence of subtle breed-related variations in certain ECG parameters, emphasizing the importance of considering breed-specific reference ranges while interpreting canine electrocardiograms.

ECG IN HEPATIC DISORDERS

The close relationship between hepatic and cardiovascular functions is well recognized. Liver disorders are frequently accompanied by cardiac complications such as cirrhosis-associated cardiomyopathy and portopulmonary hypertension [25]. Conversely, cardiac abnormalities—particularly chronic heart failure can lead to secondary hepatic injury, manifesting as cardiac-induced liver damage or congestive hepatopathy [29]. Investigation has been done on electrocardiographic alterations in dogs presenting with ascites of cardiac origin and observed low-voltage ECG patterns, characterized by reduced R-wave amplitude and left axis deviation in most cases [21, 24]. These findings were attributed to attenuation of electrical voltage by the presence of fluid. The observed ECG changes showed a positive correlation with elevated serum SGOT, SGPT, and ALP levels, indicating concurrent hepatopathy [21]. In a related study, documentation on right axis deviation suggestive of right bundle branch block in a Labrador retriever diagnosed with hepatomegaly and hepatic cyst, further underscoring the interrelationship between hepatic pathology and cardiac electrical activity [38].

ECG in Renal Diseases

Alterations in cardiac electrical activity have been documented in both acute and chronic kidney diseases in dogs [3,26]. The prevalence of arrhythmias in dogs with renal disorders was reported to be 51.2% [22]. Therefore, electrophysiological monitoring plays a vital role in early diagnosis and improving prognosis. In cases of acute kidney disease, arrhythmias are frequently observed due to the activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system [22]. Metabolic disturbances such as acidaemia, hyperkalemia, hypocalcaemia, hyperphosphataemia and azotaemia are key factors contributing to abnormal myocardial activity. Chronic kidney disease (CKD) is commonly associated with left ventricular hypertrophy [26]. Among dogs with CKD, sinus arrhythmia, first-degree atrioventricular (AV) block, and wandering pacemaker are the most frequently detected arrhythmias, followed by those seen in acute kidney injury (AKI) [22]. A positive correlation has been observed between creatinine and blood urea nitrogen (BUN) levels with the corrected QT interval, suggesting its potential as a marker for ventricular arrhythmia susceptibility and electrolyte imbalance assessment in canine renal diseases [2]. Additionally, a case was reported a negative correlation between creatinine levels and R-wave amplitude, which was associated with uremic pericarditis [36].

ECG IN ELECTROLYTE IMBALANCE

ECG serves as a vital diagnostic tool for detecting electrolyte imbalances, as the depolarization and repolarization of myocardial cells rely on the movement of key electrolytes-potassium, sodium, and calcium-across the cardiac cell membranes [42]. Among these, potassium plays a crucial role in maintaining cardiac stability [43]. Hypokalaemia, characterized by serum potassium levels below 3.5 mmol/L, has been associated with arrhythmias and can arise from renal dysfunction, inadequate potassium intake, or intracellular potassium shifts [15,34]. Hyperkalaemia, on the other hand, has been linked to atrioventricular nodal block, prolonged PR intervals, and widened QRS complexes [23,30]. Experimental studies in dogs have demonstrated that induced hyperkalaemia leads to increased T-wave amplitude (Fig 2.), decreased P-wave amplitude, and prolonged PR intervals [14]. The same study also noted notched or flattened T-waves and prominent U-waves during hypokalaemia. Severe hyperkalaemia enhances myocardial excitability, predisposing the heart to life-threatening arrhythmias such as ventricular tachycardia (VT) and ventricular fibrillation (VF). Additionally, hyperkalaemia-induced accelerated idio-ventricular rhythms were observed in our study (Fig 6.). However, contrasting results were reported no significant association between ECG changes and plasma electrolyte levels in dogs [40].

ECG IN ENDOCRINE DISTURBANCES

The activity of thyroid gland is associated with electrical and mechanical activities of the heart [33]. Hypothyroidism is associated with sinus bradycardia [39], atrial fibrillation [17,35] and atrioventricular blocks [39]. Decreased heart rate in hypothyroidism may be due to direct action of thyroid hormone on myocardium such as decreased tissue oxygenation, decreased response to β-adrenergic receptors [31]. Notable changes in the ECG parameters during hyperthyroidism includes decreased P, R and T wave amplitudes due to decrease in myocardial mass and circulating blood volume [19,31,45]. In hypothyroidism, the mean electrical axis (MEA) was also altered which may be associated with ventricular hypertrophy and intraventricular conduction disturbances [19,44]. These abnormalities were reported to be reversed by thyroxine treatment [11]. The ECG changes in abnormalities with adrenal glands have been reported in dogs. The notable alterations in the ECG parameters in bilateral adrenalectomy includes flattened and biphasic T wave and changes in ST interval [41].

ECG IN HEMOPROTOZOAN INFECTION

The anaemia caused by hemoprotozoan infections results tissue hypoxia and hemodynamic compensatory mechanism [9]. The cardiac myocytes undergo structural alterations and subjected to inflammatory changes that are reflected in ECG [20]. The study on dogs revealed that P duration, QT interval and HR increased significantly in severe anaemia due to parasites. Several authors reported notable changes in ECG parameters during canine babesiosis [8]. Several changes in the ECG parameters such as ST depression and coving (28%), large T (42%), and notched R (28%), low R-amplitude (23%), prominent Q (33%), axis deviations (40%), and prolonged QRS (32%) in canine babesiosis have been observed [13]. In addition, sinoatrial (7%) and atrioventricular blocks (4%), ventricular premature complexes (7%) and sinus arrest (7%) in Babesia infection were also found. In a related study, sinus tachycardia. ST-segment abnormalities in hemoprotozoan infections were reported [5]. In Babesiosis and Ehrlichiosis, increase in the R wave amplitude has been observed. The major ECG changes in canine Basesiosis were Sinus tachycardia however, bradyarrhythmia, atrial fibrillation 05 (5.62%) and atrial paroxysmal tachycardia were also seen less frequently [10]. In contrast to these findings, a case report showed no changes in the electrocardiography in a canine babesiosis [32].

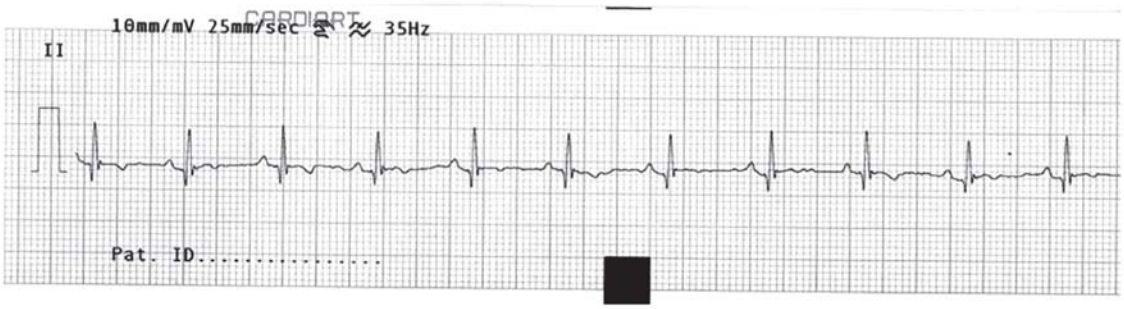

Fig 1. Low Voltage Complex in Ascites

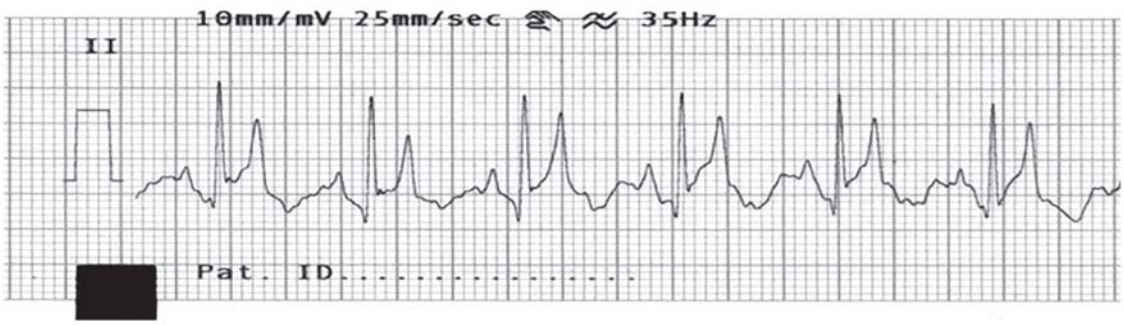

Fig 2. Tall and tented T wave in hyperkalaemia

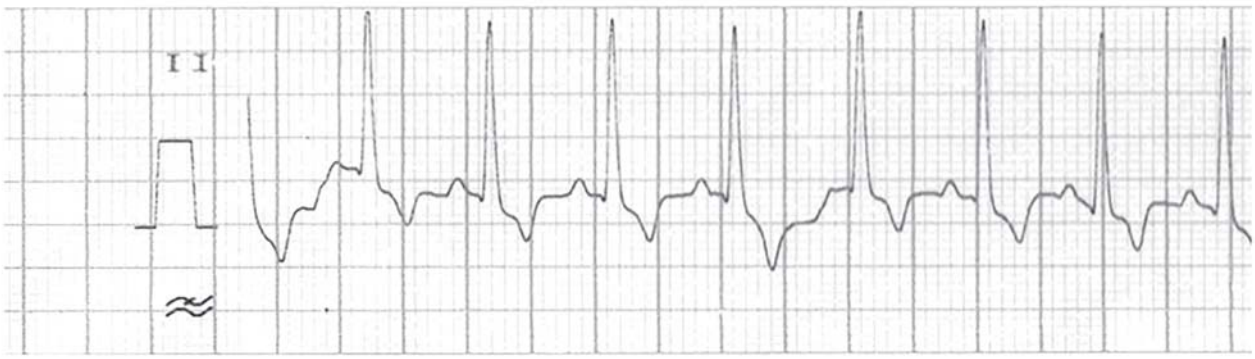

Fig 3. ST segment depression in myocardial ischaemia

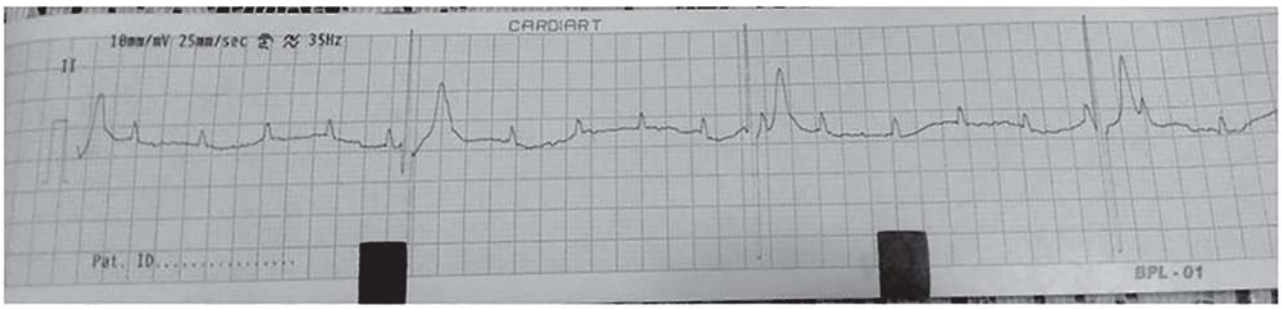

Fig 4. Seond degree AV block

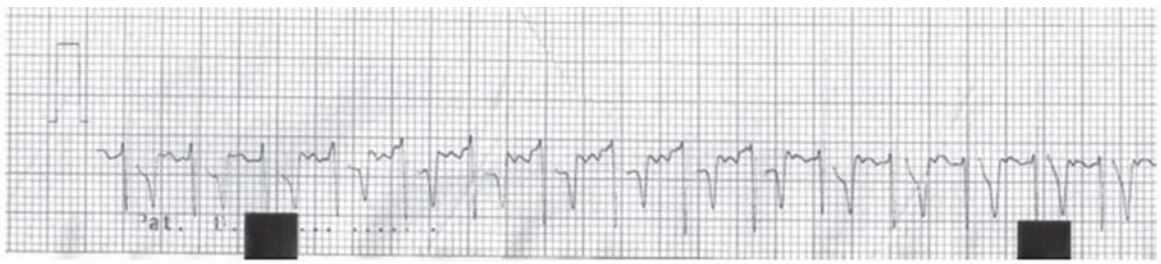

Fig 5. Bi phasic QRS complex (rS) along with right axis deviation suggestive in right bundle branch block

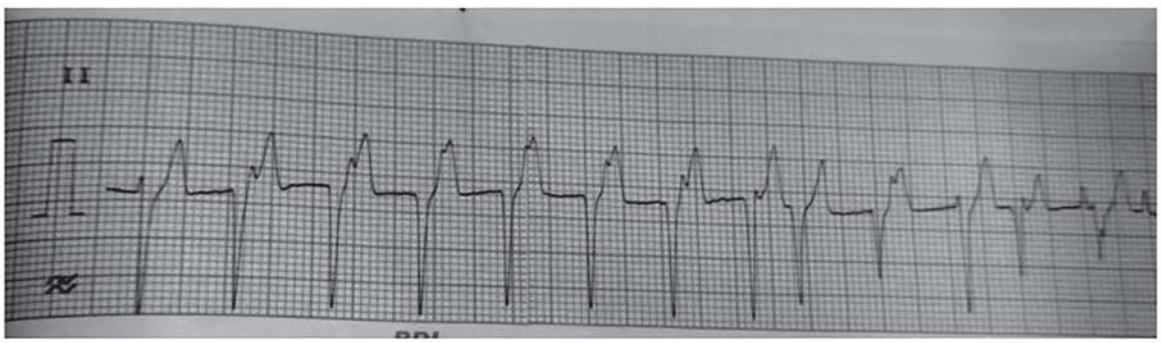

Fig 6. Hyperkalaemia induced indo-ventricular accelerated rhythm

Table 1: Breed specific reference values of different ECG parameters dog

Group	HR (bpm)	P_{amp}(mv)	P_{dur} (s)	PR interval (S)	QRS_{dur} (s)	R_{amp} (mv)	QT interval (S)	T_{dur} (s)	T_{amp} (mv)
Overall	119.24 ± 4.4	0.25± 0.01	0.04 ±0.01	0.07 ± 0.01	0.04 ± 0.01	1.31 ± 0.07	0.19± 0.01	0.04± 0.01	0.22± 0.014
Variation between breeds									
Mongrel	108 ± 18.5	0.25 ± 0.03	0.04b ± 0.01	0.09 ± 0.01	0.04 ± 0.01	1.65 ± 0.47	0.18± 0.01	0.04 ± 0.01	0.21± 0.014
Labrador	132.13 ± 8.5	0.23 ± 0.02	0.04b ± 0.01	0.07 ± 0.01	0.04 ± 0.01	1.41 ± 0.11	0.18± 0.01	0.04 ± 0.01	0.23± 0.014
Crossbred	109.6 ± 1.14	0.27 ± 0.06	0.04b ± 0.01	0.10 ± 0.02	0.04 ± 0.01	1.30 ± 0.35	0.2± 0.01	0.04 ± 0.01	0.21± 0.014
Spitz	111.00 ± 9.4	0.26 ± 0.02	0.04b ± 0.01	0.06 ± 0.01	0.04 ± 0.01	1.40 ± 0.10	0.2± 0.01	0.04 ± 0.01	0.23± 0.014
Beagle	100.00 ±7.3	0.20 ± 0.03	0.02b ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.70 ± 0.16	0.2± 0.01	0.04 ± 0.01	0.23± 0.014
Pug	125.00 ± 10.2	0.23 ± 0.06	0.04b ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.75 ± 0.26	0.16± 0.01	0.04 ± 0.01	0.24± 0.014
Dachshund	115.00 ± 12.3	0.20 ± 0.10	0.04b ± 0.01	0.08 ± 0.01	0.04 ± 0.01	1.15 ± 0.25	0.2± 0.01	0.04 ± 0.01	0.24± 0.014
German Shepherd Dog	145.00 ± 5.00	0.25 ± 0.05	0.06ab ± 0.02	0.08 ± 0.01	0.04 ± 0.01	1.43 ± 0.30	0.18± 0.01	0.04 ± 0.01	0.21± 0.014
Pomeranian	106.25 ± 3.75	0.28± 0.03	0.04a ± 0.01	0.07 ± 0.01	0.04 ± 0.01	1.07 ± 0.31	0.18± 0.01	0.04 ± 0.01	0.22± 0.014
Golden Retriever	140.50 ± 25.5	0.45 ± 0.05	0.08a ± 0.04	0.06 ± 0.02	0.04 ± 0.01	1.70 ± 0.10	0.2± 0.01	0.04 ± 0.01	0.21± 0.014
Doberman	150.00 ± 18.2	0.30 ± 0.10	0.08a ± 0.02	0.12 ± 0.03	0.04 ± 0.01	1.320 ± 0.1	0.18± 0.01	0.04 ± 0.01	0.23± 0.014
P Value	0.69	0.42	0.05*	0.1	0.45	0.08	0.43	0.06	0.6
Variation between sex									
Male	120.07 ± 7.88	0.24 ± 0.02	0.04 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	1.34 ± 0.11	0.15± 0.01	0.04 ± 0.01	0.22± 0.014
Female	118.61 ± 5.13	0.27 ± 0.02	0.04 ± 0.01	0.7 ± 0.01	0.04 ± 0.01	1.28 ± 0.10	0.18± 0.01	0.04 ± 0.01	0.21± 0.013
P Value	0.87	0.34	0.85	0.06	0.06	0.66	0.31	0.46	0.06
Variation between different age groups									
Male	120.07 ± 7.88	0.24 ± 0.02	0.04 ± 0.01	0.08 ± 0.01	0.04 ± 0.01	1.34 ± 0.11	0.15± 0.01	0.04 ± 0.01	0.22± 0.014
Female	118.61 ± 5.13	0.27 ± 0.02	0.04 ± 0.01	0.7 ± 0.01	0.04 ± 0.01	1.28 ± 0.10	0.18± 0.01	0.04 ± 0.01	0.21± 0.013
P Value	0.87	0.34	0.85	0.06	0.06	0.66	0.31	0.46	0.06
< 6 months	108.75 ± 4.20	0.20 ± 0.04	0.03b ± 0.01	0.06 ± 0.01	0.03 ± 0.01	1.10 ± 0.45	0.2± 0.01	0.04 ± 0.01	0.22± 0.0126
months.- 2.5 years	101.25 ± 5.15	0.23 ± 0.03	0.04b ± 0.01	0.06 ± 0.01	0.04 ± 0.01	1.03 ± 0.20	0.18± 0.01	0.04 ± 0.01	0.22± 0.012
2.5 - 4.5 years	134.00 ± 3.23	0.29 ± 0.02	0.04b ± 0.01	0.08 ± 0.01	0.04 ± 0.01	1.02 ± 0.12	0.19± 0.01	0.04 ± 0.01	0.22± 0.0111
4.5 - 6.5 years	109.67 ± 6.57	0.25 ± 0.04	0.03b ± 0.01	0.07 ± 0.01	0.04 ± 0.01	1.43 ± 0.24	0.2± 0.01	0.04 ± 0.01	0.22± 0.012
6.5- 8.5 years	122.32 ± 7.39	0.27 ± 0.02	0.05ab ± 0.01	0.06 ± 0.01	0.04 ± 0.01	1.57 ± 0.11	0.21± 0.01	0.04 ± 0.01	0.21± 0.01
8.5 - 10.5 years	114.67 ± 5.91	0.22 ± 0.03	0.03b ± 0.01	0.08 ± 0.01	0.04 ± 0.01	1.28 ± 0.20	0.18± 0.01	0.04 ± 0.01	0.22± 0.012
10.5 - 12.5 years	122.33 ± 36.6	0.30 ± 0.10	0.07a ± 0.03	0.11 ± 0.01	0.04 ± 0.01	1.20 ± 0.23	0.2± 0.01	0.04 ± 0.01	0.21± 0.013
12.5 - 15years	102.50 ± 2.50	0.25 ± 0.05	0.04b ± 0.01	0.06 ± 0.02	0.04 ± 0.01	1.65 ± 0.15	0.19± 0.01	0.04 ± 0.01	0.23± 0.013
P value	0.69	0.66	0.05*	0.1	0.08	0.19	0.54	0.08	0.07

Values are expressed as mean±SE. Values with different superscripts within a column differs significantly (P<0.01)

CONCLUSION

Therefore, electocardiography shows considerable potential as an initial screening and prognostic method before undertaking more specialized clinical or laboratory tests for non-cardiac disorders. When ECG results are evaluated alongside biochemical and imaging findings, clinicians can obtain a more comprehensive picture of the animal's overall health, ultimately enhancing diagnostic precision and guiding more effective treatment decisions.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Review Articles

HARNESSING THE THERAPEUTIC POTENTIAL OF SHEEP MILK

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ABSTRACT

Globally sheep are meant for meat. Only 20.8 percent of sheep are reared for milking purpose. Modern human diet and lifestyle changes are resulting in endless ailments, creating enormous scope for natural health influential foods. Sheep milk is emerging as a nutritional and sustainable food around the world. Shepherds are ignorant of the expanding prominence of it, therefore they mostly perform traditional unorganized sheep rearing. Functional consumable and non-consumable products like world-class cheese, yogurt, and several skin care formulations (lotions, soap and creams) can be developed from this milk. Enlightening farmers regarding health importance of sheep milk and creating a nexus between quality product generation and the secure market facility can significantly increase farmers' income by reshaping them from mere rearers to entrepreneurs. This article attempts to delineate an absolute sketch of sheep milk composition, health importance and aided skill to optimize milk production.

Keywords: Sheep milk, nutritional uniqueness, medicinal use, sustainability, optimising production

INTRODUCTION

Milk is a rich source of valuable nutrients that regulate variety of physiological, nutritional and functional activities in our body. It is considered as a complete food having an adequate content of human health, targeting essential nutrients like calcium, vitamins, proteins, fat and calories. Its significance in human nutrition has been accepted since evolution. It is also a prime source of all the essential amino acids needed for human health. Cows and buffaloes are considered as conventional dairy animals worldwide. Changing food habits and sedentary lifestyle compelled the existing population to choose these health bootstring animal products. Non-bovine milk and milk products are gaining significance in the world market for their bio-active potential. Non-bovine milk includes milk from goats, sheep, camel, donkeys etc. Sheep were one among the early domesticated animals because of its ruggedness, adaptability, small size, multiple utility and social natures. Owing to these qualities, management of sheep is effortless at the farmer's level [3]. Sheep husbandry plays a crucial role in the socio-economic upliftment of a large section of inhabitants in developing countries. Sheep and its products are critically important for the survival and well-being of large segments of the human population, particularly in developing countries. More than half of the world's total sheep population is found in developing countries. Sheep provides many potential outputs to mankind viz. meat, milk, wool, manure, skin etc., but most sheep in developing countries are reared for meat. Traditionally, sheep had been milked and mankind relished its taste and experienced its superiority. But slowly sheep milk was replaced by bovine milk. As per present report, cow milk is the most acceptable milk worldwide (81% of total world milk production), followed by buffalo (15.1%), goat (2.2%), sheep (1.3%) and lastly camel (0.4%) [14,29,30]. Owing to climate change, a burgeoning population and a decrease in natural resources (grazing lands and drinkable water) there is intense pressure on the

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productivity of cows and buffalos. Therefore, there is a dire need to search for alternate opportunities like milk from sheep as these animals are hardy, their milk is nutrient-rich and can be produced in resource-restricted conditions[23]. Their unique concentration of constituents makes them a suitable ingredient for dairy-based value-added products. Out of the total dairy products, about 20.8% are from sheep and goats which make up [33].

Sheep milk is rarely consumed in liquid form. More than 95% of sheep's milk is converted into cheese. Sheep milk production is mainly confined to some parts of Europe and Asia. Sheep are multi-faceted animals known for their multiple services like wool, meat, manure, etc. Due to their small size, they can be reared in restricted spaces and without specific feeding supplementation but can provide us with natural nutrient-dense food i.e. milk. Therefore, for poor landless and marginal farmers of developing countries sheep and goats are called as poor man's cows. Sheep milk is used in the production of numerous famous cheeses, including feta, pecorino, caciocavallo and Roquefort, in addition used for making yogurts. The United States is the major importer of sheep milk cheese (50-60% of annual global exports) and the market size and market demand for sheep milk cheese is growing over time [36]. It shows the growing opportunity of sheep milk.

Milk from sheep has been gaining attention in the last few years as a healthy food and is appreciated by a new consumer market [47]. Mohapatra et al reported that sheep milk has been neglected but outstanding impact as a functional food [35]. Sheep milk is a treasure of nutrients and due to human health significance, in the present day, it is considered under the umbrella of functional foods [28]. Functional milk is the milk that provides health benefits beyond what regular milk provides. It is a delicious alternative to cow milk. Its health benefits include its ability to lower blood cholesterol levels, provide strength to bone, immunity booster, reduction of inflammation, and fight cancer. Therefore, the growth of sheep milk production is exponentially increasing[48].

UNDERSTANDING THE SHEEP MILK

Currently, there are around 1.176 billion sheep in the world. China has the largest sheep population in the world consisting of 13.7% of world sheep followed by India and Australia shows the sheep in the globe are mainly concentrated in most parts of Asia, Europe, Australia and Central and South Africa. Dairy sheep breeds are predominately found in Middle East and Mediterranean countries [48]. China, Turkey and Syria top in sheep milk production. According to the FAO (2018), the world's total sheep milk production was 10.37 million metric tons per annum [18]. About 1.54 million metric tons of sheep milk is produced by China annually [38]. Syria has only 1.27% of world sheep; still, it is among the top 3 sheep milk producer countries because of its native fat-tailed Awassi breed. Awassi and Assaf are dairy sheep breeds that are predominant in Middle East Asia, whereas East Friesian and Lacaune are predominant dairy sheep breeds in Europe (Table 3.). In New Zealand the number of sheep milk producers increased by 50% between the years 2019-2021; this proves the burgeoning demand for sheep milk in the consumer's sphere. Other dairy sheep breeds are Sarda of Sardinia, Italy, Chios of Greece and Manchega of Spain, etc. are reared under an intensive system for milk production (Fig 1.).

Nutritional aspects of Sheep milk

Sheep milk has an exceptional compositional and nutritional profile which makes it ideal for different classes of people like infants, elderly, women, and athletes. Compared to cows' milk, it contains almost 60 percent more protein and fat (Table 1). The superiority of sheep milk over milk of other species is due to their different molecular weight and amino acid sequence which renders better digestibility and higher thermo-stability [8]. Easier digestibility results in better bioavailability of essential amino acids. Superior thermo-stability helps the milk proteins to retain their properties even under some degree of under-heat treatment. Sheep milk contains B and C vitamins which are good for energy, vitality and managing stress, and is also naturally low in sodium. The countries with pasture-based rearing obtain wholesome and safe sheep milk for human consumption. The higher protein, fat and calcium concentration of sheep milk makes it ideal for value-added product formulation [35].

Medicinal aspects of Sheep milk

Diet is considered one of the most compressive approaches to good health. Modern-day lifestyle is increasing the health risks of the modern world. At the

same time the developing and underdeveloped world is facing the challenges of malnutrition, therefore, in the UN's Sustainable Development Goals, good health and well-being is one among the 17 ambitious targets. Functional foods are enriched and enhanced foods, which have positive health benefits beyond nutrition. The increase in purchasing power of the middle-class population opened a new window for natural foods with health-influential properties. High mineral content, beneficial fatty acids, anti-oxidants, higher protein content, functional bioactive peptides, absorbable calcium and conjugated linoleic acids have laid the foundation to explore sheep milk as a natural therapeutic food. The beneficial properties of milk protein are attributed to its exhaustiveness (availability of all essential amino acids), digestibility and functional bioactive peptides that are released during digestion in the human gut. Nowadays the potential ingredients of milk proteins and peptides are retrieved to formulate remarkable health supplements that can be consumed as a part of the regular diet of the elderly, infants, or immune-compromised individuals. The specialty of milk protein molds sheep milk into a functional food. These days' new efforts are made by researchers to enhance its significance as a nutraceutical food. Milk proteins are a good source of bioactive peptides (BAP) [37]. BAPs positively affect human physiological processes. For this purpose, it is essential to know the sheep milk proteins, peptides and their potentials after it is transformed into bioactive peptides. BAPs obtained from sheep milk have antihypertensive, antimicrobial, opioid, antioxidant, immunomodulatory, or mineral-binding activities [2].

Sheep milk colostrums

Colostrum is the thick milk produced during the first few days of parturition and contains higher levels of protein and salts than mature milk along with naturally packaged with exclusive immunoglobulins and antimicrobial peptides that give immunologic defence, and growth factors [41]. This is vital for neonate's survival. The composition of colostrum from sheep is much higher than from cows: fat 13.0% and 5.1%, protein 11.8% and 7.1%, lactose 3.3% and 3.6%, minerals 0.9% and 0.9%, and total solids 28.9% and 15.6%, respectively [1]. shows the chemical composition and fatty acid contents of different ruminant colostrums (Table 2.). It shows that sheep milk has higher mono-unsaturated fatty acid and lower saturated fatty acid. It indicates that, health influential fatty acids are significantly high in sheep milk.

CONSTITUENTS OF SHEEP MILK

Milk composition varies with species, breed, animal age, nutrition, physiological status and also with environmental cues. shows the milk composition of various breeds of sheep found in Europe.

Seasonal changes in milk composition are the result of forage availability [44]. Sheep milk has a prodigious role in building a healthy rural society, and rural economy and for sustainable farmer's livelihood. To obtain uninterrupted sheep milk throughout the year, out-of-season lambing can be practiced, or keeping two different flocks with a farmer and practicing breeding at different times of year is a successful practice. They have a lactation length varying widely between 90-365 days [17]. Sheep milk contains higher fat (6.4%), protein (5.6%), lactose (5.1%) and salts (0.9%) compared to cow milk which has 3.3%, 3.3%, 4.7% and 0.7% respectively (Table 1.). Sheep milk is a naturally rich source of most minerals [7] making it more lucrative for health-promoting food formulations. The primary nutritional benefits of milk are from its proteins.

Water

Water is the medium in which all the other components of milk (total solids) are dissolved or suspended. Water is the principal component in all dairy products like cheese, cream, yogurt, ice cream etc. Along with temperature and pH, it influences the physical, chemical, microbiological and technological quality of dairy products [19, 20]. Sheep milk has more total solid and minimal water compared to other species (Table 1.). Total solids refer to the amount of non-water substances present in a milk sample or a milk product. These non-water substances include proteins, fat, lactose, minerals, and other solids. They determine the nutrient richness and processing ability of milk. Milk fat is the most variable component followed by proteins and minerals. Milk with high solids-not-fats (SNF) is valuable to the consumer for its flavour and nutritional value. Apart from lactose other carbohydrates include small amounts of oligosaccharides, glycopeptides, glycoproteins, and nucleotides. Milk oligosaccharides have considerable antigenic properties and are valuable in the growth promotion of the intestinal flora of the newborn.

Sheep Milk Fat

It is true that consumers now prefer fat-free, lean, or low-fat products. Sheep milk is a rich source of fat. Studies confirm that dairy-based products, regardless of fat content have beneficial fat with advantageous effects on metabolic and cardio-metabolic health [25,49]. The average size of fat globules of sheep milk (3.5 μm) is similar to goat milk (3.0 μm) and smaller than cow (4.0 μm) and buffalo milk (4.5 μm) [2,50]. This gives it a naturally homogenized consistency and makes it easier to digest. The size and dispersion of the fat globules confer greater consistency to these milks, favouring freezing without phase separation. Sheep milk is a good source of short-chain fatty acids which provides a distinct flavour to it [45].

Sheep Milk Proteins

Milk protein is considered a complete protein as it is a source of all the ten essential amino acids that cannot be prepared by the human body (provide reference). In many countries like Australia price of milk is decided based on protein content, as consumers here prefer proteinaceous milk products over fat-rich milk products. In European countries, sheep milk costs four times more than cow milk because of its reasonable protein content. Milk proteins are synthesized by the mammary gland. Casein and whey proteins are major milk proteins. In ruminant milk, the casein to whey protein ratio is 80:20 [5, 50] which was 50:50 in human milk and 60:40 in equine milk [19]. Sheep milk has the highest concentration of casein (4.18 g/100 g) and whey protein (1.02 g/100 g) compared to other ruminants [10]. The agglutinin protein is absent in sheep milk, providing better digestibility compared to cow milk [39]. The protein portion has a major impact on the nutritional and technological value of milk.

Casein

Casein protein is synthesized in the mammary gland primarily in response to lactogenic hormones. The casein portion precipitates at pH 4.6 at room temperature (Isoelectric point where a net charge is zero) whereas under the same condition, whey protein remains soluble. Casein provides a slow and sustained release of amino acids to the bloodstream [46]. Casein (CN) family includes 4 major components alpha- (αs1- and αs2-casein), beta- and kappa-casein [11]. Each casein variant phosphate group binds with calcium. Therefore, casein is referred to as secreted calcium-binding phospho-protein [26]. Phosphate and calcium ions constitute about 8% of the total mass of the casein micelles [12].

As sheep milk contains more casein (provide reference), it is an outstanding source of calcium and phosphorous for human health. Milk protein is dispersed in milk in colloidal form and exists in a micelle pattern. Micelle is roughly defined as a casein and calcium phosphate complex of milk [6]. On binding of calcium to the phospho-serine group αs1, αs2 and β casein gets precipitated (calcium insoluble caseins) [9, 16] whereas κ CN is soluble in calcium and it interacts with the calcium insoluble caseins and stabilizes them to form a stable colloidal complex [12]. The casein micelle contains approximately 40, 10, 40, and 10% (w/w) of αs1, αs2, β, and κ-caseins respectively [42] but slight variations were found species-wise (Tabl2. Different casein proteins occupy different positions in the casein micelle and have specific functions. Most scientists agree that κ-CN is present in the exterior part as a stabilizing layer in the casein micelle (provide references here). Horne (2003) proposed a casein micelle model that shows the protrusion of negatively charged particles of κ-CN at the exterior of the casein micelle [27]. These are responsible for the repulsion between micelles and the stabilization of milk preventing precipitation. Casein micelle structure is destroyed by adding acids/bases to milk. Once disrupted, micelles cannot be reformed again. The average diameter of casein micelle is 200nm (50-600nm) i.e. $1/50^{th}$ of fat globule size [51]. Blood calcium is tightly regulated; so extra dietary calcium is reflected in milk because of casein micelle. The presence of proline or histidine at the 67^{th} position of β-casein allows the distinction between two types of milk, A1 and A2 [31]. The ovine αs1-CN has 199; α s2-CN has 207; β-CN has 209 and κ-CN has 171 amino acids.

Sheep milk is mainly used for the production of world-class cheese varieties (Feta, Roquefort, etc.) and yogurt. For these products casein micelle characteristics play a crucial role. Casein micelle characteristics like diameter, hydration and mineralization, decide the economic return from a dairy industry as they affect product quality. The high levels of protein, fat and calcium present in sheep milk casein units make it an excellent matrix for cheese production [4,34]. A casein micelle consists of water and salt (calcium and phosphorous)[32]. Like goat, sheep

casein has higher mineralization and is less hydrated and heat stable compared to cow milk casein [43]. Due to the rich calcium content in sheep milk, it needs no $CaCl_2$ additive for coagulation which adds to its technological compatibility. Casein micelle size apart from important determinant for product formulation also plays a significant role in various product processing applications like stability during heating, freezing and drying [24]. Micelles of goat and sheep milk are smaller (180nm, 193nm) compared to cow milk (260 nm) [39]. The size of the casein micelles is determined by different casein fractions content, calcium phosphate ratio and κ-casein content [9]. The κ-casein content has an inverse relationship with micelles size. It has been reported that smaller micelle size increases cheese yield, without affecting the cheese gel strength [21]. Cheese yield depends upon casein and fat content of milk [15].

Whey Protein

Whey protein is composed of major albumins (55%) i.e. β-lactoglobulin, α-lactalbumin and immunoglobulins, glycomacropeptides, bovine serum albumin and minor proteins such as lactoperoxidase (LP), lysozyme and lactoferin (LF) [38]. Whey proteins are a rich source of essential amino acids like lysine, threonine, isoleucine and valine. Alpha-lactoalbumin, with the highest biological value, has relatively large amounts of cystine and tryptophan. Please provide status of these proteins in sheep milk to make this paragraph relevant.

Bio-active Peptides

Bioactive peptides are biological compounds generated after the cleavage of the parent protein compound. The natural cleavage of milk protein occurs by the action of gastrointestinal enzymes or by microbial action. Out of these generated peptides, some are biologically active [40]. Dairy-derived bio-peptides have much health promotional action. Sheep milk and fermented sheep milk products produce numerous bio-active compounds with a variety of physiological and health promotional actions including anti-oxidant, anti-microbial, anti-hypertensive, etc. [Fig 2.]. shows the potential peptide fragments obtained from various fractions of sheep milk protein with their sequence and biological activity.

Sheep Milk Minerals and Vitamins

The richness of sheep milk in minerals and vitamins is clear from (Table 5.). Higher levels of calcium and phosphorous make it ideal for the bone health of human beings, particularly young, adults and women. Higher casein level in sheep milk makes the calcium more bio-available than other ruminant milk [22]. Higher content of magnesium, zinc and copper improves the immune status of its customers. Sheep milk contains around twice as much calcium, magnesium and phosphorous than cow milk. The vitamin content of almost all vitamins is higher in sheep milk compared to other ruminants. Sheep milk is a good dietary source of fat-soluble vitamins A, and vitamin E and water-soluble vitamin B and vitamin C. Research claims that 2 cups of cow milk is equivalent to 1 cup of sheep milk [12].

The consumer acceptance and market size of sheep milk and its products have gained momentum due to the superiority of the product which gives it nutritional, therapeutic value and technological value and makes it unique from the milk of other domestic animals.

OPTIMIZING SHEEP MILK PRODUCTION

The purpose of sheep rearing varies with region. Most sheep rearers do not value their time contribution to sheep rearing and also do not give focused attention to sheep productivity as they think sheep can be reared only for subsistence. But sheep can be a good option for commercial farming. So, specialized attention needs to be given to sheep rearing. The primary considerations are good management, adequate nutrition and proper health care. In developing countries like India, sheep are raised under an extensive system or migratory system producing 50-60 kg of milk per lactation and enjoyed by shepherds and their families. Here sheep milk is used in households in the treatment of arthritis, rheumatism, fracture, etc. In Middle East countries sheep are raised under extensive systems and produce 80-90 kg of milk per lactation and most of the milk is utilized in household traditional products. In Mediterranean countries sheep are raised under commercial dairy sheep farming, produce >200 lit of milk per lactation, which is further processed into cheese and yogurt. Depending on the purpose of rearing and milk yield potential, the uses of sheep milk are also region-specific. In Greece, France, Spain, and Germany, most sheep milk is transformed into

cheese and yogurt. In Asia and Africa region, sheep milk is used for butter and ghee preparation. Countries rearing sheep for milk production need to address the four pillars of milk production to get optimized production from sheep.

The four pillars on which sheep milk production lie, include the selection of high-potential animals for selective breeding, proper nutrition to sheep as per their physiological stage, addressing the sheep's health and clean milk production, and record-keeping for monitoring the animals as well as sheep (Fig 3.).

SELECTIVE BREEDING

The story of France-based Lacaune sheep famous for world-class Roquefort cheese is a result of selective breeding. This white-colored sheep has an elongated head and rounded snout. Their ears slope downwards and are a bit floppy compared to other breeds. These sheep also have a quite bold temperament and are less docile than other dairy breeds of sheep. Sheep dairy farming is a traditional occupation of farmers in Roquefort-mountain areas of France. In 1964, the milk yield of Lacaune dairy sheep (native sheep) was 80 liters in lactation length of sheep 135 days. Manual milking of ewes was practiced at that period. But with continual selective and pure breeding, by 1998, the milk yield and lactation period were improved to 270 liters and 165 days respectively. Now rotatory milking parlor with automatic milking is practiced. About 90% of milk is processed into Roquefort cheese.

PROPER NUTRITION

Feed cost accounts 65-70% of the total cost of an animal farm. The profitability of a dairy farm therefore, relies on feeding management. Over 90 per cent of the energy for sheep comes from grass, hay or silage. Effective utilisation of grass and good grassland management is at the heart of easier management concepts. Grazing high-quality forages needs less supplementation. If forage quality is poor then supplementation can rectify the deficit. The correct nutrients (fat, carbohydrate, minerals) translate into better milk production for the ewe. In sheep pasture-based diet yields, protein and fat rich milk compared to hay or silage-based diet. Ample of roughage, adequate mineral and ad libitum water markedly affect the milk quality and quantity.

Nutrition during last trimester and early lactation greatly affects the lactation yield. Last 2 month of gestation is the most critical period as maximal foetal growth occurs during this period and body prepares itself for colostrum synthesis. In addition to this, due to increase in volume of uterus in abdomen there is increased pressure on digestive tract. So, the ewe cannot consume large meals immediately after lambing but the energy needs are very high. It needs energy rich small and frequent meals which can optimise milk production. The process of maximising milk production through quality feeding after lambing is called Steaming up.

Milk compositions are essential for quality and quantity of value-added dairy products like cheese and yogurt. In Lacaune sheep, total utilisable substances (TUS) sum of the fat and protein content in milk; expressed in g/L have been found to be an accurate predictor of Roquefort cheese yield. The milk composition depends upon nutrition. Nutrition affects the processing performance of the milk, product yield and its shelf life.

Sheep health and clean milk production

Diseases decline the performance of as sheep are reared in herd the compromised health of one animal is more likely to affect the health status of the herd. Therefore, on regular basis sheep health need to be assessed through vital signs, body coat and body conditions appropriate to production stage of sheep. To keep the herd, disease free the major precaution is to avoid the entry of new pathogen. Therefore, biosecurity is the most basic method of disease control. Internal and external parasitic diseases are common in sheep. They adversely affect the sheep health and have devastating effect on production performance of sheep. Regular deworming and timely vaccination can save the treatment cost and circumvent the losses due to diseases. Technology-driven disease detection like use of sensor based digital technology; drone-based disease identifications are some new innovations for large farms.

Cleanliness, good hygiene, and attention are essential for optimising milk production. The following things need to be taken care of for getting the best potential of an animal.

- Reports suggest that leaving the lamb with ewes for long period after lambing drop down the milk availability for human consumption and increase the chances of mastitis due to multiple suckling. Therefore, slow and weaning is an appropriate method to get higher quantity and good quality of milk.

- Udder of the sheep must be kept clean and dry which will prevent the chances entry of pathogen. After milking udder should be dipped in post dip solution. Mastitis also affects the milk composition which has negative impact on dairy product quality.
- Milking routine should be followed strictly for optimising the milk production.
- Milking staffs must be skilled and the milking parlour must be calm.
- Good communication between all farm staff, veterinarian and nutritional advisor,

Record Keeping

It is the necessary component of a dairy farm. In a farm, record keeping helps in better supervision and efficient management of a farm. It ascertains the income and expenditure from the farm. It helps in finding out the cost of milk production in the farm. Production records help the farmer to know the physiological status of animal and guide in taking decisions regarding its health, breeding and nutrition. It helps in finding the right time of culling the animal. It is the major tool for financial planning decisions and act as an official record for researchers, banking personnel's before giving a loan, for extension workers and other farmers. It ascertains pedigree and helps in taking breeding decisions. Overall, the profitability status of a dairy farm can be visualised from its records. Several software has been developed these days which can accurately maintain records of a farm in place of traditional registers.

CONCLUSSION

As we are moving towards sustainable agriculture and food security, sheep are the right animal that can sustain in extreme climatic condition and provide the nutrient rich milk to the society. It is the healthy alternative to cow milk with less space, less input and minimal environmental impact. Moreover, it has a high potential to transform into therapeutic and value-added products. There are plentiful opportunities in sheep milk processing sector. This new dimension can support the poor sheep rearer to generate an extra income with the scaling up of sheep milk production and development of novel food products from sheep milk.

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Table 1. Proximate milk composition of different species

Proximate composition	Cow	Buffalo	Sheep	Goat	Camel	Human
Water	87.8	83.2	82.1	87.7	88.2	87.5
Total protein	3.3	4	5.6	3.4	3.0	1.0
Total fat	3.4	7.5	6.4	3.9	3.5	4.4
Lactose	4.7	4.4	5.1	4.4	4.5	6.9
Ash	0.7	0.8	0.8	0.9	0.8	0.2

Source: Kalyankar *et al.*, 2016; Kula, 2016

Table 2. Chemical composition and fatty acid contents of different ruminant colostrums

Ruminant Species	Protein (%)	Fat (%)	Moisture (%)	(%)Ash	pH (%)	∑SFA (%)	∑MUFA (%)	∑PUFA
Goat	7	10.34±4.02	70.85±0.73	1.00±0.38	6.32±0.03	57.763	1.89	3.27
Sheep	17	10.92±0.93	76.57±6.84	1,99±0.78	6.13±0.01	51.24	39.79	3.56
Cow	7	4.35±0.43	82.88±1.85	0.51±0.74	6.16±0.09	58.82	29.70	3.49

Source: Koluman *et al.,* 2019

Table 3. Average milk composition (%) of well-known dairy sheep breeds of Europe

Sheep breeds of different country of Europe	Fat	Protein	Total Solids	Ash	Lactose
Lacaune(France)	7.40	5.63	18.63	0.93	4.67
East Friesian (Germany)	6.50	5.25	17.00	0.90	4.90
Sarda (Italy)	6.99	5.60	18.14	0.95	4.60
Awassi (Turkey)	6.61	5.74	18.24	0.93	4.96
Chios (Greece)	7.90	6.20	19.08	0.92	4.06
Manchega (Spain)	7.78	6.01	18.98	0.90	4.29

Source: Wendorff and Haenlein, 2017

Table 4. Major peptide fragments with sequence and biological activity

Peptide	Fragment Sequence	Biological activity
αs1-CN f(86-92)	VPSERYL	ACE-inhibitory
αs1-CN f(102-109)	KKYNVPQL	ACE-inhibitory
αs2-CN f(165-170)	LKKISQ	Antibacterial
αs2-CN f(165-181)	LKKISQYYQKFAWPQYL	AntibacterialOvine
αs2-CN f(184-208)	VDQHQAMKPWTQPKTKAIPYVRYL	Antibacterial
αs2-CN f(202-204)	IPY	ACE-inhibitory
αs2-CN f(203-208)	PYVRYL	Antibacterial
αs2-CN f(205-208)	VRYL	ACE-inhibitory
β-CN f(47-51)	DKIHP	ACE-inhibitory
β-CN f(58-68)	LVYPFTGPIPN	ACE-inhibitory
κ-CN f(106-111)	MAIPPK	ACE-inhibitory
κ-CN f(106-112)	MAIPPKK	ACE inhibitory
κ-CN f(112-116)	KDQDK	Antithrombotic
Lactoferrin f(17-41)	ATKCFQWQRNMRKVRGPPVSCIKRD	Antibacterial

Source: Park and Nam, 2015

Table 5. Mineral and vitamin contents of cow, goat, and sheep milk

Parameter	Cow milk	Goat milk	Sheep milk
Calcium (mg/100 g)	112.0±14.5	130±4.0	197.5±2.5
Iron (mg/100 g)	0.1 ±0.1	0.06±0.0	0.1±0.0
Magnesium (mg/100 g)	11.0±0.5	14.5±1.5	019.5±3.
Phosphorous (mg/100 g)	91.0±5.5	109±12.0	141.0±1.7
Potassium (mg/100 g)	145.0±11.5	185.5±4.5	138.0±2.0
Copper (mg/100 g)	Trace	0.04±0.0	0.1±0.0
Sodium (mg/100 g)	42.0±6.5	39.5±1.5	39.0±7.0
Zinc (mg/100 g)	0.4±0.0	0.43±0.1	0.6±0.1
Selenium (µg/100 g)	1.8±1.3	1.665±0.4	1.7±1.0
Manganese (µg/100 g)	6.0±0.0	8.0±0.0	7.15±1.8
Retinol (µg/100 g)	35.0±8.0	0.04±0.0	64.0±19.5
Vitamin A (µgRE/100g)	37.0±8.0	54.32±0.0	64.0±5.5
Vitamin E (mg/100 g)	0.08±0.01	0.04±0.0	0.11±0.01
Thiamin (mg/100 g)	0.04±0.01	0.059±0.0	0.07±0.01
Riboflavin (mg/100 g)	0.2±0.01	0.175±0.0	0.3±0.02
Niacin (mg/100 g)	0.13±0.05	0.235±0.0	0.41±0.05
Pantothenic acid (mg/100 g)	0.43±0.12	0.31±0.0	0.43±0.02
Vitamin B6(mg/100 g)	0.04 ±0.01	0.048±0.0	0.07±0.01
Folate (µg/100 g)	8.5±1.5	1.0±0.0	6.0±0.06
Biotin (µg/100 g)	2.0±0.5	1.75±0.3	2.5±0.0
Vitamin B12(µg/100 g)	0.5±0.3	0.065±0.0	0.66±0.05
Vitamin C (mg/100 g)	1.0±0.5	1.295±0.0	4.6±0.4
Vitamin D (µg/100g)	0.2±0.1	0.15±0.1	0.2±0.0

Source: Park *et al.,* (2007), Raynal-Ljutovac *et al.,* (2008), Wijesinha-Bettoni and Burlingame (2013).

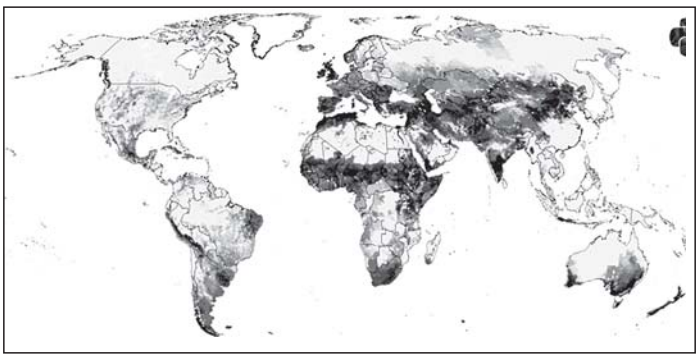

Fig 1. Distribution of Sheep across Globe
Source : Gilbert *et al.,* 2018

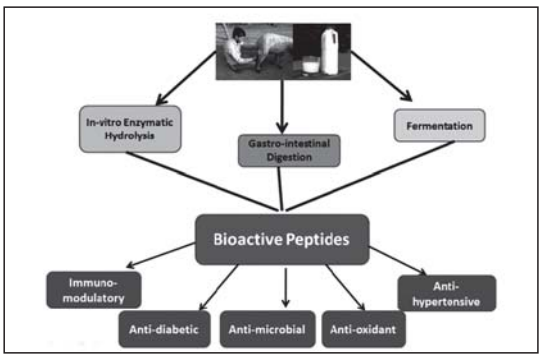

Fig 2. Bioactive peptides from sheep milk

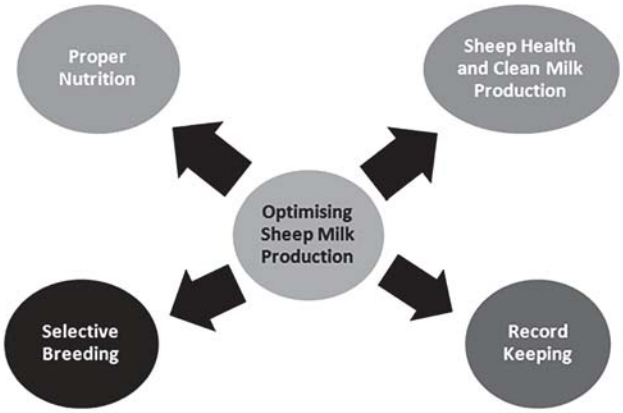

Fig 3. Strategic Pillars for Optimization of Sheep Milk Production

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CHANGES IN TESTICULAR BIOMETRY, STEROID HORMONES AND RECEPTOR EXPRESSION IN THE PERIPUBERTAL PERIOD OF INDIGENOUS TENYI-VO MALE PIGS OF NORTH-EASTERN HIMALAYAN REGION IN INDIA

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ABSTRACT

The present study was conducted to characterize the testicular changes in the peripubertal period in Tenyi-vo, a miniature-sized pig from the North-eastern Himalayan (NEH) region of India. A total of twenty-four male pigs were randomly selected and categorized for castration at different age groups (n=6/group), G1 (Days 30-45), G2 (Days 60-65), G3 (Days 80-100) and G4 (Days 150-160). Paired testes and epididymis were used for the assessment of biometry, caudaepididymal spermiogram, testicular histology and relative expression of the androgen receptor (AR), estrogen receptors (ERα and ERβ), aromatase (CYP19A1), and insulin-like growth factor-1β receptor (IGF-1R) in qPCR. Plasma testosterone (T), estradiol (E_2), tri-iodothyronine (T_3), thyroxine (T_4), and cortisol concentrations were estimated on the day of castration in each group of males using commercial ELISA kits. In pigs of G2, a greater testicular weight, volume, and epididymis weight were observed relative to G1 ($p<0.001$). The presence of live spermatozoa at 1240.9±304.2×10^6/mL concentration with 0.65% proximal droplets was recorded as early as day 60. The concentration of T increased steadily from G1 to G4, and a significantly higher ($p<0.05$) concentration was observed in G4 relative to the other categories. Among the transcripts analyzed in the testis, the relative fold change of AR was 10.8 fold in G2, which was subsequently reduced in G3 (4.87 fold) and then down-regulated in G4 (0.63 fold, $p<0.05$). CYP19A1 was abundantly expressed in the testis, and the fold change ranged from 41-54 fold, although it did not differ significantly from 60-150 days of age. Further, the presence of well-developed seminiferous tubules was evident in the Tenyi-vo male from day 60 onward, with a body weight as low as 4.28 kg. The study concluded that the male of the Tenyi-vo pig attained puberty at the earliest age of 60 days.

Keywords: Tenyi-vo pig, sexual maturity, Testosterone, puberty, testis

INTRODUCTION

Early onset of puberty is an economic attribute in the swine production system, as it ensures lower spending on extra boar care and improves reproductive life [27]. A miniature indigenous breed called 'Tenyi-vo' (Accession no. INDIA_PIG_1400_ TENYIVO_09004, National Bureau of Animal Genetic Resource, India) is raised in the backyard by the tribes of Nagaland and Manipur State in the North-East Himalayan (NEH) region of India. With broad pointed heads and relatively short legs, they are black in colour and known for premium meat quality [43]. In the smallholder pig production system prevalent in NEH India, the indigenous pigs are well known for adaptability to harsh environments and thriftiness with negligible inputs [28,29]. The indigenous pig population is decreasing, and attempts are made to conserve the germplasm [11,44].

It is difficult to ascertain the exact age of attainment of puberty, even though it can be determined based on the presence of functional sperm in the ejaculate or cauda epididymis and the observation of sexual desire [3]. Breeds used in the pig industry show initiation of

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spermatogenesis at around 115 days of age, exhibit synchronized sexual activity and first ejaculation with mature semen characteristics at six months of age [6,14,38]. Early sexual maturity was described previously in Chinese Meishan pigs at 12-weeks of age [18,24,37]. The presence of spermatozoa in the ejaculate and the ability to breed the females at 90 days of age with a mere body weight of 3.0 kg was identified in the Naga local pig [26]. In another study, the early onset of sexual maturity and impregnating ability of local boars in Northeast India was reported at 108 days based on testicular growth and pregnancy [28] compared with the reported age of 5 to 8 months in commercial breeds [20].

Testosterone (T) is necessary for the onset of sexual maturity, and peripheral T concentrations have been well associated with testicular growth, sperm production and onset of puberty [7,42]. During puberty, T concentration increased [24] and stayed at a plateau afterwards unless they were sexually stimulated. Apart from T, estradiol (E2) and its α-receptor (ERα) are important for regulating normal male fertility [19]. ERα controls the reabsorption of fluids in efferent ductules and raises the concentration of sperm prior to entry into the epididymis [22]. In adult testis, Leydig cells express CYP19A1 and successfully synthesize E2 at a far higher rate than that seen in the adult Sertoli cells [12,13,32,33]. The concentration of E2 in the peripheral blood varied in males, depending on the species [10,15,39]. Recently, transcripts of ER and androgen hormone receptor (AR) and CYP19A1 enzymes in the testicular and epididymal tissues have been investigated to explain the capacity for spermatogenesis and steroidogenesis of sexually mature boars [4,40,45]. However, information on steroid hormones and expression of their receptors in relation to peripubertal changes in Tenyi-vo boar is scanty. The present work documents the gross and microscopical changes of the testis, epididymis, cauda epididymal spermiogram, profile of thyroid and steroid hormones and relative expression of genes related to sex steroid receptors and enzymes associated with the testis of Tenyi-vo pigs to characterize the attainment of early puberty for spermatogenesis and steroidogenesis of sexually mature boars [4,40,45]. However, information on steroid hormones and expression of their receptors in relation to peripubertal changes in Tenyi-vo boar is scanty. The present work documents the gross and microscopical changes of the testis, epididymis, cauda epididymal spermiogram, profile of thyroid and steroid hormones and relative expression of genes related to sex steroid receptors and enzymes associated with the testis of Tenyi-vo pigs to characterize the attainment of early puberty.

MATERIAL AND METHODS

Study Location

The present study was conducted at the Mega Seed Project on Pig Unit, Indian Council of Agricultural Research (ICAR), Research Complex for North-Eastern Hill Region, Nagaland Centre, Medziphema, located at 25°45'24" N, 93°50'26" E of the Eastern Himalayan subtropical agro-climatic zone. The maximum air temperature ranged from 19.7°C to 37.5°C, and the minimum air temperature ranged from 6.1°C to 27°C, respectively. The area has a humid to rainy climate and receives heavy rainfall of approximately 1800-2500 mm per year.

Animals and Management

The experiment was conducted on Tenyi-vo male pig (n=24) reared under an intensive management system (Fig 1.). The breeding stock of Tenyi-vo pig was procured from the native tract and maintained in an isomanagerial condition with a commercial concentrate ration. Male piglets were selected at the age of four weeks and maintained with the dam till weaning at 45 days of age. At post-weaning, group feeding of grower ration was practiced with water ad libitum. The experiment was performed in compliance with the approved guidelines of the Institute Animal Ethics Committee, ICAR Research Complex for NEH region, Umiam.

Collection of Testes

Tenyi-vo male pigs were divided into four groups (n=6/group), viz., G1 (30-45 days), G2 (60-65 days), G3 (80-100 days) and G4 (150-160 days). The body weight of Tenyi-vo boar at birth and weaning was 0.57±0.03 Kg and 3.81±0.21 Kg, respectively. Testes were collected by open surgical castration under lignocaine local anesthesia and were transported to the laboratory in a sterile thermal flask. After washing with phosphate buffer saline (PBS, pH 7.4), the epididymis was dissected. The weight and volume of the testis and the weight of the epididymis were recorded. A piece of testicular tissue was preserved in 10% neutral buffer formalin saline for histology;

another piece of tissue weighing about 100 mg was collected in RNAlater (Qiagen, GmbH, Germany) and stored at -20°C for total RNA extraction. On the day of castration, body weight was recorded, and blood was collected in heparinized vials to assay for steroid (T and E2) and thyroid (T3 and T4) and cortisol hormones.

CAUDA EPIDIDYMAL SPERMIOGRAM

The cauda epididymis of each testis was incised, and the seminal fluid was aspirated into a micro-centrifuge tube. In the absence of free fluid in the cauda, the lumen was flushed with a few drops of PBS and the fluid was allowed to drain into the micro-centrifuge tube under gravity. The sperm concentration was estimated by measuring the absorbance of a diluted semen sample (2.9% sodium citrate buffer as diluent) at 650 nm in a spectrophotometer (Shimadzu, Japan) using the following formula: [concentration = (Dilution Factor) × (21.39 × (Absorbance) - 1.09)]. The viability and morphological abnormality were measured by eosin-nigrosin staining methods [25].

Histology of the Testis

Preserved tissue samples were trimmed and dehydrated by treatment with increasing alcohol concentration, followed by clearing with acetone and benzene. The tissues were embedded in paraffin, and the serial sections of five µm thickness were cut through a microtome. In the end, tissue sections were stained with hematoxylin and eosin and examined under a light microscope.

Hormonal Profile

Plasma T (Cat #AA-E1300), E2 (Cat # FR E-2000), T3 (Cat # TF E-2100), T4 (Cat # TF E-2200) and cortisol (Cat # MS E 5000) concentrations were assayed using commercial ELISA kits (LDN Diagnostica, Germany). The hormone concentration of the test samples was determined using four-parameter logistic equations derived from the standard curve in the Multiskan ELISA reader (Thermo Scientific Inc., USA). The sensitivity, intra-assay and inter-assay coefficient of variation of T estimation was 0.15 ng/ml, 3.6% and 7.1%, respectively. The sensitivity, intra-assay and inter-assay coefficient of variation of E2 estimation was 10.6 pg/mL, 8.97% and 10.9%, respectively. The sensitivity, intra-assay and inter-assay coefficient of variation of T3 estimation was <0.1 ng/mL, 5.58% and 6.11%, respectively. The sensitivity, intra-assay and inter-assay coefficient of variation of T4 estimation was 6.2 ng/mL, 4.1% and 6.1%, respectively. The sensitivity, intra-assay and inter-assay coefficient of variation of cortisol estimation was 7.74 ng/mL, 5.6% and 6.96%, respectively.

Differential Gene Expression Profile in Testicular Tissues

Total RNA was extracted using the RNA isolation kit (Cat # KT151A, RaflexTMGenei, India), and the concentration and purity of RNA were calculated using a micro-cuvet in a UV-VIS spectrophotometer (Shimadzu, Japan). RNA samples having A260/280 between 1.8 to 2.0 were considered for cDNA synthesis. DNase I (1U/µg of RNA, Cat # EN0521, Thermo-Fischer Scientific Inc., USA) treatment was performed to eliminate gDNA from the total RNA extracted. cDNA was synthesized using Revert Aid M-MuLV reverse transcriptase (Cat # EP0442, Thermo Scientific, USA) and oligo(dT)18 primers (Cat # SO132, Thermo Scientific, USA). A reverse transcriptase control was run in parallel to validate the effectiveness of the DNase I during cDNA synthesis. The primers for AR, CYP19A1, ERα and ERβ, IGF-1R, and endogenous control gene (GAPDH) were designed using the Integrated DNA Technology primer quest tool and custom synthesized (Europhin, India, Table 1.). The PCR cyclic condition of the target genes was optimized using Taq PCR Master Mix (Cat # K0171, Thermo-Fischer Scientific Inc, USA) in a gradient thermal cycler (Nexus gradient Master cycler, Eppendorf, Germany), and the amplicon size was verified by 1.5 per cent (w/v) agarose gel electrophoresis.

Each target gene was amplified using Maxima SYBR Green qPCR master mix (Cat# K0251, Fermentas, Thermo Scientific, USA) in a real-time qPCR system (Pico Real 96, Thermo Scientific, USA). cDNA 10 ng (1 µL) templates were added to 0.2 µL forward and reverse primers (10 pmol each) and 5 µL 2X SYBR Green Master Mix at a final volume of 10 µL. The thermal cyclic conditions included hot start denaturation at 95°C for 15 min, 40 cycles of three segmented amplification and quantification programmes (denaturation at 95°C for 15 s, annealing at a specific temperature for 30 s, an extension at 72°C for 30 s and a melting stage by heating from 55 to 95°C at a rate 1°C/s with the acquisition of fluorescent data. No template control was used as a negative control in the qPCR reaction. GAPDH was used

as an endogenous control to generate ΔCt value of the target genes, because its expression was consistent across the age group. The intra-assay coefficient of variation of each target gene in the testis was less than 3%. Gr.1 pigs served as calibrators to generate ΔΔCt, and the fold change of each gene was determined by the 2-ΔΔCt method [34].

Statistical Analysis

The variables such as testicular weight, volume, epididymal weight, cauda epididymal spermiogram and hormone concentration were analyzed by One-way ANOVA with Tukey's post-hoc test. ΔCt values of each target gene in G1, G2, G3 and G4 were analyzed by a non-parametric Kruskal-Wallis test with Dunn's post-hoc test. Significance was set at 95% and the results are presented as mean ±SEM. SPSS 16.0 was used for data analysis, and GraphPad Prism 6.0 was used for constructing the bar diagrams.

RESULTS

Body Weight and Testicular Biometry

The age-related biometrical changes of the testes and epididymis of Tenyi-vo pig are presented in Table 2. The body weight of G4 males was significantly heavier (6.280.47 kg) than the other groups ranging from 3.370.27 to 4.350.24 kg, on the day of castration ($P<0.001$). In contrast, the weight and volume of the testis were comparable among G2 to G4, but significantly heavier than those of G1 (Fig 2.). The ratio of testis weight to body weight was significantly greater in G3 as compared to G1 ($p<0.05$). The epididymal weight of G4 was significantly heavier ($p<0.0001$) than that of G1 and G2.

Cauda Epididymal Spermiogram

The result of the cauda epididymal spermiogram is presented in Table 3. Seminal fluid accumulation was evident in the cauda epididymis of G2 Tenyi-vo pig from 60 days onwards, while only one male in G1 also showed deposition of fluid with the presence of sperm as early as day 45. The amount of fluid in G4 was highest. Even though the sperm concentration varied from 1241×10^6 in G1 to 2109×10^6 in G4, the difference was not statistically significant. The proportion of live spermatozoa gradually increased with age, and abnormal spermatozoa counts, primarily spermatozoa with a distal droplet, gradually decreased in G2 to G4. The proportion of proximal droplets was up to 2% in various groups.

Hormone Profile

Changes in the hormone concentrations during the peripubertal period of Tenyi-vo boars are presented in Figure 2. The concentration of plasma T increased from G1 to G4; however, the T concentration was comparable among G1 to G3. The concentration of T was significantly higher in G4 as compared to other groups ($P<0.05$). The concentration of E2 ranged from 1.5 to 2.56 ng/ml and was comparable across the age groups. The ratio of T:E2 also remained at similar levels in G1 to G3 (from 7.09 to 8.34); however, G4 showed a significantly higher ratio as compared to other groups. The concentration of T3 remained at a significantly low level ($P<0.05$) in G1, G2 and G4 relative to G3, while the concentration of T4 was higher in G1 to G3 than that of G4. In addition, T4:T3 was significantly higher in G1 and G2 than in the other groups. Like T3, the concentration of cortisol in G3 was significantly higher than that of the other groups (Fig 3.).

Testicular Histology

The presence of differentiated seminiferous tubules with sperm cells was observed in the pre-weaning Tenyi-vo pigs of G1 (Fig 4a.). With age, the compactness of seminiferous tubules and clusters of interstitial cells increased in G2 to G4. In G3 and G4, the seminiferous tubules were more compact and rounder in shape, consisting of a central lumen with elongated spermatozoa and densely packed interstitial cells between the seminiferous tubules (Fig 4b-d.).

Expression of Steroid Hormone Receptors in the Testis

The relative expression of AR, ERα, ERβ, CYP19A1 and IGF1R in the testis of Tenyi-vo boar is presented in Fig 5. The AR expression was significantly upregulated with a relative fold change of 10.8 fold in G2 ($P<0.05$); however, the shift in fold change was reduced in G3 (4.87 fold) and subsequently downregulated in G4 (0.63 fold). The expression of CYP19A1 was most abundant in G2 to G4, ranging from 41 to 54 fold. Similarly, the expression of ERα was up-regulated in the testis in the age group, G2 to G4; however, the fold change was the highest in G4 ($p<0.05$). Similarly, the expression of ERβ appeared to be substantially higher in G4. The expression of IGF-1R transcripts tended to be upregulated in G2 and G3; however, it was moderately downregulated in G4.

DISCUSSION

The study documents the temporo-spatial changes in the testis, epididymis, hormones and their receptors during the peripubertal period in Tenyi-vo male pigs. The peripubertal changes in the testis and epididymis of Tenyi-vo boar showed that this pig breed had a greater degree of testicular weight and volume, and epididymal weight at day 60. The body weight gain was, however, non-significant during the first three months of age. The weight of male piglets of this breed ranged from 300-500 g at birth to 2.5-3.0 kg at 3.0 months of age [26] and from 9 to 32 kg at adult age [8]. The body weight gain remains unchanged during the peripubertal period, attaining a mere 6.28 kg at the age of five months. Higher testicular weight found in the present study at the young age of 60-day-old Tenyi-vo male indicates possible early sexual maturity [28]. The ratio of testis weight to body weight (g/kg) increased steadily from 30 days, reached a significantly higher level at three months of age, and subsequently decreased at around five months of age. Our finding is consistent with the earlier studies describing a significantly higher testis to body weight ratio in local pigs compared to the commercial European breeds [28]. Early attainment of puberty and sexual maturity of males was also due to faster testicular growth of indigenous pigs compared to Hampshire and Large White Yorkshire [48]. In addition, the change in epididymis weight with respect to the body weight and testicular weight also showed a positive increment; however, a distinct surge was evident from 35 to 62 days. Similarly, at the same time, the ratio of epididymis to testis weight was significantly increased, which later remained static up to three months of age, although there was a significant change in the ratio at day 150. A similar trend in higher growth rates of testis and epididymis over 4 to 6 months of age than 2 to 4 months of age in the indigenous pig as compared to the crossbred[51].

In the present study, the presence of live sperm was evident in the cauda epididymis of a Tenyi-vo boar even at day 45, but it was more distinct at 60 days, where most of the boars had at least 0.5 ml of cauda epididymal fluid consisting of sperm $>1200\times10^6$/ml with live count >60 per cent and 2% of spermatozoa with proximal droplets. The spermatozoa concentration remained unchanged between days 80 to 150, while a significant rise in live per cent and decrease in distal droplets was evident at 150 days of age. This result is consistent with the earlier reports [18,36] that the presence of sperm in the lumen of the seminiferous tubules of Chinese Meishan pigs was discernible as early as 60 days and in the cauda epididymis at around 70 days of age. Testicular size and weight are strongly associated with sperm production and total sperm reserves [35,47] and testicular size is highly heritable in pigs [21]. The evolution of the fertilizing ability of spermatozoa has been correlated with changes in progressive motility, alteration of metabolic patterns and structural status of tail organelles, changes in nuclear chromatin, changes in the nature of plasma membrane surface and movement of cytoplasmic droplets [26]. The greater testicular weight and volume, along with the presence of acceptable live count and permissible abnormality in the cauda epididymal spermiogram, support the pubertal changes of Tenyi-vo boars by 60 days of age.

Seminiferous tubules and sperm cells existence was observed in Tenyi-vo boars at the early age of day 35 in G1. The more compact seminiferous tubules, consisting of a central lumen accumulating spermatozoa and densely packed interstitial cells between the seminiferous tubules, are commonly visible in the boar on day 60. This finding supports the characteristics of histological changes that suggest the attainment of puberty [5]. Sexual precocity in male miniature pigs was previously reported [23], which is characterized by early differentiation of gonocytes as well as active development and proliferation of seminiferous Sertoli cells, which shortened the early stage of spermatogenesis. The increase in seminiferous tubule diameter during the peripubertal period suggests the ensuing testicular maturation as the diameter of seminiferous tubule, number of seminiferous Sertoli cells and tubular fluid volume increase [2,16,25].

Testicular development, initiation of puberty, sexual maturity, and sperm production are intricately associated with the production of testicular steroid hormones [7,42]. The increased concentration of plasma T found in Tenyi-vo boar from 35 to 150 days of age strongly supports the developmental changes in the testis during the process of sexual maturation. The predominant types of circulating androgens differ considerably with age as T becomes more predominant during sexual maturity [49]. T levels recorded in boars showed large variations in age, breed

and season, ranging from 0.73 ng/mL to about 50 ng/mL [7,42]. The AR expression was upregulated on day 60, but the fold change decreased on day 90 and was subsequently downregulated on day 150. Significantly higher testicular AR gene expression at a lower age may be due to early attainment of puberty as seen in spermiogram, testicular histology and steroid hormone profile in Tenyi-vo boar. Age-related progressive decline in testicular AR gene expression could not be explained convincingly.

The concentration of plasma E2 in Tenyi-vo males ranged between 1.5 to 2.6 ng/mL across the age groups. The E2 hormone also plays an important role not only in the control of male sexual activity and Sertoli cell function, but essential for normal fertility [19]. This new paradigm function of E2 in males started with the discovery that testicular germ cells and epididymal sperm express CYP19A1 and synthesize E2 [41]. The expression of CYP19A1 in the Tenyi-vo boar testis was most abundant, ranging from 41 to 54–fold across the age groups from day 60 to 150 in our study. Early studies recorded that the primary source of E2 in the immature male was the Sertoli cell [50]; however, in the adult testis, Leydig cells produce CYP19A1 and actively synthesize E2 at a much higher rate than that seen in the adult Sertoli cell [12,13,33]. The expression of ERα was up-regulated in the testis regardless of age group, although the fold change was not significant between days 60 to 150. In comparison, the expression of ERβ appeared to be significantly higher on day 150. In a recent study using in-situ hybridization and UV-single cell microdissection, ERα mRNA has been shown in the spermatogonia up to mid-pachytene primary spermatocytes of the seminiferous epithelial cycle and ERβ mRNA in the Sertoli cells. Leydig cells, however, did not show either ERα or ERβ mRNA expression. Thus, a direct effect of E2 on the Sertoli cell function through ERβ and germ cell formation via ERα was speculated in the boar testis [31]. Expression of ERα and ERβ and CYP19A1 in immature and mature boars' testis has also been documented [40].

Despite the high concentration of steroid hormones and cortisol, the lower body weight during the peripubertal phase suggests the possible involvement of the metabolic hormones in the Tenyi-vo boar. Insulin and insulin-like growth factors (IGF-1) are polypeptides that regulate growth, differentiation and survival in a wide range of cells and tissues. The effect of IGF1-IGF1R on reproductive parameters, specifically the release of gonadotropin and interactions between the IGF system and other effectors of gonadotropin release, is described [30]. In Tenyi-vo boar, the non-significant modulation of IGF-1R expression may be correlated with the poor physical growth of the Tenyi-vo breed observed in the present study. Thyroid hormones play a significant role in the regulation of the basal metabolic rate. The thyroid gland contributes to the regulation of the growth, development, adaptation and productivity of farm animals [1,46]. Knowledge on the thyroid profile in the regulation of basal metabolic rate in the Tenyi-vo boar is scanty. The concentration of T3 remained at a significantly low level between days 35 to 60 as compared to day 90, while the concentration of T4 was high on days 35 to 90 as compared to day 150. The lower growth potential in Tenyi-vo boar could be associated with reduced concentration of plasma T3 and a higher T4:T3 ratio. The level of thyroid hormone synthesis, which controls the rate and the direction of metabolic events, determines their physiological equilibrium [9]. In addition, abruptly higher sex steroid levels in males may also be associated with a slow growth rate, supporting the findings in the Tenyi-vo boar [17].

CONCLUSION

It is concluded that Tenyi-vo male pigs attained puberty at the earliest by 60 days of age with a body weight of around 4 kg. Further research on the genetic constituents of this particular breeds are required for its conservation and utilization in the commercial pig breeding program.

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CONFLICT OF INTEREST

There is no conflict of interest to declare.

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STATEMENT OF ANIMAL RIGHTS

The study was conducted following the approved guidelines of the Institutional Animal Ethics Committee (IAEC), ICAR Research Complex for NEH Region, Umiam, Meghalaya.

Table 1. Primers used in qPCR for amplification of different target genes

Target gene	Oligonucleotide sequences (5'>>>>>3')$	Amplicon size (bp) $$	Accession No.
AR	For: AACAGCAGCCTTCACAACAG Rev: TTAAGATCGGTGGAGCAGCT	208	AB052938
IGF-1βR	For: GATCAGCGGGAATGTGTGTC Rev: ACTGGTAGGGCGATGATCAG	203	U58370
Aromatase	For: TTAGCAAGTCCTCAAGTGTG Rev: CCAGGAAGAGGTTGTTAGAG	324	U37311
ERα	For: TTGTGTGCCTCAAATCCATC Rev: GACAGGATGAGGAGGAGCTG	202	AF035775
ERβ	For: CATGATGATGTCCCTGACCA Rev: GGTCTGGAGCAAAGATGAGC	200	AF267736
GAPDH	For: CGTCAAGCTCATTTCCTGGT Rev: AGTCAGGAGATGCTCGGTGT	201	NM_001206359.1

$The length of each primer was 20 bp. $$ Annealing temperature was 54 °C.

Table 2. Changes in the biometry of testis and epididymis (mean ± SE) of Tenyi-vo pigs during the peripubertal period†

Attribute	G1‡	G2	G3	G4	P value
Average age at castration (days)	35±3.16[a] (30-45)	62.67±0.92[b] (60-65)	87.90±3.09[c] (80-100)	158.67±2.11[d] (150-160)	<0.001
Body weight at castration (Kg)	3.37±0.27[a]	4.28±0.24[a]	4.35±0.24[a]	6.28±0.47[b]	<0.001
Testis weight (g)	7.76±1.01[a]	14.79±1.13[b]	16.2±1.55[b]	17.96±1.34[b]	0.0002
Testis volume (cm^3)	15.45±2.03[a]	32.85±3.31[b]	41.46±4.82[b]	39.55±1.67[b]	0.0005
Epididymis weight (g)	1.33±0.21[a]	3.65±0.14[b]	4.10±0.24[b]	7.15±0.57[c]	<0.001
Testis weight/body weight (g/kg)	2.28±0.20[a]	3.57±0.48[ab]	3.83±0.42[b]	2.93±0.32[ab]	0.035
Epididymis wt/body weight (g/kg)	0.385±0.03[a]	0.869±0.07[b]	0.958±0.06[bc]	1.137±0.03[c]	0.0001
Epididymis/testis weight	0.17±0.01[a]	0.251±0.01[b]	0.268±0.02[b]	0.405±0.03[c]	<0.001

†Average birth weight 0.57±0.03 Kg and the weaning weight 3.81±0.21 ‡
Sample size n=6/group; Figures with different superscripts across each row differ significantly.

Table 3. Cauda epididymal spermiogram (mean±SE) of Tenyi-vo pig during the peripubertal period†

Attribute	G2‡ (n=6)	G3 (n=6)	G4 (n=6)	P value
Caudaepididymal fluid volume (mL)	0.46±0.02[a]	0.53±0.06[a]	0.833±0.06[b]	0.001
Spermatozoa concentration ($\times 10^6$)	1240.9±304.2	1653.4±320.8	2109.6±55.8	0.138
Live spermatozoa (%)	63.8±3.87[a]	67.4±3.72[a]	86.5±1.92[b]	0.001
Sperm with proximal droplet (%)	0.65±0.06	2.0±0.95	0.8±0.23	0.303
Distal droplet (%)	17.9±2.49[a]	14.6±2.04[ab]	10.8±0.38[b]	0.041

†In G1, only one boar showed the presence of spermatozoa and cauda epididymal fluid, hence not included in statistical analysis. ‡Sample size n=6/group; Figures with different superscripts across each row differ significantly.

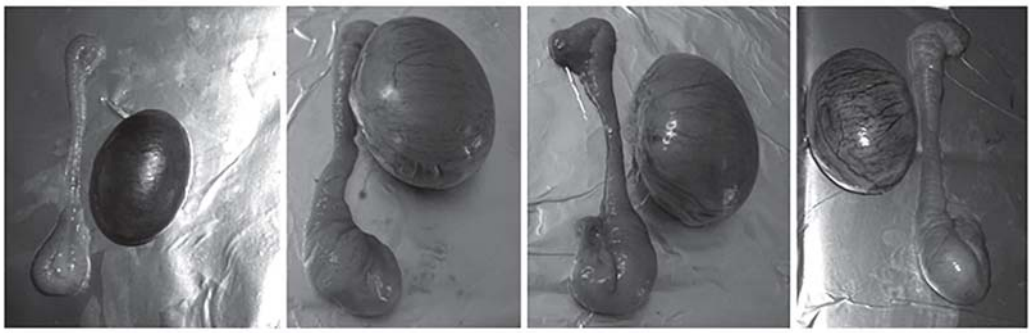

Fig 2. Representative gross photograph of testis and epididymis of Tenyi-vo pig castrated around the peripubertal period

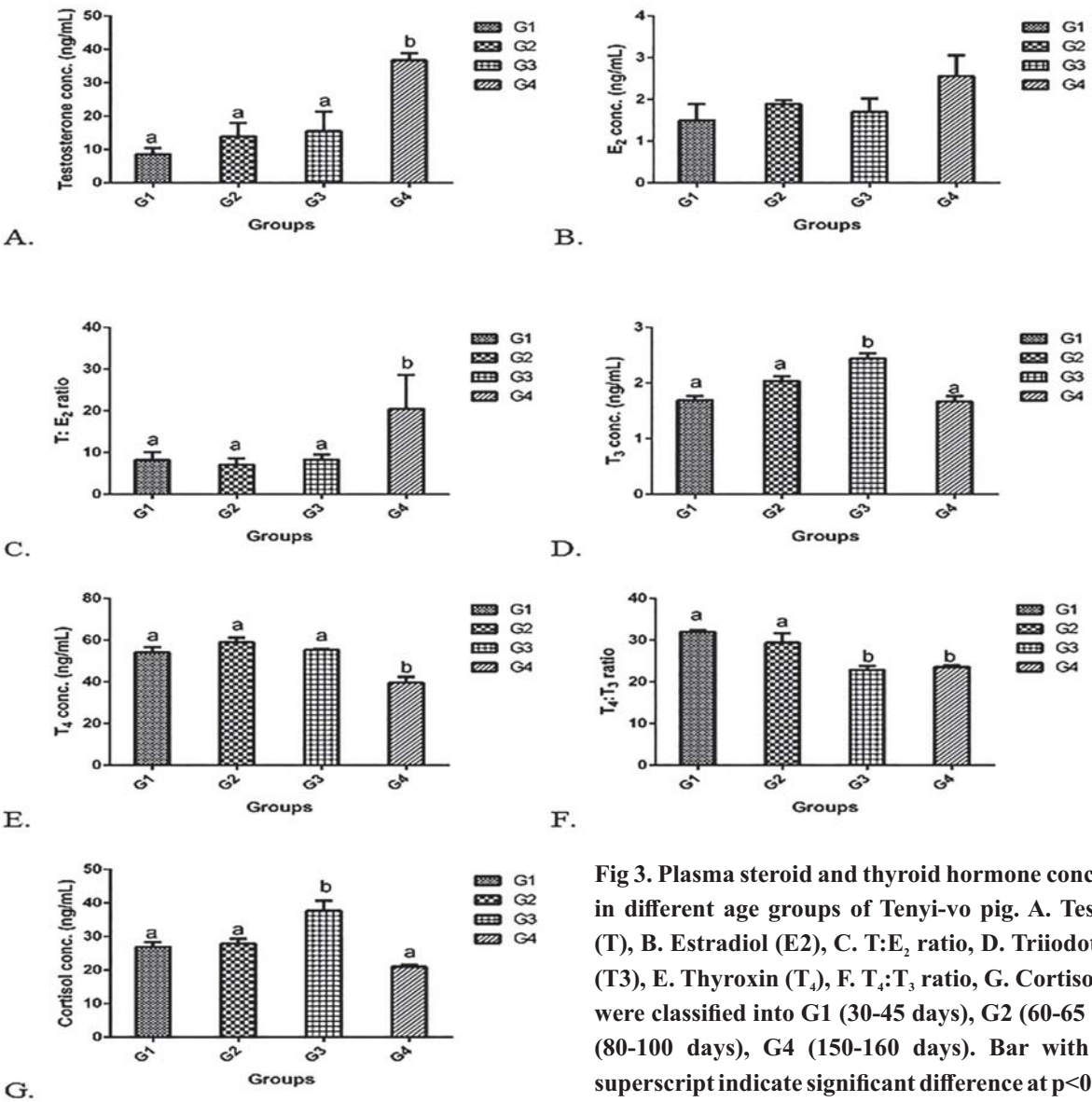

Fig 3. Plasma steroid and thyroid hormone concentration in different age groups of Tenyi-vo pig. A. Testosterone (T), B. Estradiol (E2), C. T:E_2 ratio, D. Triiodothyronine (T3), E. Thyroxin (T_4), F. T_4:T_3 ratio, G. Cortisol. Groups were classified into G1 (30-45 days), G2 (60-65 days), G3 (80-100 days), G4 (150-160 days). Bar with different superscript indicate significant difference at $p<0.05$.

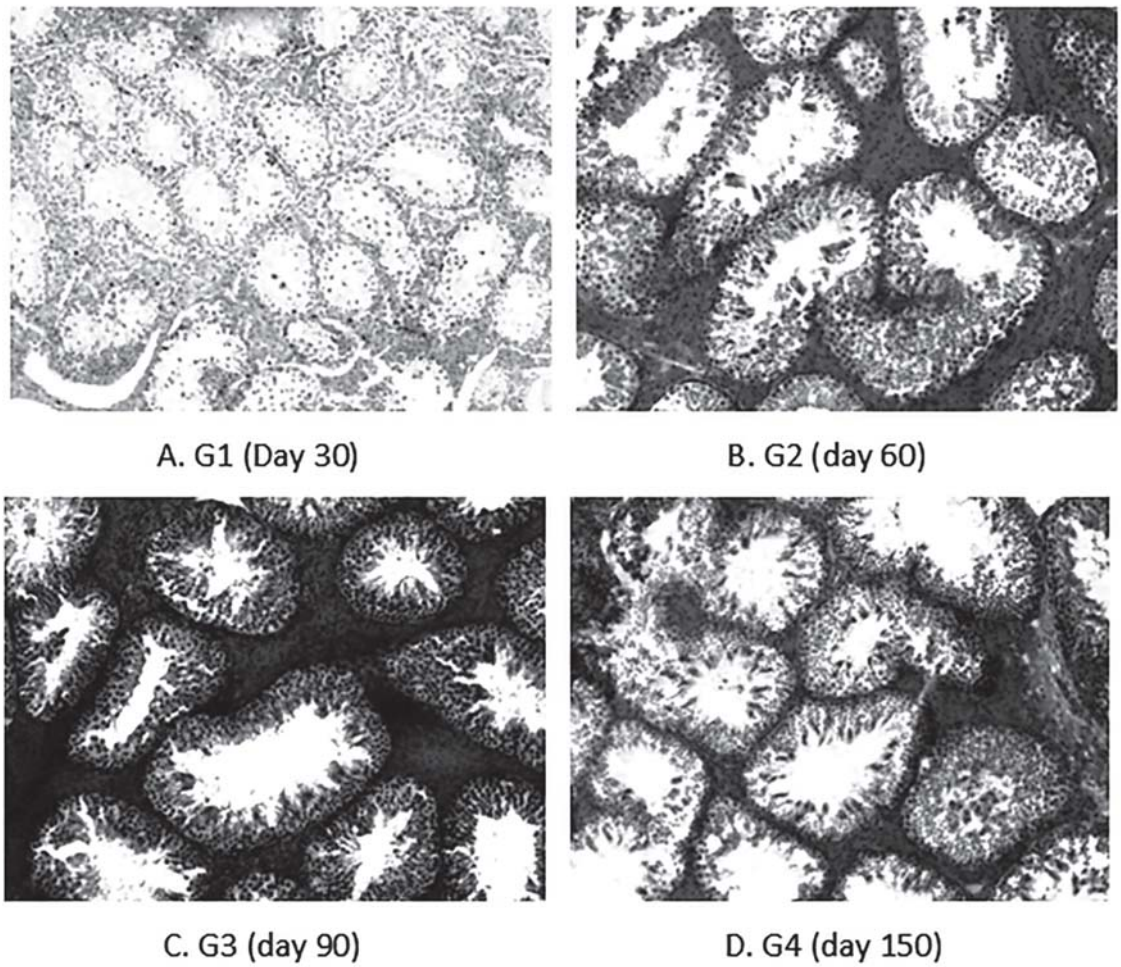

Fig 4. Representative photomicrographs of testis of Tenyi-vo pig collected around peripubertal window. Note the apparent increase in the stratification of seminiferous tubules in G2-G4 with decrease in luminal diameter along with well-defined islets of interstitial spaces with Leydig cells between the cross sections of seminiferous tubules. (Magnification 20X)

Fig 1. Tenyi-vo pigs maintained under intensive production system. A. Tenyi-vo sow with newborn piglets. B. Two months old Tenyi-vo male pigs after weaning

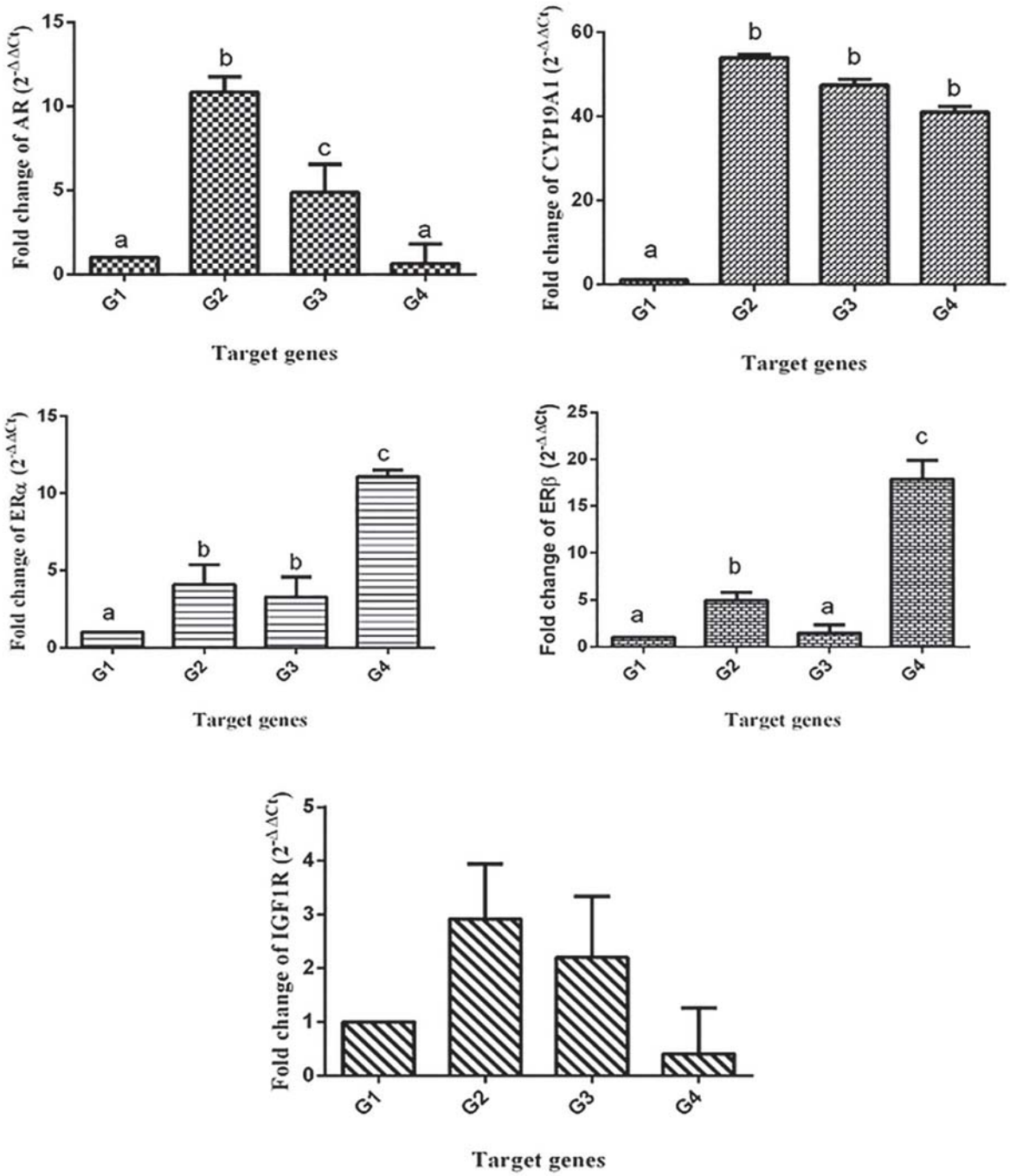

Fig 5. Relative-fold change ($2^{-\Delta\Delta Ct}$) of transcripts in the testicular tissues of Tenyi-vo male pigs during peripubertal period. A. androgen receptor (AR), B. aromatase (CYP19A1), C. Estradiol receptor α (ERα), D. Estradiol receptor β (ERβ), E. Insulin like growth factor-receptor (IGF1R). G1 served as control (calibrator group) for calculation of fold change and GAPDH was used as endogenous control. Statistical analysis was done with the Δct values. Error bar indicates standard error of difference (SED). SED = square root {(Standard deviation of gene of interest^2/n_1) + (Standard deviation of gene of reference^2/n_2)}, Where n_1 and n_2 are the number of observations. Bar with different superscript indicate significant difference at P<0.05.

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Research Article

IMPORTANCE OF EXTRACELLULAR MATRIX IN THE DEVELOPMENT OF A THREE-DIMENSIONAL BUBALINE ENDOMETRIAL STROMAL CELL CULTURE SYSTEM

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ABSTRACT

The aim of the present study was to evaluate different type of matrices on the growth and development of bubaline endometrial stromal cells (BESC) in-vitro and to select the best suited matrix. Endometrial stromal cells were isolated by double enzymatic digestion method and seeded at the density of 2×10^5 cells per well in 24-well plates. The plates were pre-coated with commercially available extracellular matrices (ECM) namely Purecol (PC), Geltrex (GT), Gelatin (GL) and Maxgel (MX). After isolation, BESC were either single or in small clumps, glistening and rounded under inverted microscope and acquired typical flat spindle shape within 24-48 hours post-seeding. The striking effect of matrix on the morphology was longer and thinner cytoplasmic processes of the fibroblasts. The order of growth rate of matrices was PC+GT > PC+GT+MX >PC > GT > GL ~ MX > control. The BESC reached confluency by 7-8 day in PC+GT, 8-9 day in PC+GT+MX, 9-10 day in PC followed by other matrices. BESC in PC expressed fibronectin but not cytokeratin. This in-vitro model would be useful in understanding the specific role of BESC during maternal recognition of pregnancy in the buffalo.

Keywords: Bubaline endometrial stromal cells, extracellular matrices, fibronectin

INTRODUCTION

Infertility due to embryonic mortality adversely affects the economy of dairy industry. About 40% embryos are lost during the early period of embryonic development. Pregnancy recognition, implantation and its maintenance involve a reciprocal interaction between the developing embryo and receptive maternal endometrium [22, 29]. Implantation related studies in domestic species like sheep, has contributed immensely to understand the molecular mechanism underlying implantation in mammalian species [28]. Animal models have their own limitations viz., variability of results, limitation in manipulating experimental conditions and ethical animal welfare issues. Therefore, development of an architecturally and functionally competent model could be an alternate way to address endometrial function.

The endometrium is a complex tissue consisting mainly of epithelial (luminal and glandular) and stromal cells. The other cell types present in the endometrium are immune and endothelial cells. Both epithelial and stromal cells produce isoforms of prostaglandins (PG) but have different morphological and physiological properties [7] with regard to growth response and presence of sex steroid receptors. Stromal cells provide a regulatory role for growth and differentiation of the overlying epithelium [8]. It is reported that progesterone receptors are present in the stromal cells during early pregnancy but not in the epithelium to mediate the effects of demonstrating the paracrine role for the stromal cells in controlling the endometrial function [3,8]. Two-dimensional culture of endometrial epithelial and stromal cells have been reported in several species viz., human [20], rat [21], rabbit [9], cattle [10,31], sheep [5] and recently in buffalo

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[6,23]. However, the limitation of the two-dimensional endometrial cell culture system is that they do not mimic the exact micro-architectural configuration of intact endometrium. This emphasizes to develop further improved techniques of endometrial cell culture system [17]. The introduction of three-dimensional cell culture techniques allows better manipulation of the culture conditions in order to study the role of different cell types and their functions. Further, these techniques also provide an opportunity to eliminate or add co-factors in a stepwise manner to determine, if an outcome is actually due to the action of a specific factor. Till date, hydrogels are the most widely used ECM for the development of three-dimensional cell culture methods [16]. Hence, defining an appropriate ECM for establishing an in-vitro environment that mimics the in-vivo situation is still a challenge. The commercially available matrices with varying composition provide representative microenvironments for these cells like basement membrane extract Matrigel for epithelial cells [18,23,30] and type I collagen or fibrin gels for stromal cells [26]; however, no single composition is ideal enough for both cell types in co-culture models. Commercially available natural matrices exhibit substantial inherent batch-to-batch variability and variations in growth factor compositions depending on the preparation protocol. To the best of our knowledge, no well-defined three-dimensional cell culture conditions are available for the development of BESC.

MATERIALS AND METHODS

Experimental Design

Primary BESC were established following isolation and culture using abattoir uterine samples. Double enzymatic digestion protocol was followed [7] with some modifications. Commercially available extracellular matrices were coated onto 24 well plates (BD Labware, USA). Cells were seeded at the density of 2×10^5 cells per well. The best suitable matrix for BESC was selected on the basis of morphological evaluation. The morphological evaluation was based on the growth pattern (day-wise differentiation %) and day of attainment of confluency of BESC in different matrices.

Coating of 24-Well Plate

Different matrices namely Purecol (Sigma, USA), Geltrex (Gibco, USA), Gelatin (Himedia, India), Maxgel (Sigma, USA) and combinations of Purecol-Geltrex and Purecol-Geltrex-Maxgel were thawed at 4°C and diluted with nuclease free water on ice. For all procedures, pre-cooled 24 well plate, tips and tubes were used. Matrices were coated uniformly in wells of plates on ice. Plates were then incubated at 37°C for 1 hour. Matrices form gel rapidly at 22-35°C.

Cell Isolation and Culture

The buffalo uteri of early luteal phase of the cycle, as evidenced by the presence of brick to cherry red corpus haemorrhagicum on the surface of ovary were collected from a local abattoir and transported to the laboratory in ice cold Hanks Balanced Salt Solution (HBSS, pH-7.3) containing double dose of penicillin (60 mg) and streptomycin (100 mg) antibiotics per litre. Genitalia were washed thrice in cold HBSS containing double dose of antibiotics. Greater curvature of the uterine horn, ipsilateral to the corpus haemorrhagicum, was dissected out and the dissected part was washed thrice in HBSS containing antibiotics. BESC were isolated from uterine endometrium by double digestion, initially with 0.3% trypsin-III (Sigma, USA) followed by a cocktail of 0.064% trypsin-III (Sigma, USA), 0.064% collagenase-II (Sigma, USA) and 0.032% DNAase-I (Sigma, USA) in HBSS and kept for incubation at 37°C in 5% CO_2 for 2 and 1 hour, respectively. After incubation, cells were subjected to centrifugation at 1200 rpm for 10 minutes. Pellets were pooled and washed with HBSS and centrifuged thrice at 1200 rpm for 10 minutes each. RBC lysis buffer (5 ml) was added to the cell pellet and centrifuged at 1200 rpm for 10 minutes. The cell pellet was suspended in RPMI (pH-7.2, Sigma, USA) containing 10% fetal bovine serum (FBS), amphotericin (0.1%) and gentamicin (0.1%). Cell suspension was strained with 70 µm mesh strainer (Genetix, Biotech Asia Pvt. Ltd.). Cell counting was performed by haemocytometer. The morphological evaluation of cells was done under phase contrast microscope (Nikon) and viability was determined using trypan blue exclusion test (Freshney, 2000). The cell concentration of around 2×10^5 cells per well were seeded. Every well was provided 1 ml RPMI at 37ºC and 5% CO_2. Cells were observed at regular interval till confluency. Media was changed on every alternate day.

Immuno-cytochemistry

Cells for immunocy to chemistry (ICC) were cultured in a 4-well chamber slide (Thermo Scientific,

usually at a concentration of 2×10^5 cells per well in a 1 ml volume of RPMI. Media was removed from the wells after attainment of confluency. Wells were washed once with cold PBS. Cells were fixed with 4% paraformaldehyde for 20 minutes at room temperature and washed twice with cold PBS (5 minutes each). Pre-heated Antigen Retrieval Buffer at 95°C was poured into wells and the slide was then kept in water bath at 95°C for 10 minutes. Slide was taken out of water bath and kept at room temperature for 10 minutes. Cells were washed thrice with cold PBS (5 minutes each) and permeabilized with 0.5% Triton X-100 for 5 minutes at room temperature. Cells were washed thrice with cold PBS (5 minutes each) and exposed to 10% goat serum in PBS (4% BSA) blocking buffer for 1 hour. Cells were then exposed to primary antibody [anti-vimentin/anti-cytokeratin (Sigma, USA)] and kept at 4°C overnight. Antibodies were diluted in 10% goat serum. Cells were washed thrice with 1% goat serum (in PBS+BSA) for 10 minutes each and exposed to IgG-FITC goat anti-mouse secondary antibody (Boster, USA) and kept at room temperature for 2 hours. Antibody was diluted in 10% goat serum. Cells were then washed thrice with 1% goat serum (in PBS+BSA) for 10 minutes each and exposed to DAPI nuclear stain (Boster, USA) for 10 minutes at room temperature. Cells were washed twice with PBS for 5 minutes each. Glass slide chamber was unloaded to expose only the glass slide. Cells were observed under fluorescent microscope for immunofluorescence. Blue light was used to visualize FITC conjugate antibodies and UV light was used to visualize DAPI nuclear stain.

Statistical Analysis

To study the effect of matrix on the degree of differentiation of BESC, mixed model repeat measure ANOVA was done with orthogonal contrast for pair-wise comparison using Bonferroni test. The difference in the day of confluency was tested by One-way ANOVA with orthogonal contrast. Graph Pad Prism Software (Version 5) was used for data analysis and chart preparation.

RESULTS

Identification and Morphological Evaluation of BESC in the Culture System

The mean yield of BESC was 1.0×10^7 cells/~40 gm of digested tissue following double enzymatic digestion. Morphological evaluation revealed that the purity and viability of BESC obtained were >90%. After isolation, BESC were either single or in small clumps, glistening and rounded under inverted microscope (Fig 1.) and acquired typical flat spindle shape within 24-48 hours post-seeding. The appearance of BESC in different matrices on day 5 and day 13 post-seeding is presented in plates (Fig 2.) and (Fig 3.), respectively. The striking effect of matrix (irrespective of the type) on the morphology was longer and thinner cytoplasmic processes of the fibroblasts. The BESC reached confluency by day 7-14 post-seeding in the different matrices. The growth rate of BESC in different matrices, based on the microscopic observations is presented in Table 1. and (Fig 4.) The findings revealed that PC+GT combination was significantly ($P<0.001$) superior for the growth and development of BESC on day of culture as compared to other matrix groups (PC+GT+MX, PC, GT, GL, MX and control). However, on day 15 post-culture, the differentiation of BESC was significantly ($P<0.001$) higher in PC, GT, PC+GT, PC+GT+MX combination than that of GT or MX (Fig 5.). The effect of different type of matrices on the time taken for confluency by the BESC is presented Table 2. and (Fig 3.) The findings revealed that BESC achieved confluency at the earliest by day 7-8 in PC+GT followed by day 8-9 in PC+GT+MX and day 9-10 in PC, which indicates that these matrices enhance growth potential of BESC. However, confluency in GL and MX was delayed and observed by day 11-13.

Table 1. Effect of different type of matrices on the differentiation (%) of BESC till confluency (Mean ± S.E.)[$]

Day post-seeding	Control	PC	GT	GL	MX	PC+GT	PC+GT+MX
5	2.3±0.3[a]	13.0±1.0[a]	10.7±1.8[a]	4.2±1.0[a]	3.5±1.0[a]	22.2±2.2[c]	16.9±6.0[b]
10	11.4±1.5[a]	33.4±3.8[c]	29.0±4.3[b]	14.9±2.5[a]	10.4±2.5[a]	50.2±5.8[c]	40.6±14.4[c]
15	31.4±2.4[a]	69.7±5.0[c]	60.6±6.0[c]	40.9±7.7[a]	23.9±4.5[a]	85.5±4.2[c]	75.5±26.7[c]

[$] Figures in the table indicate the degree of morphological differentiation (%). Repeat measure ANOVA with orthogonal contrast for pair-wise comparison was done. Bonferroni test served as *post-hoc test*. Different superscripts (a,b,c) across a row indicate significant difference with respect to control ($P<0.05$).

Table 2. Effect of matrix type on the growth rate of BESC till day 15 post-seeding (Mean ± S.E.)[$]

Matrix	Day post-seeding
Contro	114.0±1.9[a]
PC	8.9±0.4[c]
GT	10.5±0.6[c]
GL	11.6±0.7[b]
MX	12.4±0.5[a]
PC+GT	6.9±0.4[c]
PC+GT+MX	7.4±0.5[c]

[$]Figures in the table indicate the mean time required from seeding to confluency (Day). One way ANOVA with orthogonal contrast for pair-wise comparison was done. Bonferroni test served as *post-hoc test*. Different superscripts (a, b, c) in the day post-seeding column indicate significance difference with respect to control (P<0.05).

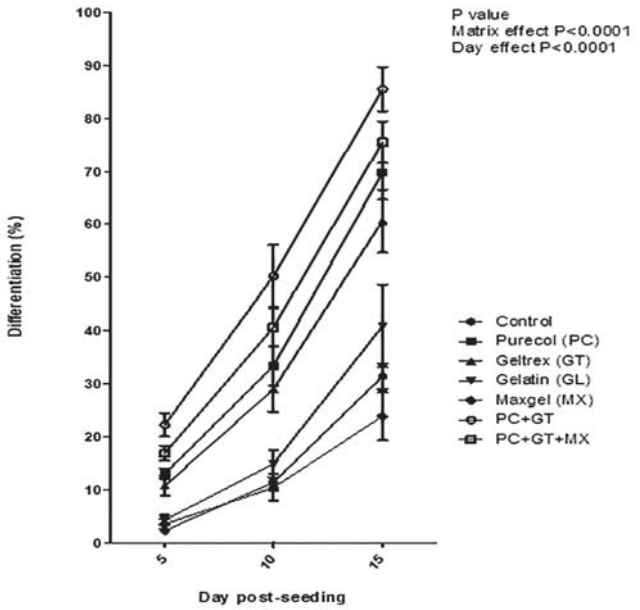

Fig 1. Effect of matrix type on the degree of differentiation (%) of BESC till day 15 post-seeding.

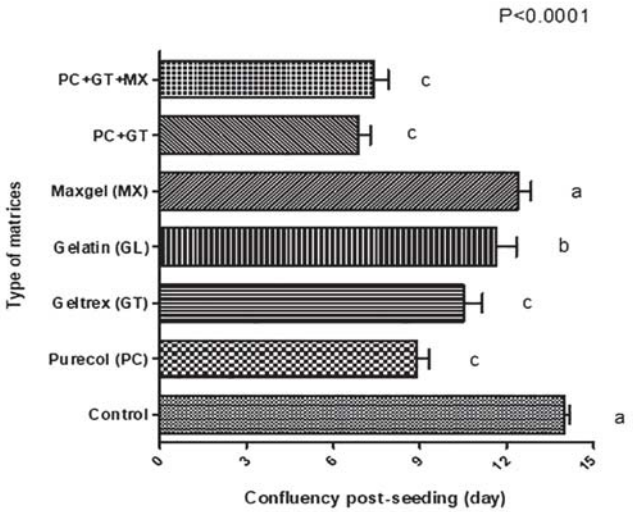

Fig 2. Effect of matrix type on the growth rate of BESC till day 15 post-seeding.

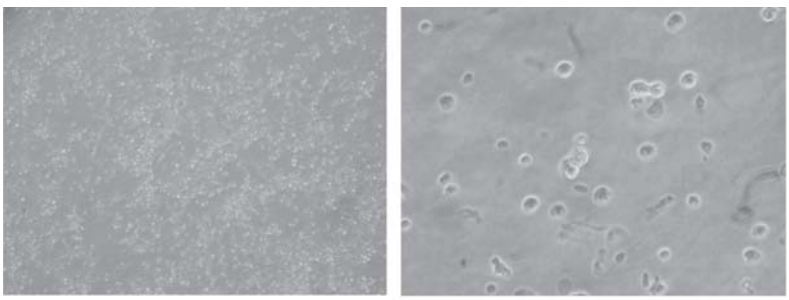

Fig 3. Representative photomicrograph of BESC at 10X (a) and 20X (b) magnifications while seeding.

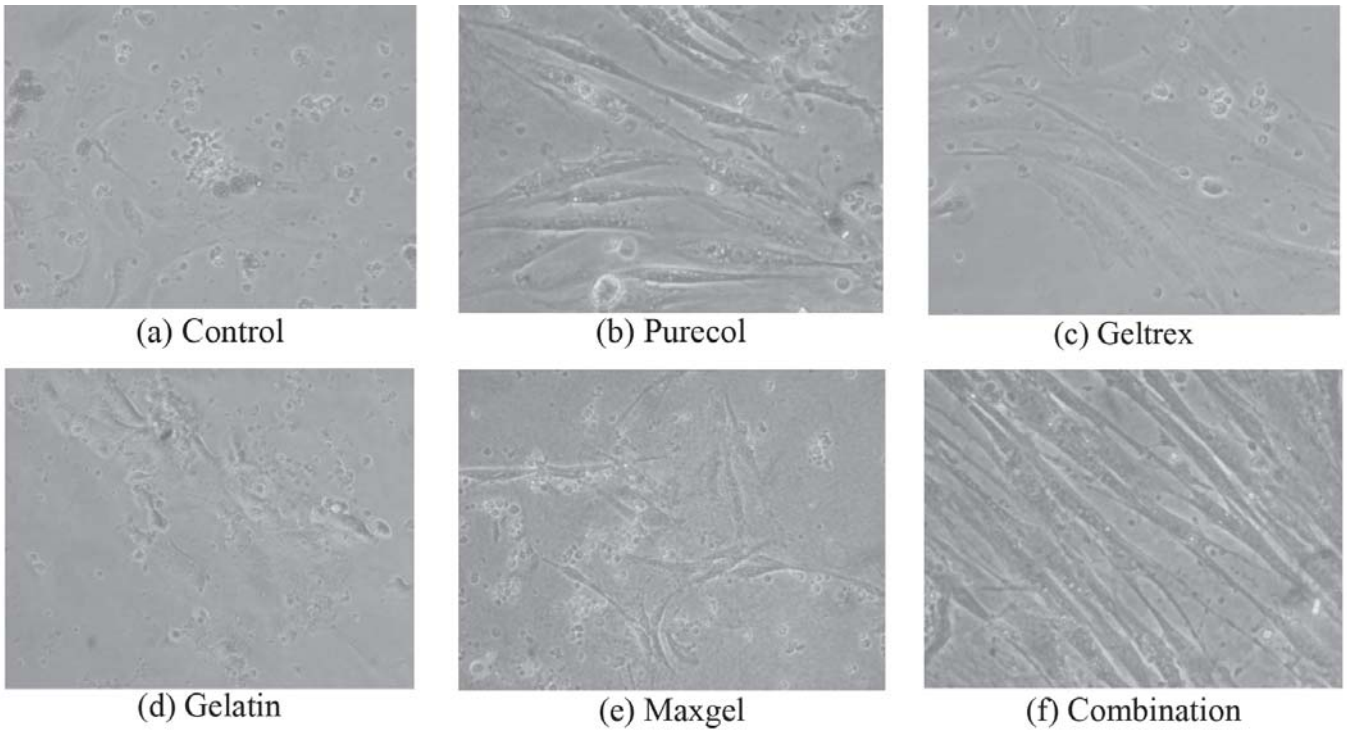

Fig 4. Representative photomicrograph of BESC (20X magnification) in different matrices on day 5 post-seeding.

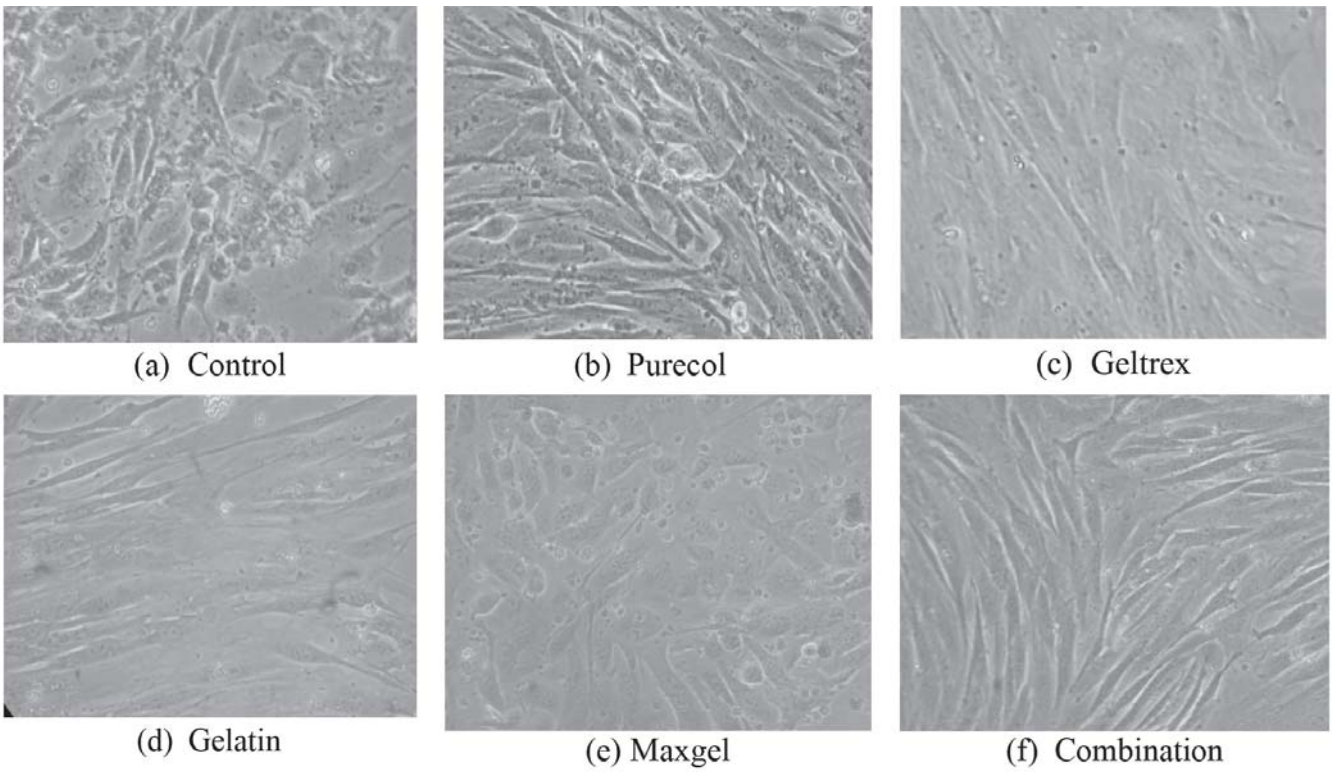

Fig 5. Representative photomicrograph of BESC (20X magnification) in different matrices on day 13 post-seeding.

Fig 6. Representative immunofluorescent images in the BESC (10X magnification) grown in PC matrix

Characterization of BESC in Tte Culture System

The BESC stained positive for fibroblast cell specific anti-fibronectin, but not with anti-cytokeratin, indicating homogenous phenotype. DAPI staining along with the secondary antibody acted as the negative control (Fig 6.).

DISCUSSION

The present study evaluated the effect of different type of matrices on the growth and differentiation of BESC. It is reported that type I collagen supports the stromal cells [26], whereas type IV collagen supports the epithelial cells [18]. In our study, type I collagen was present in PC, GT as well as MX; however, the mutually exclusive factors were L-glutamine in PC, FGF, TGF and laminin in GT and fibronectin and tenascin in MX. The importance of the growth factors and ECM proteins in the maintenance of tensional homeostasis is demonstrated in wound healing models [25]. Fibronectin regulates the organization of interstitial ECM, cell attachment and its function [27]. The choice of matrix was based on the presence of type I collagen, growth factors such as FGF, TGF, L-glutamine and ECM substances such as laminin and fibronectin. To gain advantage of specific growth factors, a few combinations of matrices was used. For instance, the PC+GT+MX combination was chosen because of the complementarity of the matrices as GT would provide FGF, TGF and laminin, whereas MX would provide fibronectin and tenascin [11]. Exogenous addition of the FGF, TGF or laminin to individual matrix was not attempted as there was no favorable trade-off between the cost and convenience, implying high cost of individual biochemical coupled with laborious preparation of specific type of matrix.

Bubaline uterus at the early luteal phase of estrus cycle (day 1-5) was used for the study based on a number of reasons. Firstly, the recognition of this stage by the corpus haemorrhagicum brought consistency in the uterine sample collected from the abattoir. Secondly, the stage corresponds to pre-implantation stage in a conceptive cycle during which the hormonal profiles are inclined towards endometrial receptivity. Total separation of individual stromal and epithelial cells was difficult. However, there was a high purity of isolated BESC (>90%) and substantial cells yield with a mean value of 1.0×10^7 cells/~40 gm of digested endometrial tissue. The typical spindle shaped morphology and the growth of BESC in culture are consistent with the results reported in the cow [10,31], ewe [5], buffalo [6,15] and bitch [2]. The striking effect of matrix (irrespective of the type) on the morphology was longer and thinner cytoplasmic processes of the fibroblasts as reported earlier [14], which is likely due to difference in the stiffness, thickness and tension of scaffold [4,13,19]. It was reported that stromal cells exhibited greater proliferation in 2D culture as compared to GT matrix-based 3D culture [1]. It was observed that PC+GT combination was morphologically superior for the growth and differentiation of BESC than any other individual matrix or combination. But statistically the PC alone, PC+GT, PC+GT+MX combinations had no significant difference among them, indicating that PC is itself sufficient enough to cater to the needs of the BESC and enhance the growth and differentiation of BESC. So, by morphological evaluation PC was preferred over other matrices.

In order to confirm the identity of the specific cell type, indirect immune-fluorescence was done with

stromal cell specific fibronectin antibody and epithelial specific cytokeratin antibody in stromal cells grown in PC matrix using DAPI as nuclear stain which binds strongly to adenine–thymine rich regions of DNA.The BESC stained positive for immune-positive fibronectin, but not with anti-cytokeratin, indicating homogenous phenotype. These markers have been reported as specific representatives of the class of cell concerned [5,7,15,24].

CONCLUSION

Thus, it can be concluded that based on the growth characteristics and degree of differentiation, Purecol matrix was observed to be superior over other matrices (Purecol+Geltrex+Maxgel, Purecol+Geltrex, Geltrex, Gelatin, Maxgel) for the culture of BESC. This in-vitro model would be useful in understanding the specific role of stromal cells during maternal recognition of pregnancy in the buffalo.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Research Article

INTEGRATED EFFECTS OF LACTATION STAGES AND REARING SYSTEMS ON MILK COMPOSITION AND SOME HEMATO-BIOCHEMICAL ENTITIES IN PANTJA GOAT OF UTTARAKHAND

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ABSTRACT

Pantja goat is an indigenous goat breed of Uttarakhand state, known for their dual purpose and unique physical features. The present study was done during late winter to spring season. Total thirteen healthy lactating Pantja goats were selected from field and farm conditions. Selected goats were then divided into two groups according to their rearing system i.e. first group with seven goats (b. wt. 20 ± Kg, 2-5 parity) from farm and second group with six goats (b. wt. 15 ± Kg, 2-4 parity) from field conditions. Various milk and hemato-biochemical parameters were studied for early (0-30 days), mid (31-60 days) and late (above 61 days) lactation stages. Milk parameters *viz*. milk fat, somatic cell count, electrical conductivity, pH and corrected lactometer reading were significantly (*P<0.05*) affected by lactation stages in Pantja goat reared in different conditions. Milk total solids percent was significantly (*P<0.05*) increased in farm goats, though the level was high in field goats but found non-significant. Haematological parameters values significantly (*P<0.05)* increased in haemoglobin, packed cell volume, total leucocytic count and erythrocytic indices in farm goats whereas haemoglobin, packed cell volume, total erythrocytic count and erythrocytic indices in field goats during different lactation stages. Osmotic fragility test of erythrocytes was significantly (*P<0.05)* altered during lactation stages. Lactation stages significantly (*P<0.05)* altered biochemical parameters values total proteins, globulin, glucose, creatinine and minerals (calcium, phosphorus) levels. significantly (*P<0.05)* higher levels of serum total protein, globulin, blood urea nitrogen, total bilirubin, minerals levels in farm goats whereas serum glucose, creatinine and phosphorus levels in field goats. Thus, it is concluded that lactation stages and different rearing systems of lactating Pantja goat living in the tarai region of Udham Singh Nagar district of Uttarakhand do influence milk composition and blood entities.

Keywords: Blood, Lactation, Milk, Pantja goat, Rearing systems

INTRODUCTION

Pantja goat is a registered indigenous goat breed of Uttarakhand state (Accession no. India_Goat_2420 _Pantja_06024). Within the state, goat husbandry is significantly increasing, especially in tarai region of Udham Singh Nagar district most of the farmers preferred to rear Pantja goat due to advantages and significant characters than other goat breed due to excellent adaptation to the hot-humid environment of tarai region, dual purpose breed, minimum inputs and more economical, commonly twins during kidding with unique physical appearance, medium sized body, beautiful brown red dorsal coat color with ebony line and lighter ventral surface and a white streak on either side of the face. Lactation period of about 156 days with some variability and average milk production per lactation is 113.89 kg (ICAR-NBAGR, 2023).

Several factors are accounted to affect milk yield and milk composition during lactation such as breed, kidding season, parity, lactation length, and lactation stage [20]. Milk components are mostly drawn directly from blood and few synthesized within the alveoli. It correlates with blood profiles and metabolic activity in lactating animals [21]. Goat milk is superior for its enriched with more selenium content, better digestibility and utilization of various metabolic minerals [10]. The present study was

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done to investigate milk composition and blood profiles affected by lactation stages and different rearing systems (under field and farm conditions) of Pantja goat, living in tarai region of Udham Singh Nagar district of Uttarakhand.

MATERIALS AND METHODS

Total thirteen lactating Pantja goats were selected from field and farm and the study was done during winter till to the onset of summer season. Prior to the experiment, permission from The Institutional Animal Ethical Committee (IAEC) of College of Veterinary and Animal Sciences, GBPUAT, Pantnagar was obtained (IAEC/CVASc/VPB/517).

Selected goats were maintained in two different rearing system as seven farm Pantja does (body wt. 20±kg, 2-5 parity) at the Department of Livestock Production Management. These goats were maintained in proper shelter and semi stalled feeding system with provision to concentrate feeding, farm-grown green fodders and tree tops (gullar, khadika, babul, ardu, jamun, berry, mulberry leaves). Another six field Pantja does (b. wt. 15±kg, 2-4 parity) were from in and around Shantipuri of Udham Singh Nagar district of Uttarakhand. Field goats were maintained in loose housing system with sufficient floor spacing for the night and during daytime provision to very less concentrate feeding and mostly allow open grazing on available grasses, forest fodders, seed and fodder crop resides. In both conditions, ad lib supply of clean and fresh water for drinking. The study location is under tarai region known for its hot-humid climate during monsoon and extreme cold during winter with short spring season. This region also have high water table, fertile soil, lush green grasses and forest fodders available throughout the year. Laboratory analysis of samples were done at the Department of Veterinary Physiology and Biochemistry, College of Veterinary and Animal Sciences, GBPUAT, Pantnagar in Udham Singh Nagar district of Uttarakhand.

Blood and milk samples were collected from each lactation stages as first (0-30 days), second (31-60 days) and third (above 61 days) in both lactating farm and field Pantja goats.

Milk sample (15ml) was collected in sterilized milk vails by following all the hygienic measures. All milk sample composition analysis was done within four hours of sampling. Each milk sample was analyzed by Ultra Scan Milch Analyzer machine (Hindustan Thermostatics, Haryana) for milk fat, solid not fat (SNF), protein, lactose, density, corrected lactometer reading (CLR) and freezing point depression (°C). Milk pH and electric conductivity (mS/cm) were analyzed by digital pH meter (HPG systems, Chandigarh). Milk total solid was determined as per IS: 1479 (1961), where milk total solid and specific gravity were calculated by following formula:

$$\text{TS (\%)} = \frac{\text{CLR}}{4} + 1.21F + 0.36$$

$$\text{Specific gravity} = 1 + \frac{\text{CLR}}{100}$$

Where, F : Fat content of milk, CLR : Corrected Lactometer Reading

Approximately 5 ml blood from each goat was collected aseptically from the jugular vein in the morning hours before feeding and watering. Blood sample was then divided into two parts - 3 ml of blood in anticoagulant-free sterile tube (JK Diagnostic, Rajkot, Gujarat) for serum harvest for evaluation of biochemical parameters and 2 ml of blood in EDTA (1mg/ml) coated sterile tube (JK Diagnostic, Rajkot, Gujarat) for evaluation of hematological parameters. Hematological parameters of hemoglobin [18], packed cell volume [5], osmotic fragility of erythrocytes [7], total erythrocyte count, total leucocyte count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, differential leucocyte count, absolute leucocyte count [13], and milk somatic cell count [4] were evaluated. Biochemical parameters of serum protein (total protein, albumin, globulin), glucose, creatinine, urea, total bilirubin, lipids (total cholesterol, triglyceride), liver enzymes (aspartate transaminase, alanine transaminase), and electrolytes-calcium, phosphorus, calcium: phosphorus (6, 25,27).

The statistical analysis was done for one-way ANOVA (for more than two groups of data) using IBM SPSS ver.22.0 software. The collected data of various hemato-biochemical parameters and milk composition from

lactating Pantja goats kept in farm and field conditions were checked for statistical significance at 5% level of significant and the data are presented as mean ± standard error.

RESULTS

Various milk parameters of milk fat, somatic cell count, electrical conductivity and corrected lactometer were found significantly ($P<0.05$) affected by lactation stages in Pantja goat reared under farm and field conditions. Milk total solids of farm goats found significantly affected by lactation stages whereas it was not in field goats though the value are much higher. Other milk compositions of water, protein, lactose, solid non-fat and pH were found non-significant during different lactation states in both farm and field Pantja goats (Table 1.).

Milk fat and somatic cell count were significant ($P<0.05$) higher at late lactation along with reduced milk yield in Pantja goats reared in both different rearing conditions. However, milk fat of field was higher than the farm Pantja goats. Pantja goat milk fat percent was found in similar report with other indigenous goat breed [8, 15, 19, 23] Another study depicted a significant positive correlation between goat milk fat with sodium content and somatic cell count [26]. Lactation stages affecting milk fat and somatic cell count of Pantja goat irrespective to the rearing system. Milk electrical conductivity and corrected lactometer reading were significantly ($P<0.05$) higher in early lactation stages and decreased with advanced lactation stage. Studies [2,24] documented similar result for milk electrical conductivity during different lactation stages. Milk total solid percent was lower in farm Pantja goats, but significantly ($P<0.05$) affected by lactation stages whereas, it was not in field Pantja goats. Earlier studied has mentioned similar results both in field [1,28] and farm goats [3,16].

DISCUSSION

Hematological parameters (Table 2.) of packed cell volume, total leucocytes count and erythrocytic indices were significantly ($P<0.05$) higher in farm Pantja goats whereas heamoglobin, packed cell volume, total erythrocyte count and erythrocytic indices were significantly ($P<0.05$) higher and better in field Pantja goats. Total erythrocyte count in farm goats and Total leucocytes count in field goats were remain unaffected irrespective of lactation stages. Field goats graze daily on forest green grasses and fodders, which are available abundantly year-round in the tarai region of Uttarakhand. This provide a good source of iron and protein requirements to the grazing goat. Hematological values when compared with other indigenous goat breeds, the significant variation in heamoglobin, packed cell volume, total erythrocyte count and erythrocytes indices during different lactation stages of Pantja goat found similar with Sirohi goat [21], while total leucocytes count values was lesser to Jamunapari goat [9]. Also significant ($P<0.05$) hemolysis was observed between different lactation stages in the saline concentration of 0.9, 0.7, 0.3 and 0.1 percent in farm goat while 0.9, 0.7 and 0.5 percent in field goat (Fig 1.). Thus, farm Pantja goat erythrocytes are more fragile than field Pantja goat when exposed to different hypotonic saline concentrations. Erythrocytes of lactating goat are less resistant to hypotonic solutions and more susceptible to oxidative stress.

Various biochemical parameters of lactating Pantja goat reared under farm and field conditions were estimated (Table 3.). Significantly ($P<0.05$) higher in serum protein (total protein, globulin), blood urea nitrogen (BUN), bilirubin and mineral (calcium, phosphorus) levels in lactating farm goats and appear better as they are mostly feeding on concentrates, cultivated green fodders and tree tops. While increase in serum glucose, phosphorus and creatinine levels in lactating field goats, it might be due to undergoing dehydration or stress as they practiced a daily routine of grazing out and increased physical activity to meet their daily requirement of nutrients. Unlike studies in other indigenous goats [14,17] lactation stages has significantly ($P<0.05$) affect serum minerals (calcium and phosphorus) in Pantja goat irrespective of rearing conditions.

CONCLUSION

Milk composition and haemato-biochemical entities varied as lactation advanced and in lactating Pantja goat reared in farm and field conditions. Therefore, in this present study it is concluded that lactation stages and managemental practices that are routinely followed under different rearing conditions can contributed to these variations. Others factors such as season, parity, breed, availability of fodders and age may also influences these variations and for which, further study may require.

Table 1. Milk parameters of lactating farm and field Pantja goats

Milk parameters	Farm condition				Field condition			
	Lactation stages			P value	Lactation stages			P value
	Early	Mid	Late		Early	Mid	Late	
Water	85.02	85.20	85.37	NS	85.42	85. 57	85.91	NS
Fat (%)	2.03a±0.57	1.91a±0.36	5.39b±0.48	0.000	4.84ab±0.33	3.94a±0.43	5.3b±0.15	0.028
Protein (%)	3.31±0.04	3.34±0.06	3.50±0.15	NS	3.47±0.21	3.94±0.43	3.37±0.08	NS
Total solids (%)	11.64a±0.79	11.42a±0.83	14.48b±0.47	0.012	14.49±0.77	13.67±0.70	14.50±0.27	NS
Solid non-fat (%)	9.21±0.12	9.02±0.16	8.97±0.13	NS	10.2±0.54	9.76±0.51	9.22±0.19	NS
Electric conductivity (ms/cm)	4.59c±0.33	2.46b±0.51	1.35a±0.13	0.000	2.97b±0.37	1.39a±0.07	1.70a±0.49	0.016
pH	6.06b±0.06	6.34a±0.18	6.08a±0.01	NS	6.67a±0.30	6.08a±0.09	6.73b±0.48	NS
Corrected lactometer reading (CLR)	35.24b±1.63	33.26ab±0.62	30.41a±0.71	0.019	36.96b±1.43	35.86b±2.03	30.95a±0.72	0.028
Temperature (°C)	26.50±0.03	30.96±1.20	33.86±0.23	-	30.1±1.21	32.92±0.19	31.08±0.15	-
Specific gravity	1.03±0.00	1.03±0.00	1.03±0.00	-	1.03±0.00	1.03±0.00	1.03±0.00	-
Freezing point depression (°C)	0.61±0.03	0.57±0.01	0.59±0.01	-	0.67±0.04	0.63±0.04	0.62±0.01	-
Density	34.99±1.56	33.06±0.59	30.60±0.77	-	35.0±1.96	33.97±1.99	30.88±0.69	-
Salinity (ppt.)	0.10±0.00	0.10±0.00	0.10±0.00	-	0.10±0.00	0.10±0.00	0.10±0.00	-
Milk SCC ($x10^5$/ml)	9.05a±0.07	10.72b±0.19	11.62c±0.16	0.000	7.94a±0.44	8.86b±0.17	9.13b±0.26	0.030

**Mean values arranged in row are differ significantly (P<0.05)*

Table 2. Some hematological parameters of lactating farm and field Pantja goats

Hematological parameters	Farm condition				Field condition			
	Lactation stages			P value	Lactation stages			P value
	Early	Mid	Late		Early	Mid	Late	
Hb (g/dl)	6.49 ±0.30	6.27 ±0.25	5.90 ±0.41	NS	7.63^{c} ±0.47	4.43^{a} ±0.20	6.62^{b} ±0.12	0.032
PCV (%)	31.14^{b} ±2.33	26.0^{a} ±1.46	29.00^{ab}±1.00	0.045	32.2^{b}±1.30	26.27^{a}±0.83	36.83^{c}±1.56	.021
TEC (10^6/µl)	9.57 ±1.14	10.07 ±0.47	10.47 ±0.41	NS	8.68^{a} ±0.24	12.2^{b} ±0.37	13.74^{c}±0.31	0.003
TLC (10^3/µl)	9.33^{a}±0.64	10.95^{ab}±1.03	12.60^{b} ±0.29	0.017	11.41 ±1.26	10.90 ±0.34	9.93 ±0.64	NS
MCV (fl)	36.56^{b} ±1.96	26.10^{a} ±1.85	28.02^{a} ±1.64	0.002	37.21^{c}±1.82	20.28^{a}±1.95	27.36^{b} ±1.38	0.011
MCH (pg)	7.13^{b} ±0.44	6.21^{ab} ±0.39	5.65^{a} ±0.38	0.019	8.85^{b} ±0.69	3.65^{a}±0.19	4.91^{a} ±0.17	0.000
MCHC (%)	22.68^{ab}±1.58	23.98^{b} ±0.92	20.24^{a} ±0.94	0.039	23.79^{b}±1.29	18.67^{a}±1.30	18.05^{a} ±0.43	0.005

**Mean values arranged in row are differ significantly (P<0.05)*

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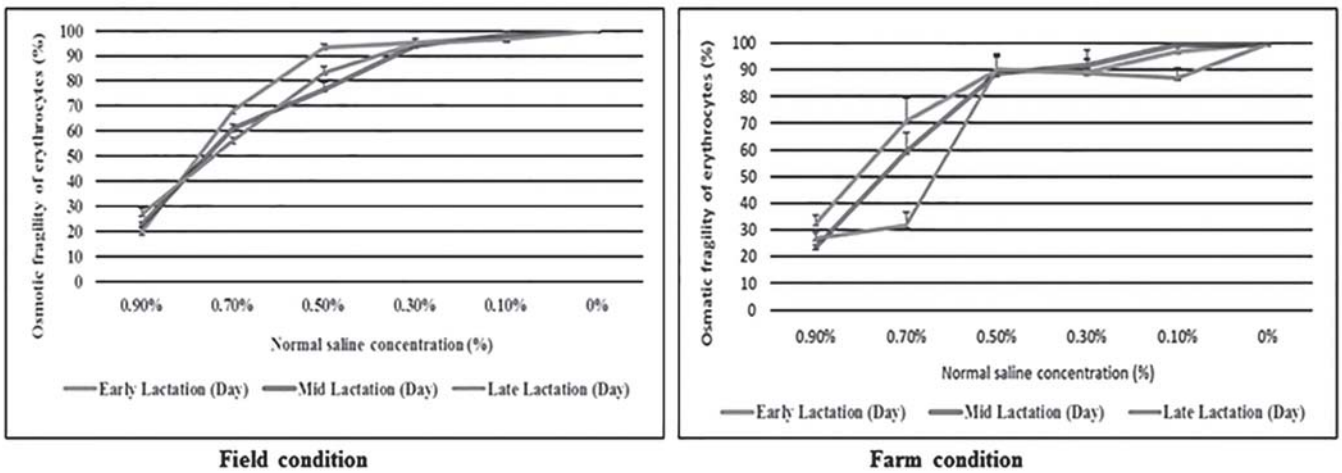
Table 3. Serum biochemical parameters of lactating farm and field Pantja goats

Biochemical parameters	Farm condition				Field condition			
	Lactation stages			P value	Lactation stages			P value
	Early	Mid	Late		Early	Mid	Late	
Total protein (g/dl)	6.23a ±0.12	5.96a ±0.13	6.83b ±0.12	0.003	6.82 ±0.25	7.27 ±0.19	6.80 ±0.52	NS
Albumin (mg/dl)	5.28±0.35	5.09±0.29	4.83±0.09	NS	5.79±0.37	5.38±0.31	5.36±0.24	NS
Globulin (mg/dl)	0.98a ±0.26	0.87a±0.24	1.99b ±0.12	0.003	1.03±0.28	1.89±0.30	1.51±0.41	NS
Glucose (mg/dl)	17.39 ±3.04	13.04 ±2.85	17.92 ±2.22	NS	23.32b±1.51	13.99a±1.72	20.03ab±3.80	0.021
Total cholesterol (mg/dl)	56.87 ±3.16	61.40 ±5.18	66.83 ±4.10	NS	59.62±2.12	62.88 ±4.17	60.42±2.66	NS
TG (mg/dl)	101.58±3.11	98.94±5.34	102.11±3.78	NS	119.13±3.37	123.46±5.12	110.49±7.31	NS
AST (IU/L)	8.62±0.29	8.55 ±0.27	8.16±0.17	NS	8.09±0.30	8.05±0.26	7.86±0.29	NS
ALT (IU/L)	10.24±0.40	10.61±0.24	10.22±0.13	NS	10.46±0.15	10.24±0.18	10.13±0.20	NS
Creatinine (mg/dl)	4.09±0.36	4.67±0.59	3.71±0.51	NS	3.67ab±0.28	2.83a±0.17	4.99b±0.56	0.003
BUN (mg/dl)	17.25b±1.56	14.44ab±1.12	11.90a±0.74	0.018	16.07±0.94	16.46±1.46	14.66±1.37	NS
Bilirubin (mg/dl)	0.46b±0.06	0.18a±0.10	0.63b±0.03	0.002	0.28±0.07	0.25±0.02	0.34±0.05	NS
Calcium (mg/dl)	12.47a ±0.80	15.60b ±0.97	15.12b ±0.70	0.033	13.09 ±0.37	13.00 ±0.27	13.35 ±0.45	NS
Phosphorus (mg/dl)	7.55a ±0.57	8.06b ±0.50	8.90b ±0.15	0.045	6.81a ±0.40	9.27b ±0.40	7.45a ±0.51	0.011
Ca: P ratio	1.68 ±0.13	1.98 ±0.17	1.70 ±0.10	NS	1.98b ±0.16	1.41a ±0.05	1.81b ±0.06	0.005

**Mean values arranged in row are differ significantly (P<0.05)*



Field condition **Farm condition**

Fig 1. Osmatic fragility test of erythrocyte at different hypotonic Saline concentration in lactating farm and field Panja goats

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Research Article

STUDY ON SOCIO ECONOMIC, COMMUNICATION AND SOCIO PSYCHOLOGICAL CHARACTERISTICS OF BENEFICIARIES UNDER KRISHI VIGYAN KENDRA IN DIFFERENT AGROCLIMATIC ZONES OF WEST BENGAL, INDIA

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ABSTRACT

The scientific agriculture in the economic development of India contributes about 38.00 per cent to the GDP on agriculture for their livelihood. Krishi Vigyan Kendra (KVK) is the important part of transfer of technology at grass-root level which has immense role in the development of Agri-animal husbandry sectors in our country. The study was undertaken with the main objectives to study the socio-economic and socio-psychological characteristics of beneficiaries in the KVKs of six different agro climatic zones of West Bengal from where one district of each zone has been selected randomly. From each district 40 numbers of respondents who were taking the service of KVK in their areas have been selected randomly. In that way total 240 numbers of sample were studied. The data were collected from pretested structured interview schedule which was compiled and analyzed statistically thereafter. The results from the study revealed that majority of the respondent farmers were belonged to middle to young age group from different joint families having medium to small size of land holding capacity and majority of stakeholders had the qualification of primary to higher secondary level. The present study stated that more than half of the farmers were dependent on agriculture and animal husbandry with their family income more than 50,000 to 1 lakh (medium level). Most of them were involved in one organization with low level of extension contact. In the end, the study also highlights that most of the respondents have mass media exposure as information source, indicating a considerable impact on KVK program among the beneficiary stakeholders in the area of study.

Keywords: KVK, agriculture, communication, socioeconomic, socio-psychological

INTRODUCTION

Agriculture is the most important sector of Indian economy. Therefore, the transformation of traditional agriculture to modern agriculture is a challenge to fulfil the requirements of over increasing population. Therefore, transfer of technology to the subsistence farmers has been the focus of Indian Planners and farm scientists. The Indian Council of Agricultural Research (ICAR) therefore, appointed a committee under the Chairmanship of Dr. Mohan Singh Mehta of Seva Mandir, Udaipur in 1973 for formulating the institutional design of Krishi Vigyan Kendra (KVK) for providing vocational training in agriculture. Soon after the submission of the report, the first Krishi Vigyan Kendra was established in 1974 itself at Pondicherry under the administrative and supervisory control of the Tamil Nadu Agricultural University, Coimbatore. Presently 732 KVK in the country out of which 494 KVK are managed by State agricultural universities (SAU'S)s and central agricultural university (CAU), 66 under ICAR institutes and 104 under NGOs, 38 under state governments, and the remaining 19 under other educational institution [7].

In West Bengal, 22 nos of KVK's are present. In view of the favourable growth areas, there is a need to conduct systematic analytical study to know the real benefit of the beneficiaries of the KVK. Therefore, the characteristics of those beneficiaries have to be understood meaningfully, so that benefits of KVK may be provided

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more successfully. With these objectives the present study has been undertaken to know the different characteristics of the beneficiaries in the selected area of study.

MATERIALS AND METHODS

The study was postulated through survey based adoptive research work. The investigation was carried out in six different agro climatic zone of west Bengal. In India, under ICAR system, where KVKs are functioning under 11 zones, out of which West Bengal belongs to zone VI. There are 732 KVKs all over the Country. In West Bengal, 22 numbers of KVKs are present under 6 agro climatic zones. From each zone, one district was selected randomly and 40 numbers of respondents from each KVK in the district were selected randomly. Thus total 240 numbers of beneficiaries were selected for the study. Prior to data collection sufficient rapport was established with the respondents during the first few days of preliminary data collection with the help of subject matter specialist at KVK with good social wealth. The field investigation was carried out April 2022 to June 2022. The pretested structured interview schedule was used by the researcher himself to collect the data. In this study 11 numbers of personal, socio-economic and 4 numbers of communication, socio psychological characteristics were taken in to consideration. After collection of data the necessary compilation and statistical analysis have been done.

RESULTS

The studies revealed that majority of beneficiary farmers (35-53%) were in the middle age group, followed by old age group respectively. The present findings are similar with the previous findings [9,3,15]. Majority of the respondents are married, except in zone-VI. Data presented in (Table 1.) indicates that majority (25-45%) of farmers were primary to HS level of education, followed by above higher level of education respectively. This finding is more or less in similar with those previous reported [2,11,12]. It is clear that almost half of the family followed by nuclear family in all zone. From the above discussion, it can be said that majority of the farmers were belonged to joint type of family. This indicates the existence of traditional system of living together in a family [13]. It is apparent from (Table 1.) that majority of farmers family had small & medium size of family, while only 25-37% large size of family. The probable cause for this might be their education and favorable attitude toward family planning. Similar findings were also reported [1]. Majority of the respondents are mainly involved in ruminant livestock farming. The (Table 1.) revealed that majority of the beneficiary farmers (25-50%) had small to medium size of land holding capacity and only 25-32.5% farmers had large capacity. Opposite findings were reported by different workers [2,14,16]. Due to lack of knowledge, majority of the respondents is low in farming experience rather high.

DISCUSSION

The presented data told that majority of respondents had kaccha house rather pakka. Majority of the farmers (32.5-50%) were dependent on agriculture and animal husbandry. It means that agriculture and animal husbandry were main occupation of the farmers. This finding is more or less in similar with those reported by other workers [2,5,15]. It said that 52 percent of the farmer had annual income between Rs. 50,001 to Rs. 1,00,000. The possible reason, as could be known during the field survey might be that majority of the farmers had animal husbandry with business income source along with agriculture which might have put them. This finding is similar to other workers [4,10,13]. Whereas, (Table 2.) revealed that majority (37.5-50%) of farmers had participation in one organization, followed by no participation in organization. The probable reason might be that activities carried out by Krishi Vigyan Kendra played a role in increasing the social participation of farmers. This finding is similar with the previous findings [2,8,11]. The data presented that slightly above two third farmers had low level of extension contact, followed by medium and high level of extension contact respectively. This finding is further similar by the work of other researchers [2,15,16]. The analysis of data showed that majority of beneficiaries (37.5-62.5%) of farmers had mass media exposure followed by local contact. This may be due to the facts that farmers might have been motivated through enormous benefits of KVK activities. This finding is similar with the finding of previous studies [6,15].

CONCLUSION

The study revealed that majority of the respondent were belong to middle to young age group and maximum stakeholders had primary to higher secondary level of education followed by maximum respondent farmers had medium to small size of land holding capacity. The present study stated that more than half of the farmers were dependent on agriculture and animal husbandry and majority of respondents belongs to joint

Table 1. Socio-Personal and Socioeconomic characteristics of KVK beneficiaries in various Agro-climatic zone of West Bengal, India.

Parameters	Zone-I (N=40)		Zone-II (N=40)		Zone-III (N=40)		ZoneIV (N=40)		Zone-V (N=40)		Zone-VI (N=40)	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Age												
Young age	15	37.5	10	25	12	30	22	55	8	20	8	20
Middle Age	20	50	20	50	19	47.5	14	35	21	52.5	19	47.5
Old Age	5	12.5	10	25	9	22.5	4	10	11	27.5	13	32.5
Marital Status												
Married	32	80	34	82	29	72.5	20	50	10	25	10	25
Unmarried	8	20	6	15	11	27.5	20	50	30	75	30	75
Education												
Illiterate	5	12.5	5	12.5	8	20	0	0	3	7.5	0	0
Up to Primary	10	25	15	37.5	12	30	12	30	12	30	12	30
Secondary&HS	10	25	15	37.5	10	25	18	45	15	37.5	18	45
Above HS	15	37.5	5	12.5	10	25	10	25	10	25	10	25
Family Type												
Nuclear	20	50	15	37.5	15	37.5	18	45	15	37.5	13	32.5
Joint	20	50	25	62.5	25	62.5	22	55	25	62.5	27	67.5
Family Size												
Small	5	12.5	3	7.5	5	12.5	2	5	8	20	3	7.5
Medium	20	50	25	62.5	22	55	25	62.5	22	55	25	62.5
Big	15	37.5	12	30	13	32.5	13	32.5	10	25	12	30
Livestock Farming												
Ruminant	30	75	22	55	24	60	20	50	28	70	25	62.5
Non ruminant	10	25	18	45	16	40	20	50	12	30	15	37.5
Land Holding												
SmallSize	15	37.5	20	50	15	37.5	12	30	20	50	20	50
Medium Size	15	37.5	7	17.5	15	37.5	18	45	10	25	10	25
LargeSize	10	25	13	32.5	10	25	10	25	10	25	10	25
Farming Exposure												
Low	20	50	20	50	25	62.5	20	50	20	50	17	42.5
Moderate	10	25	10	25	10	25	10	25	12	30	15	37.5
High	10	25	10	25	5	12.5	10	25	8	20	8	20
Material Possession												
Kaccha	28	70	27	67.5	29	72.5	25	62.5	24	60	24	60
Pakka	12	30	13	32.5	11	27.5	15	37.5	16	40	16	40
Occupation												
Agriculture	12	30	12	30	10	25	10	25	8	20	8	20
Agri and labour	5	12.5	8	20	10	25	12	30	8	20	8	20
Agri and A.H	13	32.5	15	37.5	20	50	18	45	20	50	15	37.5
Agri,A.H,Bus.	5	12.5	5	12.5	0	0	0	0	4	10	7	17.5
Agri,AH,Service	5	12.5	0	0	0	0	0	0	0	0	0	0
Occupation												
Low(>50,000/-)	5	12.5	8	20	5	12.5	8	20	3	7.5	4	10
Medium	24	60	25	62.5	20	50	15	37.5	2	5	25	62.5
High	11	27.5	7	17.5	15	37.5	17	42.5	12	30	11	27.5

Table 2. Communication and Socio-psychological Characteristics of KVK beneficiaries in various Agro-climatic zone of West Bengal, India

Parameters	Zone-I (N=40)		Zone-II (N=40)		Zone-III (N=40)		ZoneIV (N=40)		Zone-V (N=40)		Zone-VI (N=40)	
	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%	Freq	%
Social Participation												
No Participation	15	37.5	10	25	20	50	15	37.5	12	30	15	37.5
Participation In One Organization	20	50	15	37.5	15	37.5	20	50	20	50	15	37.5
Part.in More than One organization	5	12.5	13	32.5	5	12.5	5	12.5	8	20	10	25
Position Holder In Any Org.	0	0	2	5	0	0	0	0	0	0	0	0
Extension Contact												
Low	25	62.5	25	62.5	25	62.5	20	50	20	50	20	50
Medium	10	25	10	25	10	25	15	37.5	15	37.5	18	45
High	5	12.5	5	12.5	5	12.5	5	12.5	5	12.5	2	5
Source of Information												
Mass Media Exposure	25	62.5	25	62.5	15	37.5	20	60	25	62.5	25	62.5
Local Contact	15	37.5	15	37.5	25	62.5	16	40	15	37.5	15	37.5

family. Most of them were engaged in agriculture and animal husbandry for their income. Majority of the respondents had their family income more than Rs. 50,000 to Rs. 1 lakh (medium level). Most of them were involved in one organization with low level of extension contact. Finally, the study revealed that most of the respondents have mass media exposure as information source, which is indicative considering impact of KVK program among the beneficiary stakeholders in the area of study.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Research Article

SEASONAL PATTERN OF FOOT AND MOUTH DISEASE OUTBREAKS IN CATTLES ACROSS WEST BENGAL: 5 YEARS RETROSPECTIVE STUDY

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ABSTRACT

The present study was intended to find out the seasonal pattern of FMD outbreaks across the state of West Bengal. A total of 96 incidences of FMD were recorded in five years starting from 2018-19 in animal disease surveillance report published by epidemiological unit, Directorate of Animal Resources and Animal Health, Govt. of WB. The highest incidences were recorded during 2018-19(62) which dropped significantly in 2019-20(1) probably due to FMDCP programme. In 2020-21 and 2021-22 the reported FMD incidences were 15 and 17 respectively that came down to a single incidence in the year 2022-23. Irrespective of years, the highest incidences were reported in the month of August (19) followed by April (14) and September (13). But, the overall morbidity was highest in September (62.35%). Though, the highest incidence, case fatality rate (CFR) and morbidity were recorded during post-monsoon seasons but changing patterns in incidence, CFR and morbidity were seen in last couple of years. The higher incidences of FMD were shifted from post-monsoon to pre-monsoon seasons in 2020-21, 2021-22 and 2022-23. Higher CFR and morbidity% were recorded in winter in 2020-21 and 2021-22 instead of post-monsoon season. This changing pattern of pattern of FMD outbreaks in recent years should be considered to formulate preventive measures and therapeutic interventions in the State of West Bengal as important conclusions.

Keywords: FMD outbreaks, West Bengal, Season, Incidence, Morbidity, CFR

INTRODUCTION

Foot and Mouth Disease (FMD) is mainly an air-borne viral zoonotic disease of all cloven-hoofed animals. FMD is caused by apthovirus of *Picornaviridae* family [5]. FMD virus is the first virus to be identified and widely studied as the first virus in history of veterinary virology [5]. This disease is characterized by the occurrence vesicular eruptions in epithelium of buccal cavity, tongue, nares, muzzle, feet, teats and udder. FMD imposes considerable economic stress on the cattle rearers due to reduction of milk, meat and working capabilities of dairy and draught animals respectively. FMD leads to severe devastation of cattle populations across all the districts of West Bengal [3]. FMD also causes severe economic loss to cattle farmers in West Bengal. Studies of the outbreak of foot-and-mouth disease in West Bengal from 1985 -2002 have been evaluated [1]. However, data on study of seasonal influence on the outbreak patterns of FMD after 2002 in cattle across West Bengal is very scanty. So, accordingly the study was aimed to corelate the influence of season if any on the pattern of outbreak of FMD in cattle across West Bengal, as an little effort in order to minimize this devastating loss to the cattle farmers and so accordingly plan and advice the cattle farmers regarding FMD outbreaks in order to recommend them to take beforehand preventive measures and therapeutic interventions to minimize this devastating loss of cattle through this deadly disease. The overall aims of the work have been designed to find out the followings:

1. Retrospective study about the seasonal pattern of outbreaks of FMD across west Bengal (from 2018-2019 to 2022-2023).

2. Factors considered are number of incidences, population at risk, attack, death, case fatality rate (%),

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mortality (%) and morbidity (%) from 2018-2019 to 2022-2023.

This changing pattern of pattern of FMD outbreaks in recent years should be considered to formulate preventive measures and therapeutic interventions in the state of West Bengal.

MATERIALS AND METHODS

A total of 96 incidences of FMD were recorded in the last five years starting from 2018-19 in animal disease surveillance report published by epidemiological unit, directorate of animal resources and animal health, Govt. of WB in the internet. From the internet website, the author took all the data like the number of incidences, population at risk, attack, number of deaths, case fatality rate (CFR)%, Mortality% and Morbidity% of last 5 years till 2022-2023 according to month wise [Fig 1. - Fig 5.]. From the month wise data collected, author accordingly sorted out all these data collected into season wise (pre-monsoon, winter and post-monsoon seasons). Data analysis was done using SYS-STAT software by one-way ANOVA, and results were accordingly made.

RESULTS

The average number of infected animals and animal died in last five years from 2018-19 to 2022-23 reported are given in (Table 1. to Table 9.) Incidence of FMD outbreak becomes continuous throughout the year except the month of March (Table 2.). The incidence of outbreak is the highest in the year 2018-19 and the lowest in the years 2019-20 and 2022-23 (Table 1.). Month-wise morbidity is the highest in the month of September (62.35%) and the lowest in the month of March (3.56%) (Table 3.). Season-wise case fatality in the highest in the post-monsoon (Table 4.).

Table 1. Total incidence of FMD in the last 5 years

Total incidence in last 5 years	
2018-19	62
2019-20	1
2020-21	15
2021-22	17
2022-23	1

Table 2. Month wise incidence of FMD in last 5 years

Month wise incidence in last 5 years					
April	May	June	July	August	September
14	2	9	8	19	10
October	November	December	January	February	March
6	13	6	2	9	0

Table 3. Month wise overall morbidity of FMD in last 5 years

Month wise overall morbidity in last five years					
April	May	June	July	August	September
12.63	15.5	6.58	20.83	5.51	62.35
October	November	December	January	February	March
4.96	12.08	37.25	23.81	10.07	3.56

Table 4. Season wise incidences, season wise case fatality rate and season wise morbidity rate of FMD in last 5 years from 2018-2019 to 2022-2023

Season wise incidence			
	Pre-monsoon	Winter	Post-monsoon
2018-19	6	16	40
2019-20	0	16	40
2020-21	7	7	1
2021-22	13	2	2
2022-23	1	0	0
Season wise Case Fatality Rate			
	Pre-monsoon	Winter	Post-monsoon
2018-19	10.83	0	6.81
2019-20	0	0	6.81
2020-21	0	12.24	0
2021-22	0	11.76	25
2022-23	0	0	0
Season wise morbidity			
	Pre-monsoon	Winter	Post-monsoon
2018-19	9.38	32.84	73.88
2019-20	0	32.84	73.88
2020-21	3.55	23.28	1.26
2021-22	18.34	27.09	16.51
2022-23	7	0	0

Table 5. Season Wise variations of variables for the year 2018-2019

2018-19	Pre-moonson	Winter	Post-moonsoon
No of incidence	6	16	40
Population at risk	2748	5547	46275
Attack	100	212	19591
Death	5	0	363
CFR (%)	10.83	0	6.81
Morbidity (%)	9.38	32.84	73.88
Mortality (%)	0.58	0	1.28

Table 6. Season Wise variations of variables for the year 2019-2020

2019-20	Pre-monsoon	Winter	Post-monsoon
No of incidence	0	16	40
Population at risk	0	5547	46275
Attack	0	212	19591
Death0	0	363	
CFR (%)	0	0	6.81
Morbidity (%)	0	32.84	73.88
Mortality (%)	0	0	1.28

Table 7. Season Wise variations of variables for the year 2020-2021

2020-21	Pre-moonson	Winter	Post-moonsoon
No of incidence	7	7	1
Population at risk	620	766	317
Attack	22	62	4
Death0	6	0	
CFR (%)	0	12.24	0
Morbidity (%)	3.55	23.28	1.26
Mortality (%)	0	1.01	0

Table 8. Season Wise variations of variables for the year 2021-2022

2021-22	Pre-moonson	Winter	Post-moonsoon
No of incidence	13	2	2
Population at risk	18108	251	614
Attack	90	68	44
Death0	8	1	
CFR (%)	0	11.76	25
Morbidity (%)	18.34	27.09	16.51
Mortality (%)	0	3.19	0.28

Table 9. Season Wise variations of variables for the year 2022-2023

2022-23	Pre-winter	Winter	Post Winter
No of incidence	1	0	0
Population at risk	100	0	0
Attack	7	0	0
Death0	0	0	
CFR (%)	0	0	0
Morbidity (%)	7	0	0
Mortality (%)	0	0	0

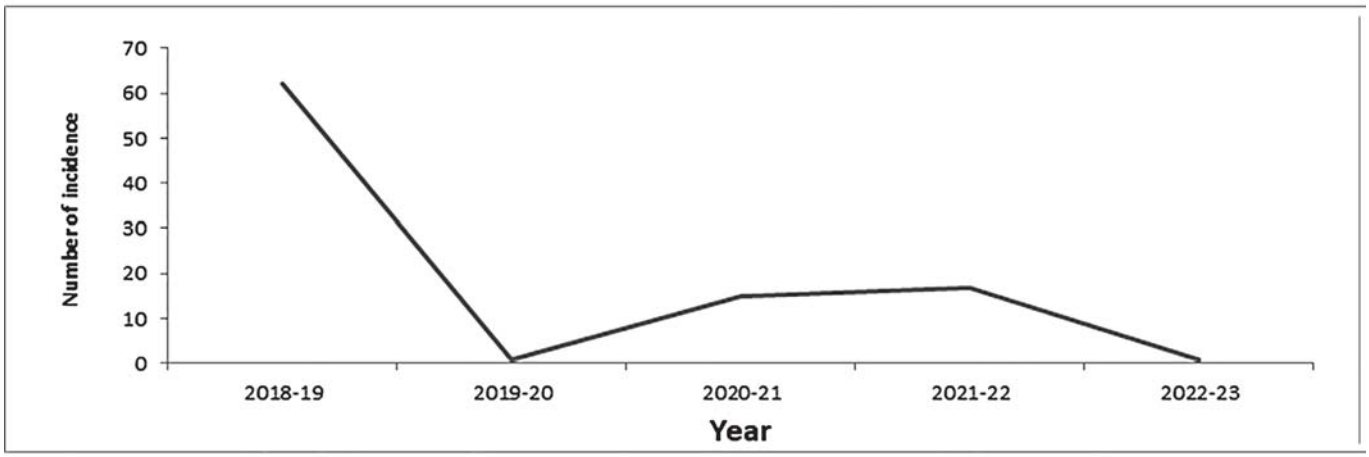

Fig 1. Number of Incidence Vs Year of occurrence

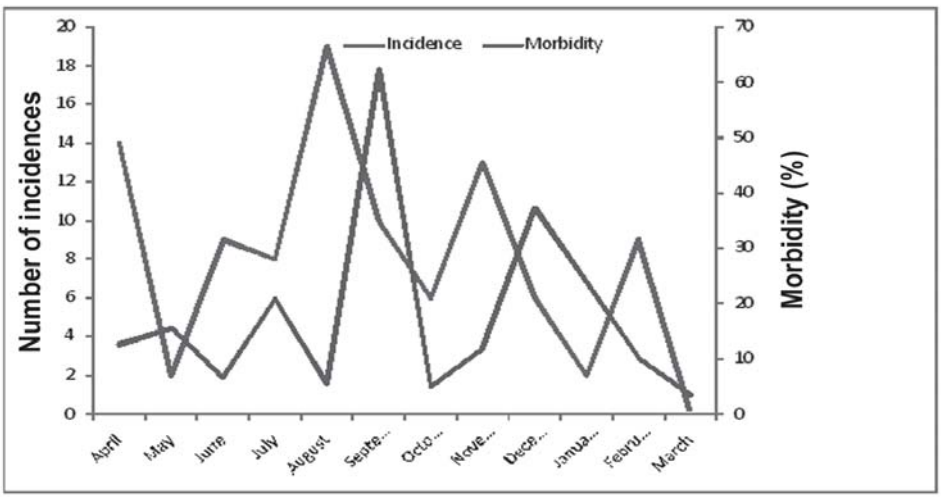

Fig 2. Number of incidences and Morbidity % Vs Month of Occurrence

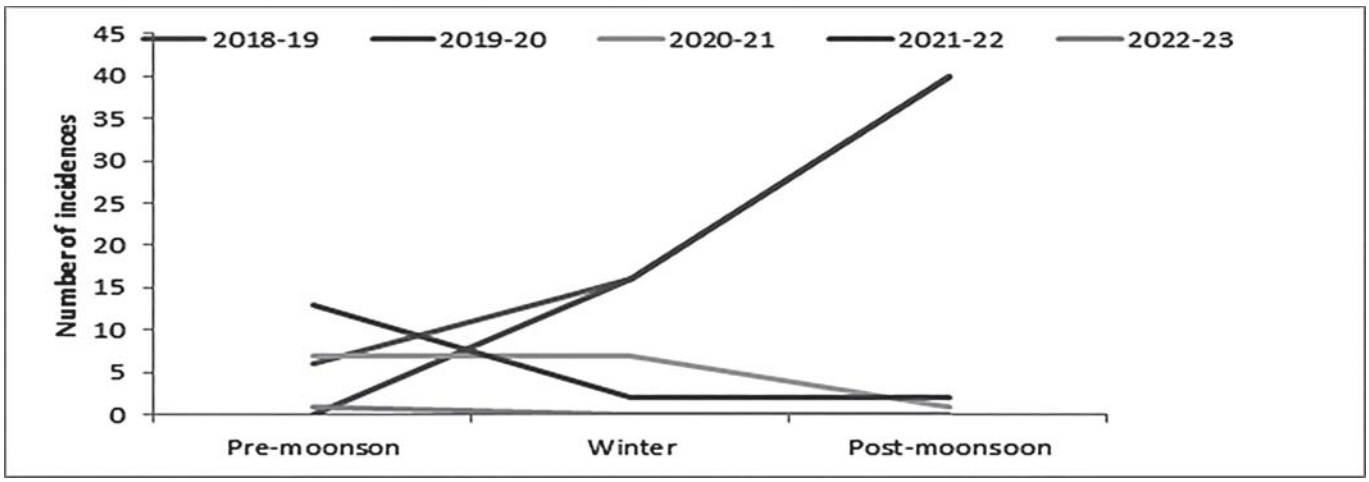

Fig 3. Number of incidences Vs Season Wise incidence

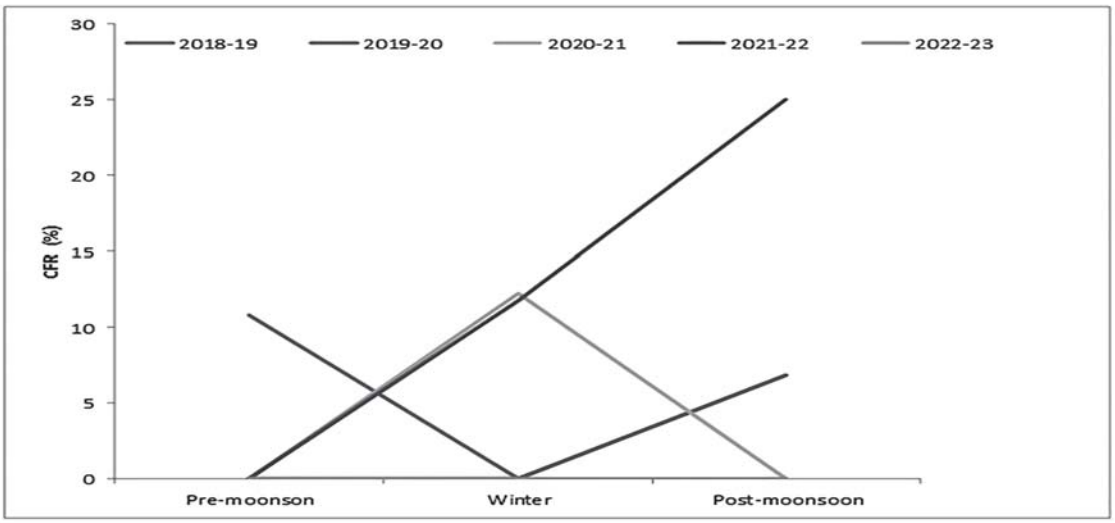

Fig 4. Case Fatality rate% Vs Season Wise Incidence

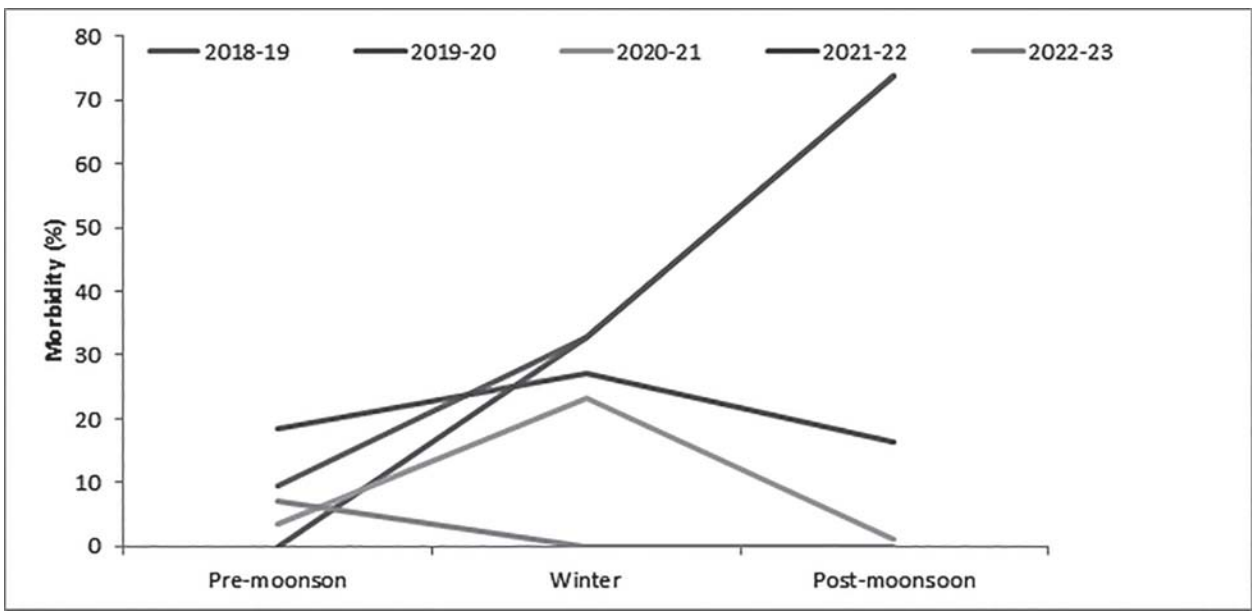

Fig 5. Morbidity % Vs Season Wise Incidence

DISCUSSION

In 5 years (from the year 2018-19 to 2022-23) retrospective study about the seasonal pattern of outbreaks of Foot and Mouth Disease (FMD) across the state of West Bengal was studied. A total of 96 incidences of FMD were recorded in last five years, in animal disease surveillance report published by epidemiological unit of directorate of animal resources and animal health under Govt. of WB. The highest incidences (62) were recorded during 2018-19, which dropped significantly to a single incidence in 2019-20 probably due to FMDCP programme. Estimation of economic losses due to FMD infection in per infected cattle is Rs.12,532, in buffalo is about Rs.21,682 and annual losses in India ranges from Rs. 12,000-14,000 crores [8]. These 5 years retrospective study of FMDCP (from 2018-2019 to 2022-2023) however agreed with the earlier studies conducted by Mathew and Menon who reported infection is about 2.42% and mortality rate 0.12% from the state of Kerela [6]. Among different component of loses the maximum loss in milk production (49.83%), opportunity cost (16.15%), reduction in growth (12.20%), loss of work power (9.35%), treatment cost (8.83%) and overall reproductive loss (14.95%) [8]. In 2020-21 and 2021-22 the reported FMD incidences were 15 and 17 respectively, that came down to a single incidence in the year 2022-23. Irrespective of years, the highest incidences were reported in the month of August (19), followed by April (14) and September (13). The overall morbidity% was highest in September (62.35%), thus agreed with the studies of Sellers and Parker who all stated that the cattle served as indicator hosts, sheep as the maintenance hosts and pigs as amplifier hosts [7].

Though, the highest incidence, CFR and morbidity were recorded during post-monsoon seasons but changing patterns in no of incidence, CFR and morbidity% were seen in last couple of years. The occurrence of higher no of outbreaks of FMD were shifted from post-monsoon to pre-monsoon seasons in the years 2020-21, 2021-22 and 2022-23, which clearly agrees with the reports that FMD disease is prevalent in all the states of india in all the seasons irrespective of rainfall [10]. Higher CFR and morbidity% were recorded in winter in 2020-21 and 2021-22 instead of post-monsoon season. Highest incidences (62) were recorded during the F.Y 2018-19, which dropped significantly (1) in 2019-20, probably due to FMDCP programme.

In 2020-21 and 2021-22, the reported FMD incidences were 15 and 17 respectively, that came down to a single incidence in the year 2022-23. The highest incidences (19) were reported in month of August followed by April (14) and September (13). The overall morbidity rate of FMD was highest in month of September (62.35%). The higher incidences of FMD were shifted from post-monsoon to pre-monsoon seasons in the years 2020-21, 2021-22 and 2022-23. Both the case fatality rate (CFR) and morbidity were recorded during the post-monsoon seasons, but changing patterns in incidence, CFR (%) and morbidity were seen in last couple of years, mainly due to anemia, over growth of body hairs, mastitis etc. as was reported [4]. Higher CFR and morbidity (%) were recorded in winter season in the financial years 2020-21 and 2021-22 instead of post-monsoon seasons, which were also reported in the Errode district of Tanil Nadu where incidence of FMDV highest in Winter season which stated that the vaccines at present available are not enough to guarantee the continuing protection of the high-grade animals against the constant risk of exposure to FMD viruses which might be circulating in the indigenous stock [9].

CONCLUSION

The changing pattern of Foot and Mouth Disease outbreaks in recent years should be considered to formulate preventive measures and the therapeutic interventions in state of West Bengal.

ACKNOWLEDGEMENT

The author is very thankful to the website of the animal disease surveillance report published by the epidemiological unit, Directorate of Animal Resources and Animal Health, Government of West Bengal for providing all the data values for making this research article.

CONFLICT OF INTEREST

The author along with the corresponding author don't have any conflict of interest at all with anybody.

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Research Article

EFFICIENCY OF UTILIZATION OF DIETARY ENERGY FOR MILK PRODUCTION IN LACTATING CROSSBRED CATTLE (*BOS INDICUS*)

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ABSTRACT

Studies were conducted on the efficiency of utilization of dietary energy for milk production in lactating crossbred cattle. Eighteen lactating crossbred cattle of early to mid lactation, same body weight (375.39±23.43 kg), milk yield, parity and stage of lactation were divided into three groups of six animals each and were fed 0, 50 and 100% diammonium phosphate (DAP) in the mineral mixture of concentrates for 120 days. The chaffed mixed roughage (berseem + wheat straw) and concentrate mixture was fed to supply about nearly 18:82 concentrate to roughage ratio on dry matter basis. Tap water was available ad lib. A metabolism trial of seven days was conducted at the end of experiment to study digestibility of organic nutrients and balances of energy. Diammonium phosphate did not affect the nutrient intake, body weight changes, digestibility of DM, CP, EE, CF, NFE and daily milk yield, From the experiment, it was concluded that the at 46.07 Mcal GE intake level the losses in faeces, urine, methane and heat production was 45.82%, 5.40%, 4.31% and 33.01% and net energy retention for milk production was 11.43%. The gross efficiency of conversion of ME for milk production was 35.69% and the net efficiency of conversion of ME for milk production was 39.56%.

Keywords: Diammonium phosphate, dietary energy, cattle, milk yield, gross energy

INTRODUCTION

Efficiency of utilization of energy for milk production is governed by a variety of factors, viz. ration composition, environmental temperature and stage of lactation [1]. Under Indian conditions, no studies have been reported to determine the efficiency of energy utilization in lactating crossbred cattle. Hence, the present study was aimed to determine the efficiency of energy utilization for milk production in lactating crossbred cattle when they were fed with concentrate and mixed roughage (berseem+wheat straw) along with the replacement of dicalcium phosphate with diammonium phosphate in the mineral mixture.

MATERIALS AND METHODS

The experiment was conducted on eighteen lactating crossbred cattle of approximate same body weight (375.39 ± 23.43 kg), milk yield, parity and stage of lactation were divided into three groups of six animals each. In the experimental groups, the DCP (dicalcium phosphate) in the mineral mixture was replaced with 50% DAP (T2) and 100% DAP (T3), respectively (Table 1.). The required amount of urea was incorporated in the mineral mixture (T1 and T2) to keep the rations isonitrogenous. Different amounts of limestone were added to all the diets to maintain the identical calcium content. The animals were fed a calculated quantity of balanced ration to fulfill their nutrient requirements as per ICAR (1998) standards [2]. Clean drinking water was offered to the animals twice daily. The ration scheduled was adjusted weekly on the basis of the milk production of the crossbred cattle. All the animals were offered weighed amounts of mixed roughage (berseem+wheat straw). Concentrate allowance was offered in two portions, one at morning milking (3.00 AM) and the other at afternoon milking (3.30 PM). The concentrate mixture consisted of 40 parts crushed maize, 22 parts wheat bran, 35.5 parts mustard cake, 2 parts mineral mixtures and 0.50 part common salt.

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Milk records were kept for individual cows throughout the experimental period.

Animals were fed experimental rations for 120 days inclusive of seven days metabolic trial, which was conducted at the end of the trial period. Faeces and urine were quantitatively collected and were preserved for further analysis. Aliquots of milk were taken during morning and afternoon for each animal. Faeces, urine, feeds, residues and milk were analyzed for proximate constituents by AOAC (1990) methods [3]. The fat content of milk was determined in Soxhlet apparatus [4]. The data obtained during experiment were analyzed by using randomized block design method as described by Snedecor and Cochran (1994) [5].

Table 1. Ingredient composition of mineral mixtures.

Ingredients	T_1	T_2	T_3
DCP	31.34	15.67	-
DAP	-	15.67	31.34
LSP	21.18	33.15	45.12
Common salt	21.66	21.66	21.66
TM*	1.87	1.87	1.87
Urea	14.26	7.13	-
Filler	9.67	4.84	-
Ca%	15.34	15.34	15.34
P%	6.58	6.42	6.26

*Trace mineral contained cobalt chloride 40g, copper sulphate 240g, ferrous sulphate 780g, manganese sulphate 780g, sodium selenite 8g and potassium iodide 24g.

GE of a feed was calculated from its chemical composition as per the formula suggested by Ewan (1989) [6].

$$GE\ (kcal/kg) = 4{,}143 + (56X\%\ EE) + (15X\%\ CP) - (44X\%\ ash)$$

DE was calculated from the TDN value obtained (1g TDN= 4.4 kcal DE). Urine (10%) and methane (8%) losses were calculated from DE.

The gross efficiency of milk production was calculated presuming 1 kg 4% FCM contained 750 Kcal and 1 kg TDN contained 3600 kcal ME [7]. The gross efficiency of ME of milk production was calculated as follows

Gross efficiency of milk production

$$= X\frac{750\ FCM\ (kg)}{3600\ TDN_{\ 1}(kg)}\ 100$$

The net efficiency of milk production was calculated by subtracting TDN or ME utilized for the maintenance from total energy intake.

Gross efficiency of milk production

$$= X\frac{750\ FCM\ (kg)}{ME_1\text{-}129kcal\ ME\ /W^{0.75}kg}\ 100$$

RESULTS

The chemical composition of the experimental diets (concentrate mixtures) and mixed roughage offered to the experimental animals are presented in Table 2. Due to replacement of DCP at 50% in T_2 and 100% in T_3 diet, the chemical composition in respect of CP, EE, CF, ash, Ca and P content did not vary as compared to the control (T_1).

Table 2. Chemical composition of concentrate mixtures and mixed roughage on DM basis (%).

Particular	Concentrate mixtures			Mixed roughage
	T_1	T_2	T_3	
DM	92.96	92.48	93.01	39.86
CP	19.96	19.88	20.15	06.24
EE	4.39	4.48	4.74	02.23
CF	6.21	6.76	6.54	30.29
Ash	11.08	11.57	11.76	09.76
NFE	58.36	57.31	56.81	51.48
Ca	1.09	0.91	1.02	0.34
P	0.82	0.77	0.86	0.22

The digestibility coefficient of various organic nutrients is shown in Table 3. There was no significant difference in the digestibility of DM, CP, EE and NFE of experimental diets,

Table 3. Digestibility coefficient of organic nutrients.

Organic nutrients	T_1	T_2	T_3
DM	58.57 ± 1.70	60.46 ± 2.90	61.51 ± 2.00
CP	60.50 ± 1.85	61.63 ± 3.67	63.19 ± 2.06
EE	51.85 ± 2.57	54.64 ± 3.55	54.20 ± 2.12
CF	74.22 ± 1.19	76.12 ± 1.25	77.89 ± 1.15
NFE	73.12 ± 1.00	73.84 ± 1.90	74.66 ± 1.28

Intake of all the nutrients (Table 4.) was similar in the control (T1) and the experimental groups (T2 and T3). During the experimental period the animals showed very little change in body weight.

Table 4. Daily nutrient intake, live weight changes and milk production in lactating crossbred cattle

Particulars	T_1	T_2	T_3	Average
DMI(g/w0.75kg)	130.94±7.55	129.90 ±7.64	126.91 ±4.84	117.78
DCP(g)	623.71±26.92	617.51±34.96	625.28±29.05	622.16
TDN(kg)	6.05±0.26	5.53±0.30	5.43±0.20	5.67
ME(Mcal)	21.07±1.49	20.01±1.40	20.66±1.38	20.58
Gain/loss(g/d)	+78.44±22.53	+41.76±34.51	+25.99±18.00	48.73
4% FCM production (kg/d)	9.85 ± 0.19	9.55 ± 0.13	9.77 ± 0.16	8.58

All the three diets (T_1, T_2 and T_3) were comparable in dry matter intake and digestibility of organic nutrient, hence the data were pooled for eighteen lactating crossbred cattle and the distribution of GE and the efficiency of utilization of energy was calculated by a factorial method.

Percentage distribution of gross energy in feeds, faeces, urine, methane, milk and heat production and tissue deposition is presented in Table 5. The energy of heat production and tissue deposition was calculated as gross energy consumed, which was not excreted in faeces, urine, methane or milk.

Table 5. Distribution of gross energy

Energy	GE (Mcal)	FE (Mcal)	DE (Mcal)	UE (Mcal)	Methane (Mcal)	Milk (Mcal)	Heat production and tissue deposition(Mcal)	NEMilk (Mcal)
Distribution	46.07	21.11	24.96	2.49	1.99	20.48	15.21	5.27
% of GE	100	45.82	54.17	5.40	4.31	44.45	33.01	11.43

DISCUSSION

In the present study out of 46.07 Mcal GE intake level, the losses in faeces, urine, methane and heat production were 45.82%,5.40%,4.31% and 33.01%, respectively, leaving behind a net energy retention for milk production as 11.43%. Similar study on various losses at the same GE intake level (53.1Mcal) as 37% in faeces, 2.3% in urine, 5.5% in methane, 14.8% in milk production and 40.4% in heat production and tissue deposition in cross bred cows [8]. The lower faecal loss in the present study may be due to higher DM digestibility. The higher heat production in our study may due to higher roughage to concentrate ratio (82:18) compared to 50:50 in [8]. The gross efficiency of ME for milk production was 35.69%, which is within the range of 19.1 to 38.6% for various types of roughages the gross efficiency of milk production from 18.54 to 20.11% with the complete feed compared with conventional type of feeding system [9].

The net efficiency of ME for milk production was 39.56%, which is similar to that (37.57%) reported earlier [10] during mid lactation. They further reported higher (52.24%) during early and lower (29.50%) in late stage of lactation. Efficiency of utilization of energy for milk production is governed by a variety of factors viz. ration composition, environmental temperature and stage of lactation. High environmental temperature caused a significant decrease in efficiency of energy utilization for milk production [11]. Similarly, low efficiency of energy utilization for milk production in our experiment was due to the high environmental temperature (average 38.31°C) in the month of June during the metabolic trial period.

CONCLUSION

From the study, it was concluded that out of 46.07 Mcal GE intake, the losses in faeces, urine, methane and heat production + tissue deposition were 45.82%, 5.40%, 4.31% and 33.01%, respectively, and the net energy retention for milk production was 11.43%.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Research Article

PREVALENCE STATUS AND PROJECTED ECONOMIC LOSSES DUE TO THEILERIOSIS IN CROSS-BRED DAIRY CATTLE IN WEST BENGAL, INDIA

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ABSTRACT

Theileriosis is a common tick -borne haemoprotozoan parasitic disease of dairy cattle in India and the world. The aim of present study was to investigate the prevalence of theileriosis and estimation of projected economic losses due to theileriosis in dairy cross-bred adult cattle of West Bengal, India. A total of 268 adult cross-bred dairy cattle were screened randomly by blood smear examination from October 2021 to September 2023. The findings revealed that the adult cross-bred dairy cattle in study area was infested with theileria is 23.13% (62 animal). During the study the affected animal was treated with specific chemotherapeutic drug bupervacquone @dose 2.5 mg / kg body weight and out of 62 affected animals, 5 animals were died (Mortality rate/ case fatality rate was 1.86 %). The total projected economic losses due to theileriosis in adult cross-bred dairy cattle was worked out as sum of mortality loss, loss in milk yield and cost of treatment of affected animal. In the study it was estimated that the projected losses due to theileriosis per affected adult cross-bred cattle was Rs. 13452.00. The study thus revealed that a huge economic losses was occurred due to theileriosis in adult cross-bred dairy cattle which may cause negative impact on livelihood security among the dairy farmers. Thus special impetus should be taken for preventive measures to control the disease in cattle.

Keywords: Prevalence, theileriosis, cattle, economic losses

INTRODUCTION

Consequent to effects of global warming, resistance to insecticides due to its indiscriminate use, wrong animal husbandry practices and poor status of nutrition it was observed that incidence of tick-borne haemoprotozoan diseases (TBHD) is on high rising trend in last 15 years in India. These TBHD like theileriosis , babesiosis and anaplasmosis are of principal economic importance [12] but the bovine tropical theileriosis caused by *Theileria annulata,* transmitted by *Hyalomma anatolicum* tick is a major constraint to the dairy industry and livestock production in India [7,14]. The impact of the disease is more in exotic and crossbred cattle and 40-60% cattle may be died [2] and mortality rate may reach up to 80% as was reported for the first times in 1970s' when cross breeding programme started in India to enhance the productivity of dairy cattle [16]. The affected animals shows the symptom like pyrexia, in appropriate appetite, conjunctival petechiation, anemia, enlargement of lymph node, anorexia, progressive weight loss and loss of milk production in lactating animal. Several studies in the Indian context are available on the prevalence of theileriosis which have numerous impacts including productivity losses, cost of treatment, market disturbances, prevention and control cost etc. In West Bengal due to ambient temperature, relative humidity, moderate rain fall, adequate water source which are conducive factor for survival and breeding of tick population which is the major factor to spread this disease [5].

In the above context, a comprehensive economic assessment of bovine theileriosis is of utmost importance before formulating the various livestock interventions effort. It was reported that economic loss due to bovine tropical theileriosis in India is Rs. 8426.7 crore annually [11] but no literature is available on economic loss due to

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theileriosis in respect of West Bengal. Hence it is necessary to focus on the estimation of projected economic losses due to theileriosis with using prevalence status of the disease to know its impact on livelihood security among dairy farmers in West Bengal.

MATERIAL AND METHODS

The study was done in two Parts: I. Prevalence Studies II. Estimation of Economic loss.

Prevalance Studies

Sample Collection

It was carried out in adult cross-bred dairy cattle of Howrah and Hooghly district in West Bengal, maintained under small dairy cattle system and were screened by microscopical examination of blood samples. The average annual temperature of these districts ranges from 26.1° C to 26.8° C, average relative humidity is around 77% although it vary from around 65% during summer to 88% during the monsoon and average rain fall (1523-1625 mm).

The investigation was carried out for a continuous period of 2 years i.e. October 2021 to September 2023. A total of 268 Blood samples were collected randomly from both apparently healthy and clinically suspected cross-bred dairy cattle above 3 years of age. Thin blood smears were prepared from the blood collected from ear vein.

Staining Method

The blood smears were fixed with methanol and stained with Giemsa's stain and examined under microscope (100X) with immersion oil for the identification of parasites following procedure as described [1,15].

Estimation of Economic Losses

The total Economic losses due to theileriosis in adult cross-bred dairy cattle was worked out with following the procedure [13] with some modification adopted in field condition.

It is expressed as:

T= A+B+C

A= Mortality Loss

B= Loss in milk yield

C= Cost of treatment of affected cattle

Loss from Mortality= (D×P) + Treatment cost

D= Number of cattle died due to theileriosis

P= Probable Market value of the Cattle.

Loss in milk yield = loss of milk per recovered cattle which are in lactating stage

C= Treatment Cost = I × Tc

I= No. Of infected animal

Tc = Average treatment cost per infected animal

For estimation of treatment cost due to theileriosis further studies were done where the affected animal were treated with specific chemotherapeutic agent Buparvaquone @ dose 2.5 mg/ kg b. wt. [8,14] with some supportive therapy

RESULTS

Microscopic examination of 268 blood smears from adult cross-bred dairy cattle revealed 62 (23.13%) samples positive for theileriosis (Table 1.). Similar type of observations reported from West Bengal (22.9%) [5] and from Chhattishgarh (23.33%) [10]. Incidentall, it was reported that *T. annulata* infection in vectors (ticks) in West Bengal was 31% by microscopy and 42.8% in PCR assay [4]. While 15.38 % occurrence of theileriosis in cattle in Rajasthan was observed [3] and the incidence of theileriosis was higher in cross-breed (63.57%) than Indigenous (36.43%) in respect of overall incidence (31.05%) in Bihar [6]. These variations might be due to different geographical distribution, dairy animal rearing systems, management practices, breed and age of the cattle or whether the sample was collected from apparently healthy or clinically suspected animal. However through systematic review and meta-analysis, the highest prevalance of theileriosis was observed in Puducherry (71%), followed Haryana (39%), Kerala (39%), and lowest prevalance was observed in Telengana (2%), West Bengal (7%), Andhrapradesh (8%) and overall prevalance in cattle (22%) [7].

DISCUSSION

During the study of economic losses it should be considered that all the estimates are approximate/ projected and exact losses can never be possible to work out. This study carried out with using some field data as well as scientific literature available on such parameter or it can be simply worked out by taking into account probable loss and cost of treatment. However estimation of projected economic losses will be help full in citing at least approximate losses occurring on the account of infection of particular parasitic species in question [11].

Table 1. Mortality Rate/Case Fatality Rate of Theileriosis

No. of animal	No. of infected	No. of died	Mortality rate
268	62 (23.13%)	5	1.86%

Table 2. Mortality losses due to Theileriosis (A)

No. of died	Total Losses due to cost of the died cattle*	Total Treatment cost**	Total losses
5	Rs.250000.00	Rs.8500.00	Rs.258500.00

*Probable market value of animal considered = Rs.50000.00; **Average treatment cost per animal= Rs. 1700.00

Table 3. Morbidity losses due to Theileriosis

Species/ breed	Total No, of Recover	No. of Lactating animal	Loss of Milk per lactated animal in Liter (Small holder dairy cattle system, Minajauw and McLeod, 2003)	Average Market price of milk per Liter	Total Losses due to loss of Milk Production (B)	Total Treatment cost (C)	Total Losses
Cross-bred (cow)	57	31	386	Rs. 40.00	Rs. 478640.00	Rs. 96900.00	Rs.575540.00

Total projected economic losses due to loss in milk yield per recovered animal= Rs. 8397.00

Total projected eonomic losses due to theileriosis: (A+B+C)= Rs. (258500+478640+96900).00

= Rs.834040.00

Toatal projected economic losses per affected animal due to Theileriosis = Rs. 13452.00

Mortality Losses due to theileriosis in adult cross bred dairy cattle were Rs. 258500.00 in which treatment cost contributed 3.29% [Table 2.). In the study total projected economic losses due to loss in milk yield per recovered animal Rs. 8397/- under small holder dairy cattle system. Projected economic losses due to loss in milk yield due to theileriosis in cross-bred cattle in Gujrat, Karnataka,Kerala, Tamilnadu and Uttarakhand were Rs. 1785.62, Rs.854.20, Rs.772.16, Rs. 627.38 and Rs.2191.00 respectively [11] where loss of milk per affected animal in liter =127 (under crop-livestock farming system [9]. Rate of milk per liter considered as Rs. 38.00 and loss per animal was calculated total loss in milk in Rs. / total population as per 19th livestock census, whereas in this study total projected economic Losses due to theileriosis was Rs. 834040.00 in which share of loss due to milk production & mortality losses were 57.39 % and 30.99 % respectively. Total morbidity losses due to theileriosis was Rs. 575540.00 in which share of loss due to milk production and cost of treatment were 83.16% and 16.84% respectively (Table 3.). The study thus revealed that there is a huge economic losses due to theileriosis and thus may hamper the sustainability of livelihood of the dairy farmer in West Bengal. Earlier it is reported that the estimated annual economic loss due to bovine tropical theileriosis in India is US$ 384.3 million per annum [9] and due to tropical theileriosis in India Is US$ 1,295 million (Rs. 8426.7 crore) per annum [11].

CONCLUSION

The Occurrence of theileriosis in dairy cattle specially in cross-bred cattle may results in loss of milk production over time, cost of treatment, loss due to morbidity can trigger economic loss to dairy farmers. So preventive measures should be taken to control the disease by means of vector control, to take surveillance programme in sustainable manner and also by using novel diagnostic method for early detection of the disease so that loss due to mortality, production and cost of treatment may be minimized.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Short Communication

PREVALENCE OF *Haemoproteus columbae* INFECTION IN PIGEONS: A STUDY ACROSS FOUR DISTRICTS OF WEST BENGAL

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ABSTRACT

The present study was carried out to know the prevalence of *Haemoproteus columbae* infection in captive pigeon in four districts viz. Kolkata, South 24 Parganas, North 24 Parganas and Malda. The sample was collected from sicked pigeon, smear was prepared, stained with Giemsa's and seen under 100X magnification. The presence of micro and macrogamotocyes inside the RBC was detected which showed the positive in the *H. columbae* infection in pigeon. The prevalence was 5.56% (South 24 Parganas), 9.38% (North 24 Parganas), 13.33% (Kolkata) and 20% (Malda). The sicked pigeons recovered completely after treated with Bupervaquone @ 5mg/kg body weight intra-muscularly at 72 hours interval with multivitamins. Further investigation season-wise, sex-wise and age-wise by molecular study is required to get more complete data about haemoprotozoan load in pigeon throughout West Bengal.

Keywords: *Haemoproteus columbae*, Giemsa's, Prevalence

INTRODUCTION

Pigeons were the most ancient domesticated birds in the world [1]. Pigeons and dove comprise 50% of all the birds kept in captivity for food, hobby, entertainment and display [6]. Parasitic infection affects the growth rate, egg production, weight gain and immune status [3]. Haemo proteus is an apicomplexan, intracellular parasite of birds, reptiles & amphibians [5]. *Haemoproteus columbae* is a haemotozoon that commonly infects pigeons in tropical countries [2]. The vector responsible for the transmission of *H. columbae* are haematophagus, Hippoboscid fly, Pseudolynchia canaviensis [2]. It is fatal diseases of young and stressed birds but adults are non-pathogenic. The clinical sign includes anorexia, lethargy, depression, dyspnea, circling movement and diarrhea [15]. The prevalence study of *H. columbae* infection in pigeons is higher in Indian states namely Kerela and Gujrat [11]. Prevalence of *H. columbae* in pigeon popularly named as pseudo malaria or pigeon malaria because the parasite resembles as plasmodium species [4]. Haemoproteus infection is commonly diagnosed based on microscopic examination of thin blood smear where gametocytes are seen within the erythrocytes. The blood of Haemoproteus revealed gametogenic stages the male (microgametocyte) is distinguishable from female macrogametocyte by its larger and more diffuse nucleus. Usually the concentration was spare (1-6 pars/100 RBC) but occasionally a high degree of erythrocytes parasitization was visible (10-20 pars/100 RBC). Occasionally, the parasites infect two adjacent cells. The young and immature forms develop lateral to the host cell nucleus and have no contact with the host cell membrane or the host cell nucleus [12].

Mature forms could be differentiated into macrogametocytes (randomly scattered granules, nucleus with clear margin), and microgametocyte granular polar, nucleus diffused with cytoplasm.

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Macrogametocytes are broadly sausage shaped, slightly halteridial and usually laterally situated to the erythrocytic nucleus. The full-grown parasite reached the poles of the infected erythrocytes but never encircled its nucleus. Cytoplasm of the parasite was moderately coarse and stained pale blue with Giemsa's stain. The granules are medium or small sized and dispersed randomly in all parts of the cytoplasm averaging 20 per granules. Macrogametocytes are broad at one end and narrow at other end, curved around the host cell nucleus, sometimes cytoplasm of host cell becomes completely filled with macrogametocyte. Microgametocyte is slightly larger adhere to the cell membrane at the polar zone. Cytoplasm of the mature form is slightly stained with Giemsa's or occasionally colorless [12].

MATERIAL AND METHODS

Collection of Blood Sample

Blood was taken from winged vein of sicked pigeons and immediately poured into EDTA-coated collection tubes. The tubes were gently coated through inversion and appropriately labelled. The blood samples were taken from four districts viz. North 24 Parganas, South 24 Parganas, Kolkata and Malda. The pigeons were reared in domesticated manner.

Preparation of Blood Slide

A thin blood smear was prepared on a clean, grease-free glass slide. Firstly, a drop of blood was taken on the slide. Using second slide on top of the first holding at an angle of 45°, the blood was spreaded, air dried completely and flooded with absolute methanol (100%) for 3-5 minutes. The smeared was air-dried and flooded with Giemsa's stain diluted with PBS (pH 6.8) and kept for 45-60 minutes. The stained smear was washed in distilled water & examined under high-power oil immersion lens (100X). Parasite identification was carried out based on pigmentation pattern, stage-specific morphology, shape of gametocytes in cytoplasm of parasite infected RBC.

RESULTS AND TREATMENTS

The blood samples were collected, smeared with Giemsa's and seen under 100 X magnification for detection of microgametocytes and macrogametocytes based on the pigmentation of parasites, position of gametes in the erythrocytes (Fig 1-4.). The prevalence of *H. columbae* was 5.56% in South 24 Parganas, 9.38% in North 24 Parganas, 13.33 % in Kolkata and 20% in Malda (Table 1.).

The treatment of sicked pigeons was initiated with Bupervaquone @ 5mg/kg body weight intra-muscularly 72 hours apart [7,11]. The multivitamins @0.2 ml per bird twice daily for 2 weeks in drinking water was supplemented to remove stress and enhance immunity [9]. The vectors of *H. columbae* were controlled by changing the litter regularly and treating the pigeons' shelter with Deltamethrin @ 2 ml/litre of water [11]. Regular review and monitoring revealed complete recovery of the pigeon in two week and no infection was traced by follow-up blood smear examination.

DISCUSSION

From the present study, it was found that the prevalence of *H. columbae* infection was 5.55% (South 24 Parganas), 9.38% (North 24 Parganas), 13.33 % (Kolkata) and 20% (Malda). The systemic study conducted in four districts concluded that favorable climatic condition and presence of vectors are the contributing factors towards prevalence of haemo protozoan parasite in west Bengal. Sex-wise prevalence of Haemoproteus was recorded more in female (34.81%) than male (25.30%), age-wise prevalence was recorded in squab (<30 days) 18.75%, young (30-90 days) 34.32% and adult (>90 days) 33.72 % in Assam [12]. Occurance of *H. columbae* in Khulna district of Bangladesh has also been reported [10]. Haemoprotozoan infection was recorded highest during pre-monsoon season (72.22%) and lowest during post monsoon (46.15%). However, the infection was more or less present through-out the season [12]. Molecular detection of *H. Columbae* targeting cyt b gene was carried amplifying 207 bp band which shows the occurrence of *H. columbae* infection to the tune of 54.7% in Giemsa's-stained blood smear and 63.63% in PCR amplified cyt b gene in Kerala [14]. The prevalence study of *H. columbae* was 23.8% from Hooghly district of West Bengal [8]. Therapeutic management of pseudo-malaria in a flock of pigeons with chloroquine has been tried giving promising results [13].

CONCLUSION

The present study reveals that the overall prevalence of *H. columbae* is about 9.68 % in four districts of West Bengal. Microscopic examination is a gold-standard and cost-effective technique for diagnosis

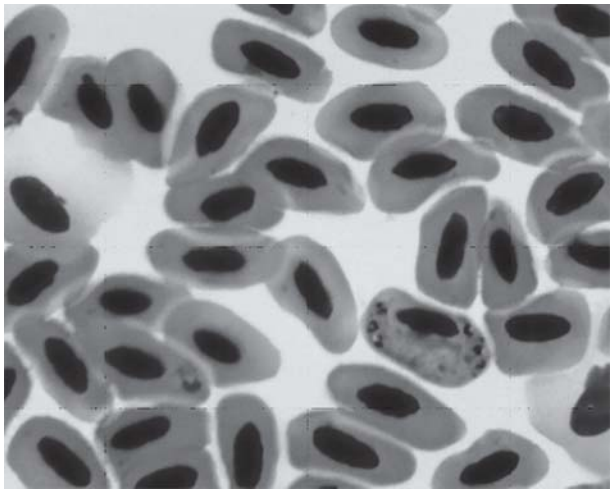

Fig 1. *H. columbae* from Kolkata

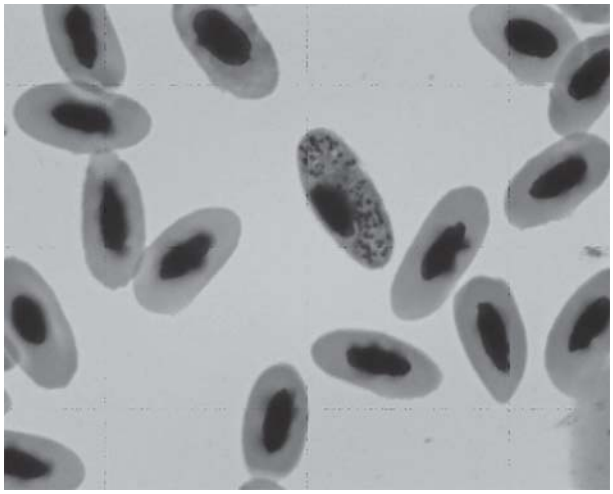

Fig 2. *H. columbae* from South 24 Pgs

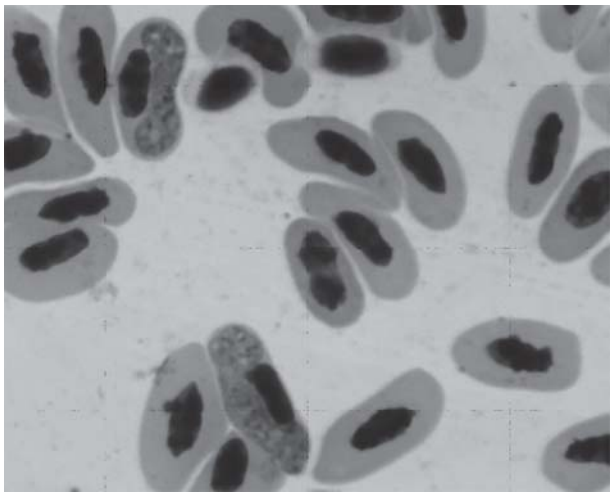

Fig 3. *H. columbae* from North 24 Parganas

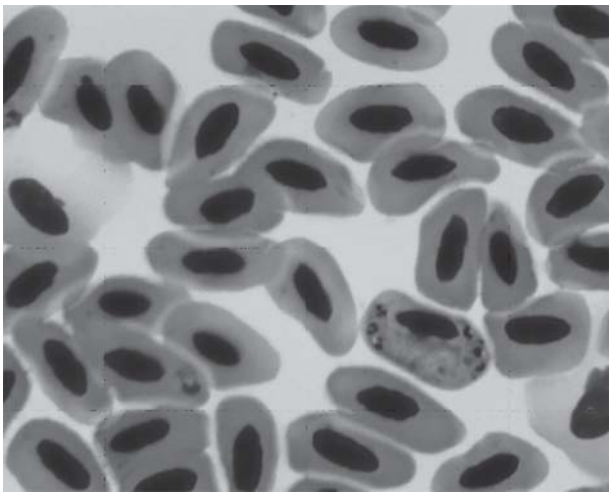

Fig 4. *H. columbae* from Malda

Table 1. Prevalence study of *H. columbae* in four districts of West Bengal

Sl. No.	District	Total no. of pegion	Sicked pegion	Total no of birds tested	% prevalence
1	South 24 Parganas	36	20	02	5.56
2	North 24 parganas	32	10	03	9.38
3	Kolkata	15	04	02	13.33
4	Malda	10	02	02	20.00
	Over all	93	36	09	9.68

of *H. columbae* infection. However, Realtime based PCR technique may be incorporated to validate the degree of infection showed from blood smear examination.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Short Communication

DIAGNOSIS AND THERAPEUTIC MANAGEMENT OF *PSOROPTIC* MANGE IN A YOUNG GOAT

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ABSTRACT

A three-months-old Sirohi kid was presented to the Referral Veterinary Polyclinic & Veterinary Clinical Complex, ICAR-IVRI, Izatnagar, with a history of alopecia under the ventral neck region and ear margins with scab formation and intense pruritus. The owner reported progressive development of crusted lesions and hair loss over the past few weeks. Pruritic skin lesions with pus discharges were also observed on the right forelimb and right hindlimb, respectively. Skin scrapings revealed the presence of a parasitic mite which was morphologically identified as *Psoroptes mites*, causing psoroptic mange in goats. Swabs from pus discharge showed positive growth of *Staphylococcus* spp.Based on diagnostic investigations, the case was diagnosed as psoroptic mange with concurrent *Staphylococcus* infection. Treatment with antibiotics, endectocide, antioxidants and supportive care led to complete recovery within 21 days.

Keywords: Dermatitis, Ivermectin, Mange, Psoroptes, Scabies

INTRODUCTION

Mange is a highly contagious and widespread skin condition, especially prevalent in tropical and subtropical regions [2]. Ecto-parasite infestation poses significant economic challenges to goat production worldwide. Among these, *Psoroptes cuniculi* (ear mite) commonly affects the ears of goats but may extend to the head, neck, and body, leading to severe irritation and dermatitis. This results in major economic losses due to poor meat quality, reduced milk production, and trade restrictions in the leather industry [3]. Psoroptes mites are superficial skin parasites that predominantly inhabit hair-covered areas of the body [4]. As obligate, non-burrowing mites, *Psoroptes spp.* feed on the surface of the skin by abrading the outer epidermal layer using their toothed chelicerae, ingesting a mix of lymph, red blood cells, skin cells, exudates, and surface bacteria [1]. Mite infestations are linked to oxidative stress, which can lead to immunosuppression and increase vulnerability to secondary infections caused by opportunistic microorganisms. Free radicals and oxidative stress play a crucial role in the development of various allergic and inflammatory skin disorders in humans. Being the body's first line of defence, the skin is constantly exposed to both internal and external pro-oxidants. The impact of free radicals on the skin includes oedema, erythema, inflammation, wrinkling, autoimmune reactions, hypersensitivity, and keratinization disorders [4]. Psoroptic mange results in significant economic losses due to weight loss, reduced milk yield, and heightened susceptibility to secondary infections in affected animals [5]. This paper reports the clinical manifestations and successful therapeutic management of Psoroptes infestation in a young goat kid.

CASE HISTORY AND CLINICAL OBSERVATIONS

A three-month-old Sirohi kid was presented to the Referral Veterinary Polyclinic, ICAR-IVRI, Izatnagar, with a history of alopecia around the ventral neck and ear margins, accompanied by scab formation and severe pruritus [Fig 1. & Fig 2.]. The owner reported progressive development of crusted lesions and hair loss over the previous few weeks. Clinical examination revealed pruritic skin lesions with pus discharges on the right forelimb and right hindlimb,

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Fig 1. Alopecia over the ventral neck region

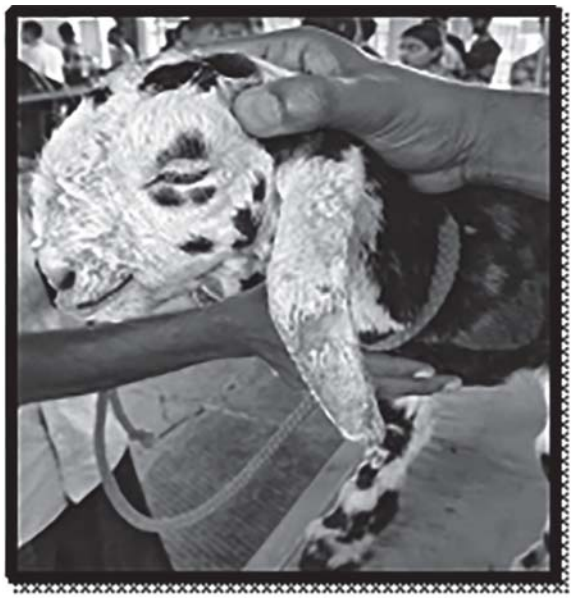

Fig 2. Alopecia at Ear margins, with scab formation

Fig 3. Alopecia and pruritic skin lesion with pus discharge on the right fore limb

Fig 4. Alopecia and pruritic in right hind limb

Fig 5. Photomicrograph of *Psoroptes* mite

Fig 6. Culture plate positive for *Staphylococcus* spp.

respectively (Fig 3. & Fig 4.). Haematological examination revealed mild neutrophilia. Microscopic examination of skin scrapings confirmed the presence of Psoroptes mites (Fig 5.), identifying psoroptic mange as the causative condition, while Giemsa-stained smears were negative for haemoprotozoan infections. Swabs from pus discharge showed positive growth of *Staphylococcus* spp (Fig 6.). Based on the clinical and diagnostic findings, the case was diagnosed as psoroptic mange with concurrent Staphylococcus infection.

TREATMENT

The treatment protocol consisted of cefotaxime at a dosage of 25 mg/kg body weight administered intramuscularly twice daily for five days to address the secondary bacterial infections. A 1% ivermectin preparation was given subcutaneously at 200 µg/kg body weight once weekly for three weeks to eliminate the mite infestation. Chlorpheniramine maleate (1.0 ml, IM) was administered for three days to alleviate pruritus. Meloxicam was administered intramuscularly at 0.2 mg/kg SID for 3 days to alleviate pain and discomfort associated with the lesions. To mitigate oxidative stress, vitamin E and selenium (Repronol) were administered subcutaneously at a dose of 1.0 ml per 50 kg body weight. Pus was drained, antiseptic dressing was done using 5% povidone iodine, and the lesions were advised to be kept dry. The kid demonstrated an uneventful clinical recovery, with gradual improvement and complete resolution of clinical signs within 21 days.

RESULTS AND DISCUSSION

The mites observed in the skin scrapings were oval, relatively large, and characterized by legs extending beyond the body margin. Based on these morphological features, they were identified as Psoroptes mites, aligning with the observations reported earlier. Hepatological analysis revealed mild neutrophilia (Table 1.), which is consistent with the findings and may be attributed to secondary bacterial infections. Following treatment, skin scraping examinations showed a gradual reduction in mite count; however, the presence of a few live mites on day 7 necessitated two additional doses of ivermectin. Concurrently, cefotaxime therapy was continued for five days to address secondary bacterial infections. The animal exhibited an uneventful recovery with progressive improvement after treatment with cefotaxime, ivermectin, antioxidants, and supportive care.

Table 1. Haemato-biochemical profile of the affected goat before and after treatment

Parameter	Prior treatment	Post treatment
Hb (gm/dl)	10.2	12.8
PCV (%)	36.7	44.6
TEC ($x10^6/mm^3$)	5.1	5.8
TLC ($x10^3/mm^3$)	18.5	10.7
Neutrophils (%)	66	30
Lymphocytes (%)	30	67
Monocytes (%)	1	3
Eosinophils (%)	3	0
Platelets ($/mm^3$)	1,54,000	1,67,000

CONCLUSION

This case report highlights the importance of early diagnosis and management of *Psoroptes* infestation to prevent severe health complications.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Short Communication

THERAPEUTIC IMPACT OF HOMEOPATHY AND NUTRITIONAL SUPPLEMENTATION ON REPEAT BREEDING IN CATTLE WITH EARLY PREGNANCY DETECTION VIA RAPID TEST KIT

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ABSTRACT

Repeat breeding represents a significant reproductive challenge in dairy cattle, with its prevalence differing across various management systems, environmental conditions, and geographical areas. Repeated breeding leads to increased culling rates and a reduced number of calves, thereby affecting overall productivity. Repeat Breeding is typically attributable to reproductive tract anomalies, improper artificial insemination (AI), hormonal disorder, inadequate nutrition, and poor management. The indiscriminate application of hormonal treatments has also not given expected outcomes. Consequently, there is a necessity for an alternative approach to address the issue of repeat breeding. In this pilot study, we explored the efficacy of a homeopathic medicine in alleviating repeat breeding and detected the pregnancy in early stage by a rapid pregnancy detection kit.

Keywords: Repeat breeding, Pregnancy detection, Fertisule, BovEasy

INTRODUCTION

Repeat breeding cows are a varied group of sub-fertile cows who do not have any diseases or structural defects, but even after at least two attempts, they are unable to conceive. They have clinically normal estrous cycles and periods for the reproductive system [4]. It results in a financial catastrophe because of a decrease in lactation production, an increase in medical expenses, calving time, insemination costs, and the mortality rate of cattle and buffalo [1]. Compared to buffaloes (12.74%) and native cows (8.64%), crossbred cows have a far higher rate of repeat breeding (17.57%) [3]. Buffaloes are better acclimated to the hot heat than cows, so productivity issues with buffaloes are uncommonly found, and only a small number of cases of Repeat Breeding syndrome are looked into. During the first 42 days of gestation, when organ development and growth occur, early embryonic death occurs. Embryonic mortality occurs at day 17 up to 50%, at day 17 to 42 approximately 10-15%, and 5% after day 42. Hormonal therapy and antibiotics are frequently used in conventional treatments; however, they can have withdrawal symptoms and not always produce the desired results. Because of this, alternative methods like homeopathy are being investigated for their potential to safely and naturally correct underlying hormone imbalances and increase conception rates. Pregnancy detection in the early stages after artificial insemination (AI) is important for the future economy of cattle breeders [2].

MATERIAL AND METHODS

Animals and Study Design

The study was conducted on 4 clinically healthy repeat breeder cows (cows failing to conceive after three or more consecutive inseminations despite regular

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estrous cycles and no apparent reproductive tract abnormalities) at Tatarpur, Arambagh block, Hooghly. All animals were of similar age, breed (HF), body condition score, and parity. Cows were randomly assigned into two groups: **Control Group (n=6):** Did not receive any fertility-enhancing treatment. **Treatment Group (n=6):** Received Fertisule™ (Goel vet Pharma, India) homeopathic tablets and mineral mixture supplement (Chelated Agrimin Forte™, Virbac, India) Powder 50 gm twice daily).

Treatment Protocol

The treatment group (n=6) was administered Fertisule™ (Fig 1.) tablets at the dose recommended by the manufacturer - 5 tablets daily as marked in a line at the same hour. Control group cows were maintained without any fertility-related intervention. The Fertisule™ (a combination of Homoeopathic drugs) contains saturated tablets with Alteris Farinosa 30, Aurum Met.30, Apis Mel. 30, Borex 30, Calc. Phos. 30, Colocynthis 30, Folliculinum 30, Iodine 30, Murex 30, Oophorinum 30, Palladium 30, Platinum 30, Pulsatilla 30, Sepia 200. The 6 cows were artificially inseminated (AI) once during natural standing estrus using semen from the same bull to ensure uniformity.

Pregnancy Diagnosis

Early pregnancy diagnosis was conducted using a commercially available bovine pregnancy test kit (BovEasy™, Prompt Equipments Pvt. Ltd., India) (Fig 2.) on Day-28 post-insemination, based on the detection of pregnancy-associated glycoproteins (PAGs) in blood as per the manufacturer's protocol.

RESULTS AND DISCUSSION

Out of the four clinically healthy repeat breeder cows enrolled in the study, both cows in the Treatment Group (n=6), which received Fertisule™ homeopathic tablets along with Chelated Agrimin Forte Powder, were confirmed pregnant on Day 28 post-insemination using a bovine pregnancy test kit detecting pregnancy-associated glycoproteins (PAGs). In contrast, no pregnancy diagnosis was conducted in the Control Group (n=6) as no fertility treatment was applied, and this group served as a non-intervention baseline. The results from this small-scale pilot study suggest a positive influence of the combined homeopathic and mineral supplementation protocol on fertility restoration in repeat breeder cows. The 100% conception rate (6/6) observed in the treatment group, though based on a limited sample size, is encouraging and supports the hypothesis that the use of Fertisule™, along with balanced mineral supplementation (Chelated Agrimin Forte), may enhance reproductive performance in cows with a history of breeding failure.

Repeat breeding in cattle is a multifactorial condition, often associated with subtle hormonal imbalances, mineral deficiencies, or subclinical reproductive tract disorders. The ingredients of Fertisule™ comprising multiple homeopathic agents such as Folliculinum, Pulsatilla, Sepia, and Oophorinum—are traditionally indicated for hormonal regulation and ovarian stimulation in veterinary practice. Supplementation with chelated minerals may have further addressed latent mineral deficiencies, contributing to an improved uterine environment conducive to conception and embryo survival.

While the observed outcome in the treatment group is promising, it is essential to note the limitations of the study, primarily the small sample size and lack of pregnancy diagnosis or follow-up data in the control group. Therefore, the findings should be interpreted cautiously. Further large-scale, randomized controlled trials with appropriate diagnostics for all groups are warranted to validate the efficacy and reproducibility of this fertility-enhancing approach.

CONCLUSION

This pilot case study presents preliminary evidence suggesting that a combination therapy involving Fertisule homeopathic tablets and Chelated Agrimin Forte Powder may support fertility restoration in clinically healthy repeat breeder cows. The 100% conception rate (6/6) in the treatment group, while notable, must be interpreted with caution due to the study's small sample size and absence of pregnancy assessment in the control group. The positive response observed may be attributed to the synergistic effects of hormonal modulation through homeopathic agents and correction of underlying mineral deficiencies via chelated supplementation. These findings underscore the potential of integrative therapeutic protocols in addressing complex reproductive challenges in cattle.

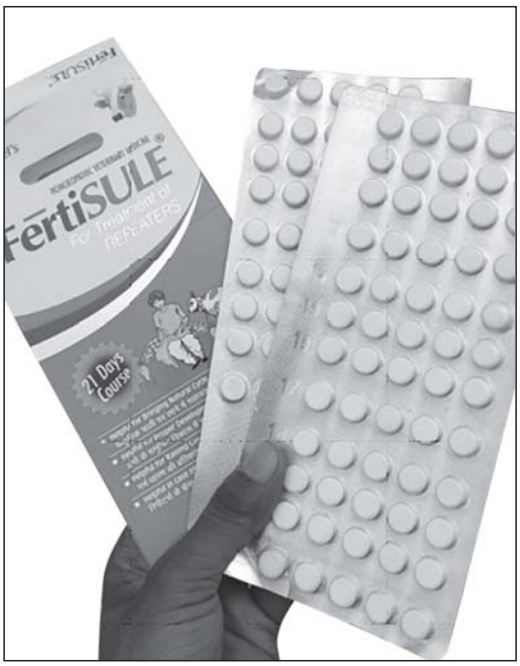

Fig 1. Fertisule™ Tablets

Fig 2. BovEasy™ kit showing positive result for pregnancy

However, larger, well-controlled studies with comprehensive diagnostic follow-up are essential to confirm the efficacy, safety, and reproducibility of this approach in broader field conditions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Short Communication

CO-INFECTION WITH *Theileria* sp. AND *Trypanosoma* sp. IN A BUFFALO: A CASE REPORT

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ABSTRACT

A six-year old buffalo was presented with high fever (107°F), anorexia, and marked weakness underwent clinical and hematological examination. Giemsa-stained peripheral blood smears revealed mixed infection with Theileria sp. and Trypanosoma sp. Initial treatment was started with Buparvaquone for theileriosis, followed by diminazene aceturate administration upon detection of trypanosomes. The animal showed marked recovery with normalization of temperature and appetite. This case highlights the importance of thorough diagnosis and sequential therapeutic management in mixed haemoprotozoan infections in bovine.

Keywords: Co-infection, Buffalo, *Theileria* sp and *Trypanosoma* sp

INTRODUCTION

Bovine tropical theileriosis is a tick-borne haemoprotozoan disease primarily caused by *Theileria annulata,* and it poses a significant limitation to dairy cattle and buffalo production in India and worldwide. First theileriosis case was reported in India by Lingard in 1905, the disease affects a massive bovine population of over 300 million, with India leading global milk and buffalo production [6, 9]. Economic impacts are severe, with global tick-borne disease losses ranging from US$13.9–18.7 billion annually and tropical theileriosis alone causing US$384.3–1,295 million in India yearly through mortality, reduced milk yield, and abortions [2, 5]. Field diagnosis relies on clinical signs and microscopic examination of Giemsa-stained blood or lymph node smears, the conventional "gold standard," which detects schizonts in acute cases but fails in subclinical carriers due to low parasitemia (<1 infected cell per 10,000). These limitations are low sensitivity (26–73%) and technician dependence, underscore the need for advanced molecular diagnostics like PCR, which offer superior specificity (up to 100%) [3].

The rate of *T. evansi* infection in buffaloes varies a lot depending on the region and the diagnostic method, with reported rates between 4% and 67% [4]. For instance, high rates have been found in the Mumbai suburbs (67% at Karjat), Bareilly district (18.42%), Jabalpur (15.25%), and Assam (23.3%). Older buffaloes and females are more likely to be infected, and cases often peak after the monsoon season. The disease shows up as fever, anemia, swelling, neurological symptoms like head pressing and circling, pale mucous membranes, loss of appetite, nosebleeds, and lower milk production. Many buffaloes have mild or subclinical infections and act as reservoirs. Blood tests in affected animals often show higher levels of urea nitrogen, creatinine, gamma-glutamyl transferase, and bilirubin, which point to kidney and liver problems. Diagnosis relies on finding the parasite in Giemsa-stained blood smears or using molecular methods like PCR, which can detect more cases [8, 10]. Drugs such as quinapyramine sulphate and oxytetracycline are effective in treating the infection, but the disease still causes significant economic losses due to illness and reduced productivity [1].

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CASE HISTORY AND CLINICAL SYMPTOMS

A six-year-old buffalo from the Hooghly district of West Bengal, India, was presented with a three-day history of high fever (107°F), anorexia, nervous disorder and marked weakness. A significant reduction in milk production was also noted. Clinical examination and hematological analyses were performed to assess the animal's condition. The buffalo exhibited an enlarged prescapular lymph node and respiratory distress. Microscopic examination of Wright-Giemsa-stained peripheral blood smears revealed intra-lymphocytic macroschizont stage (Koch's Blue Bodies) stage of Theileria sp. and extracellular trypomastigote forms of Trypanosoma sp., indicating a concurrent mixed hemoprotozoan infection (Fig 1.).

TREATMENT AND OUTCOME

Treatment was initiated with buparvaquone (Butalex®, MSD Animal Health-2.5 mg/kg, deep intramuscular injection, repeated after 72 hours) to manage theileriosis. Subsequent diagnostic evaluations confirmed trypanosomiosis by light microscopy of Giemsa' stained blood smear, leading to administration of diminazene aceturate (Berenil®, MSD Animal Health) at 3.5 mg/kg body weight by deep intramuscular injection. Sequential, parasite-specific therapy resulted in rapid clinical recovery, including normalization of body temperature and appetite. Supportive therapy with crystalloid fluids, iron preparations, multivitamins, and cyanocobalamin were also provided, as these interventions may accelerate recovery in buffalo infected with Theileria and Trypanosoma species.

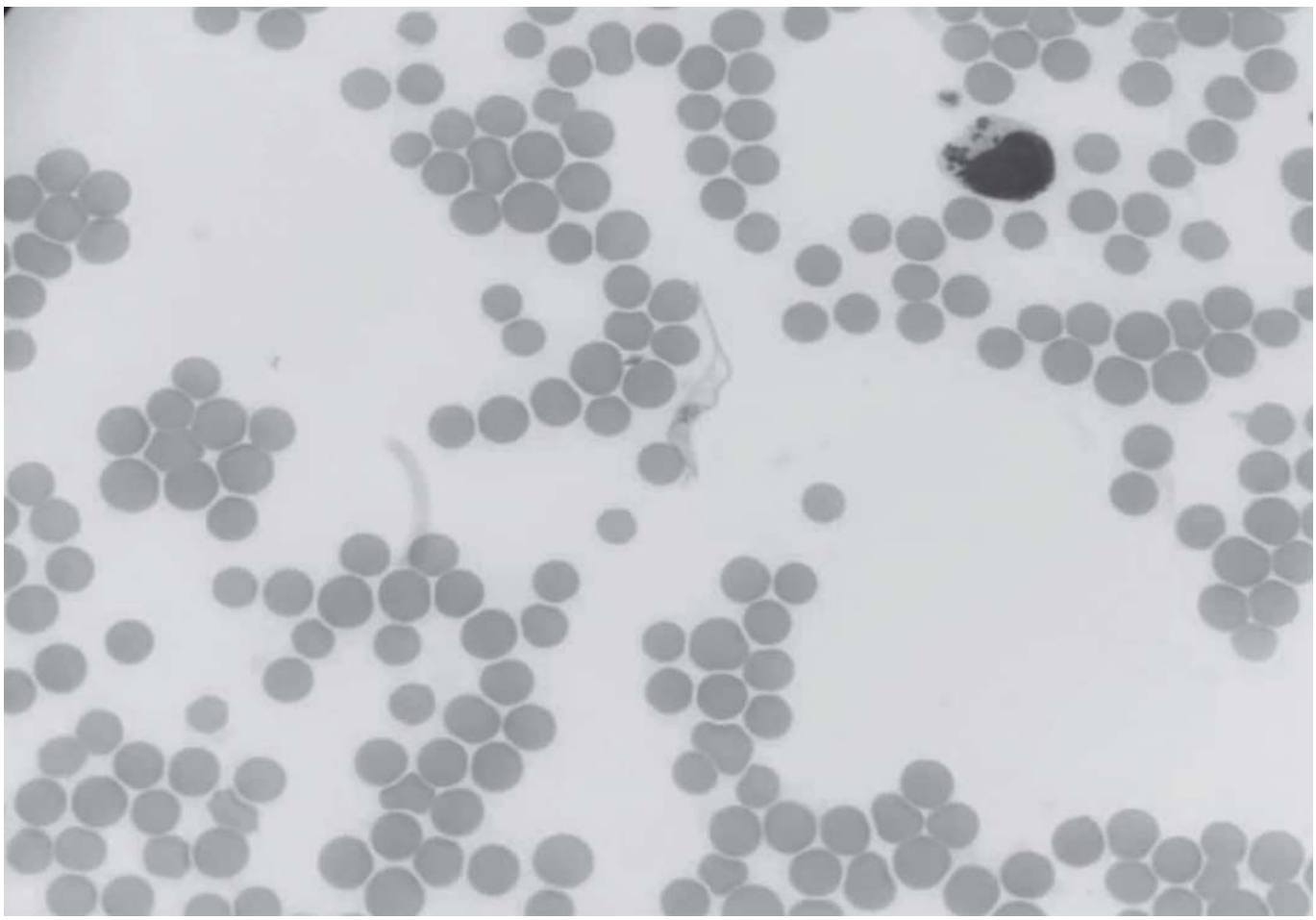

Fig 1. Blood smear illustrating intra-lymphocytic schizonts stages of *Theileria sp.* and extracellular trypomastigote stage of *Trypanosoma sp.* in the microscopic field, visualized using the Write-Giemsa staining method.

DISCUSSION

Mixed hemoprotozoan infections can make diagnosis difficult because their clinical signs often overlap, especially in areas where these diseases are common. This case highlights the importance of repeating and carefully examining blood smears to find co-infections. A similar case was reported from Mathura, Uttar Pradesh [7]. Treating each pathogen in sequence is important for successful management and better patient outcomes.

CONCLUSION

Co-infections of *Theileria* sp. and *Trypanosoma* sp. are common vector-borne diseases in buffalo, particularly in tropical regions. These concurrent infections often cause severe clinical illness, high fever, anemia, and significant economic losses, requiring specialized and combined therapeutic intervention. Early and proper diagnosis plays important role to select actual therapeutic agent.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Case Study

SUCCESSFUL THERAPEUTIC MANAGEMENT OF ORAL PAPILLOMATOSIS IN A DOG

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ABSTRACT

A five months old, black and white female Spitz puppy weighing 5 kgs(approx.) was presented to the State Animal Health Centre, Naxalbari, District Darjeeling with a history of multiple cauliflower like outgrowths on the oral mucous membrane, gums and lips which progressively developed over a period of one month. These growths were pinkish-white in colour and were diagnosed as Canine Oral Papillomatosis based on clinical examination. The animal was treated with a combination of oral antibiotic Azithromycin once daily for 10 days and a nutritional supplement with recognized antioxidant and immunomodulatory properties (Viusid) twice daily for 15 days, which was found to be effective. The animal started showing rapid improvement and the lesions were completely regressed.

Keywords: Canine, oral papilloma, warts, Azithromycin, viusid, immunomodulator

INTRODUCTION

Canine Oral Papillomatosis (COP) is a contagious, self-limiting and spontaneous regressing neoplastic disease of young dogs caused by Canine Oral Papilloma virus [15]. The causative agent is a double-stranded, non-enveloped DNA virus of the *Papovaviridae* family which is usually species-specific and has a strong tropism for cutaneous squamous or mucosal epithelium [9]. Canine Pral Papilloma virus mainly affects young dogs, approximately 1 year old in age, and there is no difference in prevalence between sex and breed [10]. COP in puppies is characterized by multiple, invasive, cauliflower like hyperkeratotic masses typically in the oral mucosa, including lips and muco-cutaneous junctions. Occasionally, tongue, pharynx and esophagus can be affected [17]. COP has a specialized ability for spontaneous regression due to development of cell mediated immune responses that may develop over a period up to 12 months in naturally infected animals [14]. In spite of auto-regression, treatment of COP cases is extremely important due to the physical challenges the animal faces while eating, the restriction of social interaction with other dogs, anorexia, drooling, halitosis, bleeding and secondary bacterial infections [5]. Various treatment methods have been tried in order to shorten the recovery period. Autogenous vaccine, homeopathy, immuno-therapy, various antibiotic and antiviral drug trials have been reported [11]. The present study describes about the therapeutic use of oral antibiotic Azithromycin along witha nutritional supplement with recognized antioxidant and immunomodulatory properties (Viusid) in management of canine oral papillomatosis.

MATERIALS AND METHODS

A five months old black and white female Spitz puppy weighing 5 kgs (approx.) brought to the State Animal Health Centre, Naxalbari, Darjeeling District with the chief complaint of several abnormal nodular growths in the gums and lips which developed progressively over a period of one month. The owner reported that the animal was having troubled feeding because of the lesions. On physical examination the animal revealed presence of pinkish white cauliflower like nodular growths of various sizes, dispersed over the oral mucous membrane involving gums and lips, which were visible externally (Fig 1.). Otherwise, the animal was in apparent general health. Based on clinical

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examination, it was diagnosed as suspected case of canine oral papillomatosis. Treatment was started with antibiotic Azithromycin @ 10 mg/kg body weight PO once daily for 10 days along with anutritional supplement with recognized antioxidant and immunomodulatory properties (Viusid Pets Oral Solution by Vivaldis Animal Health) @ 1ml/ 5kg body weight PO twice daily for 15 days.

RESULTS

Following commencement of treatment, the dog was examined after 10 days and it was observed that there was a significant reduction in extension and numbers of papillomas, the growths were shrunken and partial regression was evident and after 20 days, all the growths disappeared completely (Fig 2). There was no recurrence of papillomatosis in the treated dog during a follow-up period of 6 months and no adverse effects were recorded.

DISCUSSION

Although canine oral papillomatosis is a self-limiting disease, with spontaneous regression of the lesions that usually occurs within 12 months, there is a need for safe therapeutic protocols that promote the regression of the lesions more quickly and effectively, considerably reducing the inconvenience to the affected animals, and returning the animal's well-being quickly. This is particularly important to prevent the prolonged course of the disease, which can increase the spread of infection to other canines [3]. The use of a specific and effective drug for treatment of canine papillomatosis is debatable. However, various drugs have been prescribed for treatment of canine papillomatosis with varied level of effectiveness.

Azithromycin, a macrolide antibiotic, has been reported as an effective well tolerated therapeutic option for the treatment of papillomatosis in humans [1,2,16], cattle [4] as well as dogs [3,17]. In present study, the lesions disappeared following Azithromycin therapy, suggesting that Azithromycin therapy is a safe and effective therapy of canine oral papillomatosis. The observations in the present case are in accordance with the findings o, who reported that with the use of Azithromycin, lesion disappeared at approx. 10-15 days after treatment commenced and there was no recurrence of papillomatosis after 8 months. The combined treatment of Azithromycin and Meloxicam was effective for the rapid resolution of Canine Oral Papillomatosis within a 10 days period, without recurrence [3].

Immunity is an important factor that determines development of papillomatosis in dogs. Mostly disease is seen in young dogs which are yet to develop effective antibodies able to prevent the establishment and development of virus [12]. Most of the canine oral papilloma spontaneously regresses and the regressed dogs do not develop new papilloma because of specific cell mediated immunity. However, recurrent papillomatosis may be associated with defective cell-mediated immunization [17].

Viusid (Catalyis laboratory, Madrid, Spain) is a nutritional supplement that contains different molecules (ascorbic acid, zinc and glycyrrhizic acid) with recognized antioxidant and immunomodulatory properties. Glycyrrhizin, the most important active ingredient of the supplement, is known to have various immune-modulating, antiviral and biological response-modifier activities [7]. Hence Viusid has been taken into consideration as a form of immunotherapy in treatment of canine oral papillomatosis and as an adjunctive therapy to enhance the efficacy of Azithromycin. Immunomodulatory properties of Viusid has well been documented in various case studies in human as well as animals [6,7,13].

In present study, the combination of Azithromycin and Viusid was found to be effective in the treatment of canine oral papillomatosis resulting in complete regression of papillomatous lesions. The observations in the present study are in accordance with the findings [1,8] who also reported that the combination of Glyzigen and Viusid was effective in the treatment of external anogenitalwarts (AGW) caused by human papillomaviruses (HPV).

CONCLUSION

In conclusion, oral administration of antibiotic Azithromycin along witha nutritional supplement with recognized antioxidant and immuno-modulatory properties (Viusid) are found to be effective for the therapeutic management of canine oral papillomatosis

Fig 1. Multiple cauliflower like outgrowths on the oral mucous membrane, gums and lips at the time of presentation

Fig 2. Complete recovery after 20 days of treatment

without any side effects. Further research and clinical trials are warranted to validate and extend these findings.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Case Study

TUBE CYSTOSTOMY IN A JAMNAPARI KID SUFFERING FROM OBSTRUCTIVE UROLITHIASIS: A CASE REPORT

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ABSTRACT

A one-year-old Jamunapari goat was presented with acute straining and inability to urinate, with the last normal urination reported the previous day. Clinical examination revealed discomfort, tachycardia, hyperventilation, an extended stance, and visible calculi on the prepuce, indicating obstructive urolithiasis. Following diagnosis of complete urinary obstruction, tube cystostomy was selected as the surgical intervention. The animal was recovered after 15th day post-surgery.

Keywords: Tube cystostomy, goat, obstructive urolithiasis

INTRODUCTION

Obstructive urolithiasis is a significant condition in male ruminants, causing notable economic losses and serious welfare issues. Obstructions most commonly occur at the distal sigmoid flexure, leading to urine retention [4]. If untreated, the condition can become fatal, with urinary bladder rupture possible within 72 hours of complete blockage [5]. Medicinal management generally yields poor outcomes, and once obstruction is complete, surgical intervention becomes essential [2]. Calculi may be removed directly or bypassed, with tube cystotomy being the preferred long-term surgical solution. Despite advances in understanding dietary, hydration, and urinary factors, the condition continues to be frequently encountered in clinical practice [1]. This report presents a case of urinary bladder rupture secondary to obstructive urolithiasis in a Jamunapari kid, successfully managed using tube cystostomy.

MATERIALS AND METHODS

Case Presentation

A one-year-old Jamunapari goat kid was brought to the Additional Block Animal Health Center (ABAHC) in Ramnathpur under Chanditala-I Block of Hooghlydistrict due to straining and an inability to urinate. The owner reported that it had last urinated normally the day before. During examination, the animal was quiet, alert and responsive, but noticeably uncomfortable. The kid stood in an extended posture and frequently cried out in pain while being evaluated. Several small caliculi were observed on the prepuce, which were presumed to be uroliths-urinary stones or calculi. The animal had tachycardia (HR: 120 bpm) and hyperventilation (respiration rate 30/minute). Rectal temperature of the animal was within the reference range.

The case was diagnosed as complete urinary obstruction and it was decided to perform tube cystostomy.

Surgical Procedure

After placing the animal in right lateral recumbency, the general anaesthesia was performed by intravenous combination of butorphanol (0.1–0.5 mg/kg) and midazolam (0.2 mg/kg), with ketamine (2–4 mg/kg) added as needed [7].

A 3-cm linear skin incision was created cranial to the rudimentary teat. Sequential dissection through the skin, fascia, musculature, and peritoneum was performed to locate the bladder. The enlarged urinary bladder was palpated directly by inserting two fingers.The flushing of the urethra was done by saline and a temporary tube cystostomy was performed. A Foley catheter was advanced through the created tunnel, and using a K-wire passed through the catheter eye, it was directed into an

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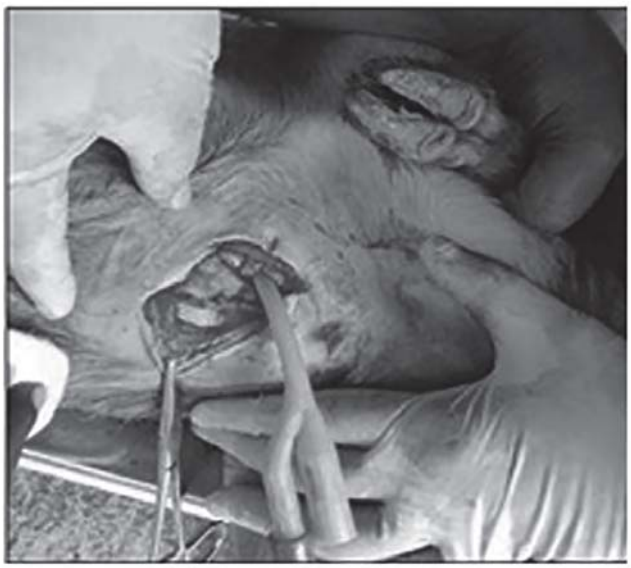

Fig 1. Insetion of Foley chtheter

Fig 2. Fixed catheter at ventral abdominal wall

Fig 3. Normal urination after clipping the catheter

Fig 4. Normal urination after removal of catheter

avascular region of the urinary bladder (Fig 1.). The catheter balloon was then inflated with 10 mL of sterile saline to secure its position within the bladder, after which the K-wire was carefully withdrawn. The peritoneum, muscles and skin were closed properly and the catheter was fastened to the ventral abdominal wall with interrupted silk sutures (Fig 2. & Fig 3.).

RESULTS AND DISCUSSION

Post-Operative Care

The kid recovered well from anesthesia. Postoperative management included daily wound dressing with 0.5% povidoneiodine solution until complete healing was achieved. The patient received an

intramuscular course of Amoxicillin-cloxacillin (500 mg) for 5 days, Meloxicam (0.25 mg/kg, i.m.) for 3 days, and oral ammonium chloride (300 mg/kg) for 15 days. The owner was advised to flush the catheter every four hours for first three days. To assess and maintain urethral patency, the Foley catheter was intermittently clamped for progressively longer periods to encourage the animal to resume normal spontaneous urination (Fig 3.). The Foley's catheter was removed after 15 days post operation when the obstruction was relieved completely (Fig 4.).

Although several surgical options exist for managing complete urethral obstruction in cases of urolithiasis-including urethrostomy, marsupialization, penile catheterization and amputation, tube cystostomy has been reported as an effective technique for ruminants [6, 9, 10, 11]. The overall success rate of surgical tube cystostomy in small ruminants was reported to be 65-76% [3, 7]. The re-occurrence of the obstruction was not reported after one months which was in line with the observations of previous study [8]. But, in contrary, some cases of re-obstruction within one-month post-surgery were also reported [3].

CONCLUSION

The observations from the present case report indicate that animals with obstructive urolithiasis can achieve a favorable outcome when managed surgically using tube cystostomy.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Case Study

POST-PARTUM UTERINE PROLAPSE IN INDIGENOUS CATTLE-TREATMENT AND MANAGEMENT

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ABSTRACT

A five-year-old post-partum Sahiwal cow was presented at the Block Animal Health Centre (BAHC), Chopra, Uttar Dinajpur, on 11th March 2024 with a history of protruding uterine mass accompanied by significant straining. The uterine prolapse was observed four days after parturition. The animal exhibited signs of depression and lethargy, characterized by subnormal body temperature, cessation of urination, rumination, defecation, and congested mucous membranes. The history and clinical assessment indicate no substantial uterine trauma or damage. A favourable prognosis was established, and it was recommended that the animal must undergo correction of the prolapse. Uterine prolapse constitutes an emergency that necessitates prompt reporting to ensure a favourable prognosis for the animal.

Keywords: Uterine prolapse, pluriparous, matress sutured

INTRODUCTION

Uterine prolapse refers to the condition where the uterus protrudes from the vulva, exposing the uterine mucosa [6]. It is also called as "wethers" or "casting of calf bed". Uterine prolapse is a prevalent condition observed in dairy farming. The condition entails the total prolapse of the uterus, cervix and vagina [5]. It typically occurs immediately following parturition and may also arise several hours thereafter [4,5]. Differences exist among various cattle regarding the contributing factors linked to uterine prolapse; however, uterine inertia, dystocia, and hypocalcaemia are among the most prevalent causes [1]. Uterine prolapse during the postpartum period, resulting in expulsion through the genital passage, is a common consequence of prolonged dystocia. Uterine prolapse has been observed across all animal species, with the highest incidence in pluriparous dairy cows. Following prolapse, the tissues initially present as nearly normal; however, within a few hours, they exhibit enlargement and edema. This condition is considered as a veterinary emergency, as untreated cases may result in the cow's death [9]. An uncomplicated case of uterine prolapse typically exhibits a favourable prognosis when addressed promptly; thus, it should consistently be regarded as a veterinary emergency. The approach to addressing uterine prolapse involves cleansing and disinfection of the organ, reduction of the size if edema is present using glycerol, repositioning of the organ, and the application of stay sutures [1, 8, 11].

CASE HISTORY

A five-year-old pluriparous (second parity), post-parturient (four days) upgraded Sahiwal cow, given birth to a female calf after forceful traction (Fig 1.), was presented to the Block Animal Health Centre (BAHC), Chopra, Uttar Dinajpur.

CLINICAL ASSESSMENT

During the clinical examination, a prolapsed mass (gravid horn) was observed protruding from the vulva. The mass exhibited a bright red color, with uterine caruncles distinctly visible. The animal exhibited excessive traction, showing reluctance to move. Rectal temperature measured at 100.8°F, pulse rate 84 bpm, respiratory rate 18 bpm, conjunctival mucous membranes appeared pink, and the animal displayed a decreased appetite for feed and water.

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Fig 1. An upgraded Sahiwal cow presented with total uterine prolapse

Fig 2. Prolapsed uterus repositioned and retention by horizontal mattress (Halstead) technique

The uterine mass exhibited signs of edema, was engorged, felt cold upon palpation, and was contaminated with bedding materials. No rupture of blood vessels or prolapse of the urinary bladder was observed. The prognosis appeared to be fair given the absence of shock and the limited extent of laceration and contamination observed (Fig 1.).

Fig 3. Favourable outcome after 7 days of treatment

TREATMENT AND DISCUSSION

Upon arrival, the animal was restrained, and a regional nerve block was administered through caudal-epidural anaesthesia using an injection of 7 ml of 2% lignocaine HCl (Xylocaine) at the sacro-coccygeal (S5-Cx1) intervertebral space. Subsequently, the uterus was elevated to the level of the vulva, and the prolapsed mass was rinsed with cold water and a 1% $KMnO_4$ solution prior to repositioning. Magnesium sulphate and Dicrysticin-S (5 gm) powders were sprinkled all over the prolapsed mass to cause shrinkage of the mass and prevent infection respectively. The prolapsed organ was elevated to the level of the vulva and subsequently repositioned into the vagina through manual pressure, assisted by another person, to restore its normal anatomical position. The body of the uterus was initially pushed, followed by the horns; restoration to the normal position was achieved by inserting a hand to the tips of both uterine horns to confirm the absence of invagination. Horizontal mattress (Halstead) suture technique (Fig 2.) was employed on the vulvar lips to stabilize the uterus and mitigate the risk of recurrence.

Post-operative treatment involved Inj. Ceftiofur (Xceft 1g S/C) @ 2.2 mg/kg BW, along with Inj. Flunixin meglumine @ 1.1 mg/kg BW (Megludyne Inj. 8 mL I/M), Inj. Chlorpheniramine maleate (Cadistin-vet 8 mL I/M), and Inj. Tribivet (12 ml I/M) for 3 days. The suture was removed after seven days resulting in a favourable outcome (Fig 3.).

The current instance suggests that eversion of the gravid uterine horn of and cervix may have happened following forced traction of the foetus and foetal membrane, a prevalent predisposing factor for organ eversion [10]. In another finding it was reported that 12 out of 44 cows (27%) with uterine prolapse experienced milk fever, dystocia, or retained foetal membrane[3]. Several techniques have been introduced to prevent recurrence, including rope truss[2] horizontal mattress suture , and Buhner's suture [7, 13]. The favourable prognosis in this case and the successful outcome may be attributed to prompt repositioning and timely intervention prior to the onset of damage, mutilation, necrosis, or gangrene.

CONCLUSION

A five-year-old pluriparous upgraded Sahiwal cow was presented to the Veterinary Hospital with total uterine prolapse following parturition. The case was corrected through reduction, repositioning, and retention. Uterine prolapse may occur in the periparturient period. Diagnosing and treating uterine prolapse are critical responsibilities. Prolonged correction may result in severe complications, including edema, fibrosis, necrosis, and septicemia. The outcome of this case resulted in a favorable prognosis, as farmers promptly reported the incident.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Case Study

CLINICAL EVALUATION OF A SHRIMP-BASED PHOSPHOLIPID AND OMEGA-3 DIET IN THE MANAGEMENT OF CANINE DERMATITIS AND PYODERMA

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ABSTRACT

Canine dermatological disorders such as dermatitis and pyoderma are commonly managed with long-term pharmacological interventions, which may lead to adverse effects and antimicrobial resistance. This field-based clinical study evaluates the efficacy of a natural shrimp-based dietary intervention, rich in phospholipid-bound omega-3 fatty acids and astaxanthin, in managing dermatological problem in human without pharmacological treatment. Five dogs of different breeds, ages, and body weights diagnosed with dermatitis, pyoderma, or associated conditions were fed a standardized shrimp-based diet once daily for one month. Clinical improvement in skin condition, coat quality, and inflammation was observed in all cases, with no adverse effects. The findings suggest that shrimp-derived omega-3 fatty acids may serve as an effective, palatable, and bioavailable nutritional strategy for canine skin health.

Keywords: Canine dermatitis, omega-3 fatty acids, shrimp phospholipids, astaxanthin, nutritional therapy

INTRODUCTION

Dermatological disorders are among the most frequently encountered clinical conditions in companion animal practice. Conventional treatment often relies on antibiotics, corticosteroids, and medicated shampoos, which may cause systemic side effects and recurrence of symptoms [3]. Nutritional modulation, particularly supplementation with omega-3 fatty acids, has gained attention as a safer and sustainable alternative. While fish oil is the most commonly used source of omega-3 fatty acids, its triglyceride form shows limited absorption and poor palatability in dogs. Sea shrimp, however, provides omega-3 fatty acids in a phospholipid-bound form, along with astaxanthin [1], a potent antioxidant with anti-inflammatory properties [4]. This study aims to clinically evaluate the effect of a shrimp-based dietary regimen on canine skin disorders under field conditions.

MATERIALS AND METHODS

Design of Experiment

Five client-owned dogs of different breeds and ages presented with dermatological conditions were included in the study under the jurisdiction of Block Animal Health Centre (BAHC), Patrasayer, Bankura, West Bengal, India. The age and body weight of dogs under investigation was also recorded for proper dosing of shrimp diets. Fresh shrimps without any preservatives were procured from the market and the outer covering was removed and dosing was finalized according to the body weight of the dog (Fig1.).

Dogs were fed one morning meal per day consisting of boiled sea shrimp, pumpkin with seed, black pepper, turmeric, carrot and cooked rice (Other than two major meal). The diet was prepared fresh, boiled, and cooked thoroughly. The shrimp dosages are 15–30 kg body weight @ 60 g shrimp/day and 30–35 kg body weight@ 100 g shrimp/day for 30 days. Normal (non-medicated) shampoo once weekly for 4 weeks, No systemic or topical medications.

Clinical Observations

The study was included with five types of breeds i.e. Labrador, German Shepherd, Indian Pariah, Golden Retriver having symptoms of dermatitis and pyoderma.

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Table1. Clinical study of different breeds with shrimp-based meal treatment

Breed	Age (Years)	Indications	B.Wt(Kg)	Outcome After 1 Months
Labrador Retriver	5.2	Dermatitis	31.2	Improved
German Shepherd Dog	3.8	Pyoderma	34.8	Normal
Indian Pariah	2.7	Dermatitis	19.0	Normal skin
Golden Retriver	8.0	Dermatitis & Osteoarthritis	25.9	Remarkable improvement
Indian Pariah	2.9	Dermatitis	21.3	normal

Fig 1. Sea Shrimp from local Market

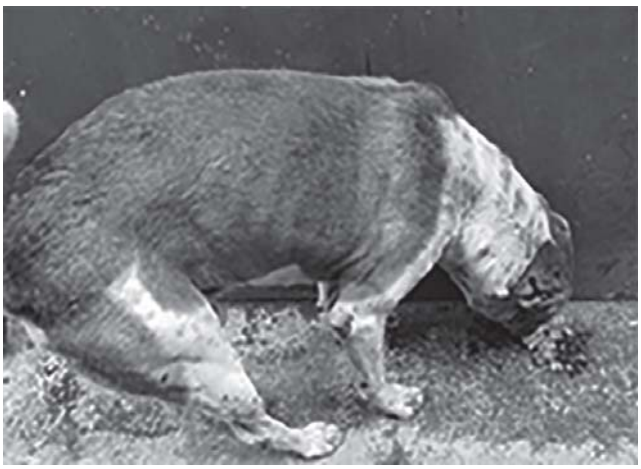

Fig 2. Recovered Indian-pariah after treatment

The dogs were kept with a close clinical observation with recording of skin condition on weekly basis. The final observations were documented for each breed of dog which showed improvement in Labrador and Golden Retriver and recovered completely in German Shepherd and Indian Pariah (Table 1.).

RESULTS AND DISCUSSION

All dogs showed significant clinical improvement within one month [Fig 2.]. Reduction in pruritus, erythema, and skin lesions was evident, along with improved coat texture. No adverse reactions were reported. The shrimp-based diet was highly palatable and well accepted by all dogs [2]. The shrimps are enriched with anti-oxidant and anti-inflammatory biomolecules i.e. carotenoids and phenolic with high concentration of (3,3'-dihydroxy-β, β -carotene-4,4'-dioxe) is axanthophyll carotenoid [1]. The superior clinical outcomes observed in this study may be attributed to the high bioavailability of phospholipid- bound omega-3 fatty acids and the presence of astaxanthin, which enhances anti-inflammatory and antioxidant activity. Unlike fish oil, shrimp omega-3 fatty acids are easily incorporated into cell membranes, improving skin barrier function and immune modulation [1]. The absence of pharmacological agents further highlights the potential of dietary management as a standalone or adjunct therapy for canine dermatological disorders [4]. Topical treatment with glycosaminoglycans for canine atopic dermatitis in canine showed promising result [2]. In another study ophytrium has been used in the form of lotion or shampoo by blocking the Staphyloccocal induced dermatitis in canine [5].

CONCLUSION

A shrimp-based omega-3 phospholipid diet appears to be a safe, effective, and economical nutritional intervention for managing canine dermatitis and pyoderma. Larger controlled studies are recommended to further validate these findings and establish standardized dietary protocols.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Case Study

SUCCESSFUL NEW THERAPEUTIC MANAGEMENT OF NEONATAL TETANUS IN A GOAT : A CASE REPORT

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ABSTRACT

A thirty-nine days old Black Bengal kid was presented to Additional Block Animal Health Centre, Shyamnagar, Tehatta-1 Block, Nadia with history of anorexia, stiffness in hind limb and whole body, lock jaw, opisthotonus, anuria and absence of defecation. After taking detailed history, it was revealed that they did open method of castration by local untrained person. On clinical examination kid showed stiffness of body including head and neck, trismus, opisthotonus, wooden horse appearance and proplapse of third eye lid. The physiological parameters revealed that the rectal temperature was 103°F, respiration rate 24/minute and pulse rate125/minute. On the basis of history and clinical examination, the case was diagnosed as Neonatal Tetanus. The kid was treated with Tetanus Toxoid and procaine penicillin thrice a day and supportive therapy of flunixin meglumine, Diazepam, vitamine B-complex and saline dextrose for seven days. Clinical improvement was seen on second day onwards after treatment as movement in limbs, improvement in feeding, normal urination and defecation.

Keywords: Kid, neonatal tetanus, unhygienic practice , open method castration

INTRODUCTION

Tetanus is a non contagious, non-febrile, infectious disease of mammals affected by exotoxins. It is characterized by spasmodic contraction of skeletal muscles and death in affected animals. It is caused by *Clostridium tetani* which is spore forming, Gram positive, anaerobic bacterium. Two types of toxins are produced by the causative agent viz. Tetanlysin and tetanospasmin. The diseases is characterized by stiffness of body including head and neck, tail is look like pump handle due to stiffness, trismus, ophisthotonus, wooden horse appearance and prolapsed in third eye lid. All age group are susceptible to this infection-disposing factors of tetanus in in small ruminants including hearing, punctured wound, dehorning, disbudding, dystokia,tattoing, parturition, trimming of hoof and castration [1]. Goats are very susceptible to the disease [3].

CASE HISTORY

A thirty-nine days old Black Bengal kid was presented to Additional Block Animal Health Centre, Shyamnagar, Tehatta-1 block, Nadia having body weight 7.6 kg was presented with the symptoms of stiffness of body including head and neck (Fig 1.) rectal temperature was 103°F , unable to open mouth, absence of rumination. Moderate tympany of rumen was noticed. Animal was presented in lateral recumbency (Fig 2.).

TREATMENT

The goat was treated with tetanus toxoid 0.5 ml IM 0 day (initial dose) 3 rd (booster dose) and 7th day (booster dose) procaine penicillin @22000-44000 IU/Kg IM for 8 hours interval/ day for 7 days. Diazepam @0.1-0.5 mg/kg IM for 12 hours interval/day for 7 days. Neurobion forte 1 ml IM for 12 hours interval/day for 7 days. Flunixin meglumine-1.1 mg /kg IM 12 hours interval/day for 7days and saline Dextrose 5% @ 10 ml/kg IV for 7 days [2,4,5]. After 7 days of treatment the animal recovered successfully and return to normal posture (Fig 3.).

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Fig 1. Symptoms of stiffness in head and neck

Fig 2. Kid presented in lateral recumbency

Fig 3. Kid recovered completely

RESULTS AND DISCUSSION

Clinical improvement was noticed after one week of treatment. The animal was totally cured after 3 weeks. The main principles in the treatment of tetanus are eliminating the causative bacteria, control muscle spasms until the toxin is eliminated or destroyed, maintain hydration and nutrition and provide supportive treatment [2].

Tetanus toxoid stimulates antibody production to neutralize the toxin. Procaine penicillin is given for elimination of the organism from body. Diazepam acts as mild sedative and intercostals muscles and diaphragm resulting in a normal respiration. Neurobion was given as nervine tonic and fluid therapy helped in survival of animal as it was not able to consume feed orally due to locked jaw condition as well as for rehydration and neutralization of the circulating toxin. Flunixin meglumine act as a anti-inflammatory to control endotoxicosis.

CONCLUSION

The present study clearly shows that treatment of neonatal tetany in goat can be completely cured by using flunixin meglumine, diazepam and procaine penicillin.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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Case Study

UTERINE PROLAPSE IN A DOE GOAT: A CASE REPORT

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ABSTRACT

This study reports a case of uterine prolapse in a doe goat. The animal was brought to the hospital with a complaint of prolapse of the uterus. The everted organ was carefully assessed, and gross debris was removed by washing with dilute chlorhexidine solution. Epidural anaesthesia was achieved using lignocaine solution. The prolapsed uterus was replaced, and a retention suture was placed on the vulva to prevent re-prolapse. Oxytocin, dexamethasone, and broad-spectrum antibiotics (penicillin and streptomycin) were administered intramuscularly. There was no recurrence. Sutures were removed after 7 days.

Keywords: Doe, uterine prolapse, epidural anaesthesia, suture, antibiotics

INTRODUCTION

Postpartum uterine prolapse occurs in all large animal species. It is most common in the cow and ewe, less common in the doe goat, and rare in the mare. It is simply an eversion of the uterus, which turns inside out as it passes through the vagina. Prolapse of the uterus generally occurs immediately after or a few hours of parturition when the cervix is open, and the uterus lacks tone [2]. Prolapse that occurs more than 24 hr. post-partum is extremely rare and is complicated by partial closure of the cervix, making replacement difficult or even impossible [1]. The prolapse is visible as a large mass protruding from the vulva, often hanging down below the animal's hock. The placenta may likely be retained during this period [6]. It normally occurs during the third stage of labour at a time when the foetus has been expelled, and the foetal cotyledons have separated from the maternal caruncles [5].

The aetiology of uterine prolapse is unknown, but many factors have been associated [2, 4]. These include conditions such as poor uterine tone, increased straining caused by pain or discomfort after parturition, Excessive traction at assisted parturition, the weight of retained foetal membranes, conditions that increase intra-abdominal pressure, including tympany and excessive oestrogen content in the feed.

Animals with uterine prolapse treated promptly recover without complication, while delayed treatment could result in death of the animal in a matter of hours or so from internal haemorrhage caused by the weight of the organ, which tears the mesovarium and artery [5]. The success of treatment depends on the type of case, the duration of the case, the degree of damage, and contamination. This study, therefore, aims at highlighting the management of uterine prolapse in small ruminants.

CASE REPORT

A one-and-a-half-year-old West African dwarf goat weighing 13 kg was presented for evaluation and treatment of a prolapsed uterus [Fig 1.], which the owner noticed soon after the goat had kidded 7 hours ago. History further revealed that this was her third pregnancy, and the flock size is 10 goats. The owner normally allowed the goats out in the morning for grazing, but locked them up in the pen at night. A thorough physical examination was carried out, and the vital parameters were: Temperature 39.9ºC, Heart rate 126 beats/min, Respiratory rate 79 cycles/min, and pulse rate 126 beats/min. The ocular mucous membrane was pinkish,

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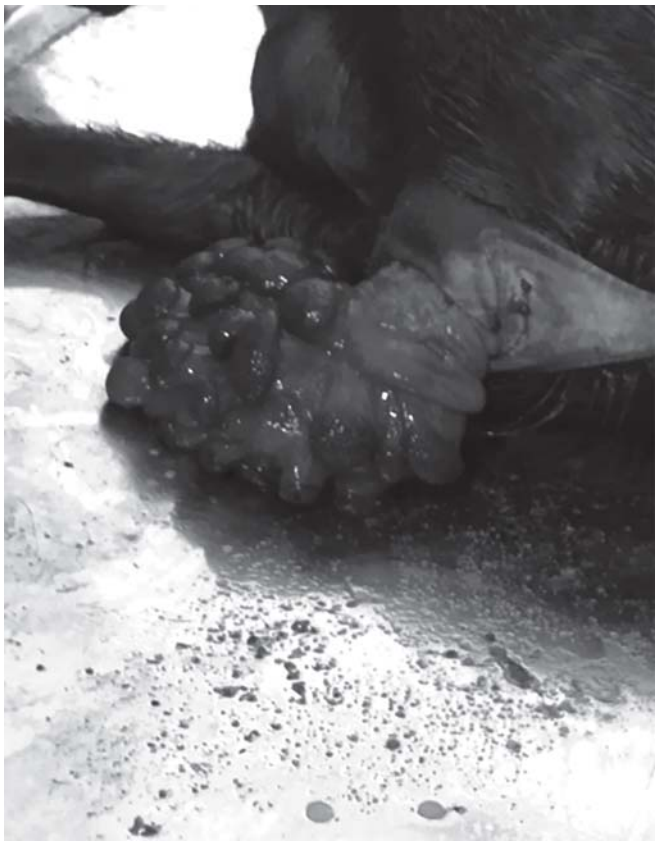

Fig 1. Uterine Prolapse of She-goat

Fig 2. Surgical Correction of uterine prolapse in Goat

and the prolapsed uterus was swollen, necrotic, and stained with faecal materials and debris.

Epidural anaesthesia was achieved by infiltration of 2 mL of lidocaine solution into the first intercoccygeal vertebrae to prevent straining during replacement of the prolapsed organ. After allowing 5 min for the anaesthetics to take effect, sensitivity around the perineal region was assessed by pricking with a needle. The debris and faecal materials were gently removed, and the prolapsed uterus was washed with warm dilute povidone iodine solution, and then washed with Metrogyl i/v solution 10%. The necrotic area was debrided. The doe was then placed on sternal recumbency, and the two hind limbs were pulled out behind her. Then, using both hands with moderate force, the prolapsed uterus was gently pushed in through the vagina. The body was first pushed in, followed by the horns. Horizontal mattress sutures using braided silk were placed in the vulva as a retention technique to hold the uterus in place (Fig 2.).

Oxytocin 10 I.U., Procaine penicillin 20,000 I.U./kg, and Streptomycin 10 mg/kg were administered for 5 days. Dexamethasone 1 mg/kg was given for 3 days. The vulva retention suture was removed after 7 days.

The vital parameters, which were above normal values when the animal was first presented, were monitored on a daily basis, and normal values were attained on the third day of treatment.

DISCUSSION

Prolapse of the uterus normally occurs during the third stage of labour at a time when the foetus has been expelled, and the foetal cotyledons have separated from the maternal caruncles [5]. The goal in the treatment of uterine prolapse is replacement of the organ, followed by a method to keep it in the retained position.

A full clinical examination of animals with uterine prolapse must be undertaken, as signs of toxaemia, like in appetence, an increased respiratory rate, a raised pulse, and congested mucus membranes, may be consistent with metritis. Vascular compromise, trauma, and faecal contamination may also increase toxin intake across the uterine mucosa. However, careful removal of these

materials, after soaking with warm dilute antiseptic solution, is usually successful, causing only minor capillary bleeding. Vigorous attempts to remove superficial contamination should be avoided as they may prove to increase toxin uptake. A caudal epidural anaesthesia is essential before replacement of a uterine prolapse as it decreases straining and desensitizes the perineum [2, 3]. This requires the area over the tail head to the second coccygeal vertebrae to be clipped and surgically prepared. The space between the first and second intercoccygeal vertebrae is then identified by digital palpation during slight vertical movement of the tail. The lignocaine is then injected into the space between the first and second intercoccygeal vertebrae.

The uterine prolapse can be replaced with the animal in a standing or recumbent position [2]. Once the uterus is replaced, the operator's hand should be inserted to the tip of both uterine horns to be sure that no remaining invagination could incite abdominal straining and re-prolapse [1]. If the uterus is completely and fully replaced all the way to the tips of the uterine horns, the prolapse is unlikely to occur [2]. Once the uterus is in its normal position, oxytocin 10 I.U. intramuscularly should be administered to increase uterine tone. It has also been reported that most animals with uterine prolapse are hypocalcaemic [1]. Where signs of hypocalcaemia are noticed, such animals should therefore be given calcium borogluconate.

An injectable broad-spectrum antibiotic, once administered for three to five days after replacement of the prolapsed, will prevent secondary bacterial infection. Dexamethasone is normally given to reduce the uterine swelling. Animals with uterine prolapse that were properly managed can conceive again without problems. Complications develop when lacerations, necrosis, and infections are present or when treatment is delayed.

Shock, haemorrhage, and thromboembolism are potential sequelae of a prolonged prolapse [5]. The high vital parameters witnessed in this case when the animal was first brought could be a result of metritis caused by secondary bacterial infection, especially as the animal was brought for treatment after three days of occurrence of the prolapse.

Treatment with broad-spectrum antibiotics (penicillin 20,000 I.U./kg and streptomycin 10 mg/kg) was responsible for the lowering of the vital parameters to the normal values after three days of treatment.

CONCLUSSION

The present study show that uterine prolapse in doe can be managed by treating horizontal mattress suture followed by procaine penicillin.

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CONFLICT OF INTEREST

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