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Edited by Kiro R. Petrovski



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Volume 28

Aims and Scope of the Series

Paralleling similar advances in the medical field, astounding advances occurred in Veterinary Medicine and Science in recent decades. These advances have helped foster better support for animal health, more humane animal production, and a better understanding of the physiology of endangered species to improve the assisted reproductive technologies or the pathogenesis of certain diseases, where animals can be used as models for human diseases (like cancer, degenerative diseases or fertility), and even as a guarantee of public health. Bridging Human, Animal, and Environmental health, the holistic and integrative “One Health” concept intimately associates the developments within those fields, projecting its advancements into practice. This book series aims to tackle various animal-related medicine and sciences fields, providing thematic volumes consisting of high-quality significant research directed to researchers and postgraduates. It aims to give us a glimpse into the new accomplishments in the Veterinary Medicine and Science field. By addressing hot topics in veterinary sciences, we aim to gather authoritative texts within each issue of this series, providing in-depth overviews and analysis for graduates, academics, and practitioners and foreseeing a deeper understanding of the subject. Forthcoming texts, written and edited by experienced researchers from both industry and academia, will also discuss scientific challenges faced today in Veterinary Medicine and Science. In brief, we hope that books in this series will provide accessible references for those interested or working in this field and encourage learning in a range of different topics.

Meet the Series Editor



With more than 35 years of academic experience, Dr. Rita Payan-Carreira is now a Full Professor at the University of Évora (UÉ). She specializes in animal theriogenology, with a particular focus on the determinants of female fertility and fetal-maternal interaction during implantation. Dr. Payan-Carreira is dedicated to promoting a culture of collaboration and knowledge-sharing within the scientific community. In addition to her extensive publication record, she frequently collaborates with various publishing houses on peer-review activities. She further contributes to the scientific advancement of students and professionals by supervising academic theses and offering technical and scientific courses, including scientific communication, critical reasoning and discussion, and the ethical aspects of research.

Meet the Volume Editor



Kiro Petrovski is an Associate Professor at the University of Adelaide, where he has served as Head of Department and Program Director for 4 years each. With a career spanning Europe, New Zealand, and Australia, he is a distinguished production animal veterinarian with a special interest in bovine mastitis and cattle fertility and health. Dr. Petrovski holds a DVM, MVSc, and Ph.D., and has published extensively in international journals on subjects ranging from antimicrobial resistance to precision technologies in dairy farming. As an educator, he is a leading voice in modernizing veterinary pedagogy, specifically in clinical reasoning and metacognitive skill development. His global consulting work underscores his commitment to bridging the gap between advanced diagnostics and practical ruminant herd health management.

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Preface

Bovine medicine is a cornerstone of veterinary education and a vital field for mixed and production animal practitioners. Traditionally, the approach to bovine practice was often described as an “art”, a term once used to define the complex, intuitive processes of the experienced clinician. Today, we recognize that this “art” is actually a sophisticated metacognitive skill: the ability to monitor and regulate clinical reasoning. As bovine medical education evolves to keep pace with other medical specialties, it is essential to use tools that facilitate the development of metacognitive competencies in learning, practicing, and teaching. This volume provides a comprehensive exploration of bovine medicine, with a dual focus on clinical practice and educational methodology. The book is organized into four sections: I. Alternative Veterinary Medicine, II. Theriogenology, III. Educational and Extension Approaches, and IV. Ancillary Diagnostics.

Section I: Alternative Veterinary Medicine explores ethno-veterinary science, specifically focusing on alternative approaches to antimicrobial use. This section highlights a collaborative initiative between the University of Trans-Disciplinary Health Science and Technology and the National Dairy Development Board (NDDB) in India. An impact study (2016–2019) demonstrates how reducing conventional chemical treatments can lower antibiotic residues in milk while successfully managing conditions such as cowpox, enteritis, mastitis, and repeat breeding.

Section II: Theriogenology presents three chapters on reproductive health and neonatal care:

- Chapter 2 investigates infectious causes of abortion in N'Dama cattle in Guinea, emphasizing the economic toll on traditional farming and the urgent need for better extension systems and government support.
- Chapter 3 provides a clinical framework for managing premature calves (born between 230 and 260 days of gestation), using blood biochemistry and pathophysiology to guide holistic, multimodal treatment.
- Chapter 4 presents a case study on managing herd-level leptospirosis through a combination of diagnostic testing, streptomycin treatment, and strategic vaccination.

Section III: Educational and Extension Approaches addresses the dissemination of knowledge in a changing industry:

- Chapter 5 provides a critical update on Bovine Tropical Theileriosis, a tick-borne protozoan disease that remains a major threat to ruminant populations.

- Chapter 6 outlines a case-based teaching model designed to facilitate clinical reasoning. By integrating structured frameworks with deliberate teaching strategies, it offers a roadmap for developing reasoning skills in the next generation of veterinarians.
- Chapter 7 examines the health challenges of high-producing dairy cows and buffaloes in intensified production systems, advocating for integrated health programs that combine early diagnosis with context-specific interventions.

Section IV: Ancillary Diagnostics highlights technological advancements in the laboratory:

- Chapter 8 reviews the use of metagenomics to understand the bovine gut microbiome and its response to internal parasitic infections.
- Chapter 9 addresses the standardization of flow cytometry for bovine immunohematology, proposing a new methodology to overcome the common challenge of non-specific binding.
- Chapter 10 details a novel thermocoagulation method for the purification of Bovine Serum Albumin (BSA), offering a cost-effective and practical alternative to chromatography when high purity is required.

As the Editor, I am confident this book will be a valuable addition to the bovine medical literature. By bridging the gap between laboratory diagnostics and clinical reasoning, and between traditional practice and modern education, this work serves as a vital resource for students, practitioners, and scientists dedicated to the health of cattle and buffaloes worldwide.

Kiro Petrovski

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Section 1

Alternative Veterinary Medicine

Chapter 1

Ethno-Veterinary Science and Practice as an Alternative Approach to Antibiotics and Other Chemical Veterinary Drugs in Veterinary Care

*Balakrisnan Mannoor Narayanan Nair, A.V. Harikumar
and Natesan Punniamurthy*

Abstract

India leads the world in milk production with 239.2 million metric tons produced in 2023–2024. The unintended side effect of the introduction of crossbreeding with exotic breeds in India was a high incidence of diseases in crossbred animals. Antibiotics and other chemical veterinary medicines had to be extensively used to treat these animals, which led to high antibiotic residues in food products of animal origin. The University of Trans-Disciplinary Health Sciences and Technology, along with the National Dairy Development Board (NDDB), India, has been promoting validated, cost-effective, and efficacious ethno-veterinary medicine (EVM) as an alternative approach since 2017 to rationalize the use of antibiotics and other veterinary drugs for managing several common ailments in bovines. In this approach, the reduction of antibiotic residue in milk is significant. Empirical data from over 1,060,000 cases treated for 34 disease conditions using EVM alone show an overall recovery rate of above 80%. The intervention impact study indicates an 87.78% reduction in antibiotic residue in milk and reduced incidence of mastitis (83.3%), enteritis (63.6%), repeat breeding (96%), and cowpox (100%) from 2016 to 2019. The cost of purchase of antibiotics was reduced from Rs. 18.8 M (2017–2018) to 5.44 M (2020–2021) and the other veterinary drugs from 48.4 M (2017–2018) to Rs. 34.31 M (2020–2021).

Keywords: ethno-veterinary medicine, herbal formulations, animal health, antibiotic residue, antibiotic resistance, public health

1. Introduction

The livestock sector supports the livelihood of over a billion people worldwide, mostly in developing and underdeveloped countries. Dairying plays a major role

in building resilience to natural disasters, ensuring food and nutritional security, generating employment, reducing poverty, and empowering women. For many farmers in India, livestock keeping plays a major role in food and nutritional security, poverty reduction, and women's empowerment. Livestock provide liquidity and build resilience for over 70% of the poor households in the villages during natural disasters.

1.1 Rationale of ethno-veterinary herbal formulations for animal health

The crossbreeding with exotic breeds was initiated to increase the production of milk [1, 2]. However, the hybrid cows were susceptible to diseases, and therefore several antimicrobials were extensively administered to treat these diseases in crossbred animals. The higher risk of antimicrobial residue in milk in developing countries may, partly, be due to the lack of rigorous testing of supplied milk [3]. This led to an increase in antimicrobial residues in animal products like milk above the permitted level, which has the potential for allergic reactions and side effects in people consuming them [4–15], creating a public health problem. It may also result in the development of antimicrobial resistance (AMR). Antimicrobial resistance (AMR) is a global problem today. It is reported that, due to AMR, by the year 2050, an additional 10 million people will die globally, and the loss of gross domestic product (GDP) will increase to \$100 trillion [16, 17]. A major portion of the antimicrobials used ends up in the environment, contaminating water, soil, and biodiversity, thereby increasing the risk of the emergence of AMR. Therefore, it is urgent to focus on an alternate approach for replacing antimicrobials in veterinary care. To ensure food safety and public health, it is important to save the available antimicrobials [18–23].

1.2 Traditional veterinary science in India

Ancient Hindu, Babylonian, Hebrew, Arab, Greek, and Roman civilizations have records in animal medicine. Two hundred fifty medicinal plants and more than 120 mineral salts were known to ancient Egyptians for the treatment of animal diseases [18, 24]. Ancient Indian literature, like the Rigveda and Atharvaveda, has extensive information on the rearing and treatment of cattle, horses, elephants, birds, and many other animals [18]. As cultural norms were strongly related to cattle, they were included in many of the ancient veterinary texts, including Brāhmaṇa, Pashu, Purāṇa, and Veda. These texts also contained regulations for the protection of animals and a list of punishments for cruelty to wildlife [18, 19, 25, 26]. In addition, ethno-veterinary practices and medicines are part of the regular life in Indian villages. Indeed, many of these ethno-veterinary approaches are not documented and are passed on from one generation to another by word of mouth. The local community consults these knowledge holders, and they use the locally available medicinal plants for the treatment of animals. The ethno-veterinary practices are an ecosystem and are ethnic-community specific. Therefore, approaches to ethno-veterinary practices are different according to the healers and their locality.

In post-independent India, the ancient veterinary science and the local breeds have gone into eclipse. The lack of transmission of the ethno-veterinary knowledge ceased, resulting in the risk of the disappearance of the Mrugayurveda. The literature available is in Sanskrit, and it is only present in a few libraries. Yet, the ethno-veterinary practices and knowledge are still alive across India. Additionally, there are

serious limitations, like high treatment costs, indiscriminate use of antimicrobials, and a lack of accessibility to modern veterinary management in many rural areas. Ethno-veterinary science and practice have great potential to address these current challenges. However, they are facing the threat of rapid erosion and loss. There is an urgent need for the revival of these traditional veterinary practices.

The Food Safety Act 2006, Chapter IV, item 21, indicates “no article of food shall contain insecticides or pesticide residue, veterinary drug residues, antibiotic residues, solvent residues pharmacologically active substance and microbiological counts in excess of such tolerance limit as may be specified by regulation.” In this context, it is necessary to revitalize our ancient veterinary knowledge and rediscover its role in sustainable agriculture. In the year 2000, the Indian Council of Agricultural Research recorded 595 veterinary traditions from different sources [18, 24], and 48 of them were recommended for scientific validation. It was found that they showed therapeutic and ameliorative potential. One hundred and fifty-eight plants were cataloged, and 50 have been evaluated for their anti-parasitic activity [27].

2. Alternate approach

Validated, safe, and cost-effective ethno-veterinary practices will be able to rationalize the use of conventional veterinary medicines, not only in developing countries like India but also in developed countries [10]. In this approach, the reduction of antimicrobial residue in milk is significant. Ethno-veterinary practices also lead to the production of safer and cost-effective dairy products, improved animal welfare and human health, and a sustainable environment.

3. Indian systems of medicine

Indian systems of medicine have two streams. Codified systems (Ayurveda, Siddha, Unani, and Tibetan) are based on the theory of physiological functioning, disease etiology, and clinical practices and are legal in India. The non-codified, oral, or folk traditions are location- and ethnic-community-specific knowledge on rearing animals, spread across 4639 ethnic communities all over India. The University of Trans-Disciplinary Health Sciences and Technology and the Tamil Nadu Veterinary and Animal Sciences University have documented 441 formulations of ethno-veterinary medicine from the knowledge holders and assessed their safety and efficacy [21, 25]. A participatory assessment of ethno-veterinary medicine was done using local experience and Ayurveda/Mrugayurveda, as well as Western pharmacology, involving the community, healers, and medical practitioners from various systems of medicine, pharmacology, botany, nongovernmental organizations (NGOs), and people's organizations (**Figure 1**). The effectiveness of ethno-veterinary practices has been reported earlier [22, 23, 28–34]. The transdisciplinary validation of the traditional formulation for mastitis is described earlier [29, 33].

TDU and the National Dairy Development Board (NDDB), India, in collaboration with various milk-producing institutions, have been promoting ethnoveterinary medicines as alternatives to antimicrobials and other veterinary drugs since 2017. They have documented and validated guidelines for the use of ethno-veterinary medicine (EVM) for managing around 30 conditions in cattle.

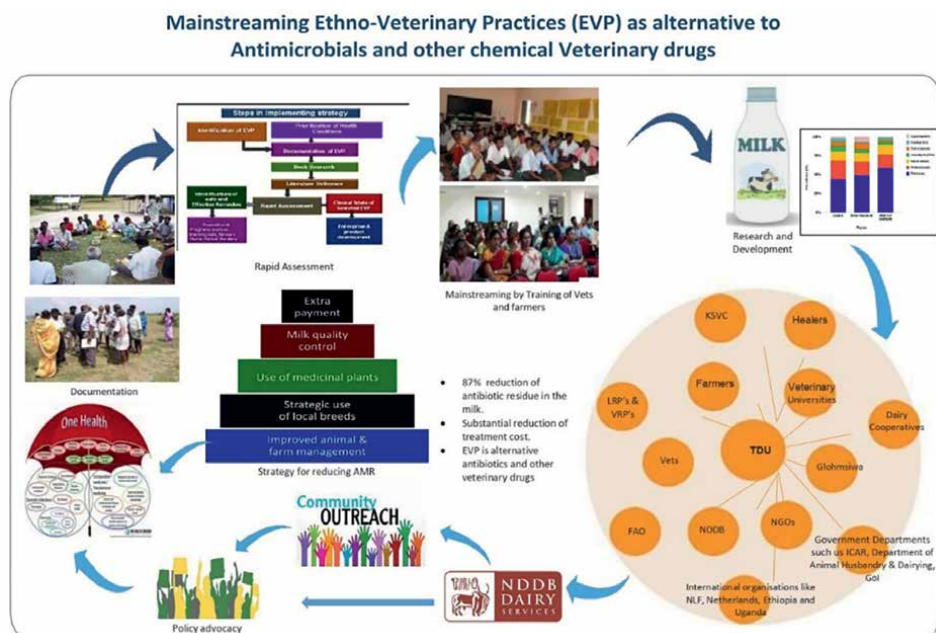


Figure 1. The schematic presentation of the approach to increase the use of ethno-veterinary approaches to the treatment of animal diseases taken by the University of Trans-Disciplinary Health Sciences and Technology and the Tamil Nadu Veterinary and Animal Sciences University.

A simple, cost-effective, and efficacious EVM has been used to manage ailments in livestock in India as an alternative to the Western treatment options, providing an instant management option to those in remote areas who cannot afford conventional veterinary care. We have trained over 3134 vets, 10,076 animal health (AH) personnel, and around 30,000 dairy farmers and established 613 demonstration plots across the country (TDU-NDDB data). A total of 79,832 veterinary service calls from farmers have decreased in 2019–2020 compared to 2017–2018 (NDDB data). The feedback data from over 106,000 individual cases for 29 conditions have been recorded with an above 80% recovery rate (**Table 1**). The treatment with ethno-veterinary herbal formulations has shown an 87.9% reduction in antimicrobial residues in milk. The intervention impact study using Ethno-veterinary practice (EVP) indicates reductions in the incidence of mastitis (83.3%), enteritis (63.6%), repeat breeding (96.0%), and cowpox (100%) from 2016 to 2019 [35].

The cost of purchase of antibiotics was reduced from Rs.18.8 M (2017–2018) to 5.44 M (2020–2021, saved Rs. 13.42 M for farmers) and the other veterinary drugs from Rs. 48.4 M (2017–2018) to Rs.34.31 M (2020–2021, saved 13.81 M for farmers) (**Figures 2 and 3**). The intervention has created a double-fold increase in the awareness of EVP among stakeholders (NDDB), a double-fold increase in the awareness of antimicrobial residues in milk (NDDB), and a six-fold reduction in the preference for antimicrobials for mastitis among farmers. All the validated and field-tested herbal formulations were included in the Standard Guidelines for the treatment of animals released by the Government of India, Department of Animal Husbandry and Fisheries (2024).

S.no	Ailments	# treated	# apparently cured	Cure %
1	Deworming	31,064	29,418	95%
2	FMD mouth lesion	14,191	12,229	86%
3	Diarrhea	188,279	158,482	84%
4	Indigestion	45,534	38,231	84%
5	Udder edema	15,623	13,115	84%
6	Blood in milk	22,643	18,865	83%
7	FMD foot lesion	9950	8167	82%
8	Wound	13,489	11,024	82%
9	Fever	215,245	175,847	82%
10	Ectoparasites/ticks	8308	6624	80%
11	Metritis (all forms)	17,939	14,166	79%
12	Bloat	9075	7121	78%
13	Mastitis (all forms)	344,085	268,139	78%
14	Leukoderma	316	242	77%
15	Agalactia	5332	4036	76%
16	Retention of placenta	12,621	9487	75%
17	Wart	5185	3815	74%
18	Swelling/ Joint pains	3800	2790	73%
19	Milk fever	1174	860	73%
20	Lumpy skin disease	6536	4757	73%
21	Anestrus	26,005	18,774	72%
22	Hygroma	2185	1561	71%
23	Prolapse	2517	1735	69%
24	Poisoning	1113	767	69%
25	Teat obstruction	6784	4651	69%
26	Downer	4079	2733	67%
27	Cystic ovary	1669	1112	67%
28	Repeat breeder	20,550	13,546	66%
29	Thelitis	1792	1049	59%
Grand total		1,037,083	833,343	80%

Table 1.
Empirical data on treatment with ethno-veterinary medicine alone over a million cases for 29 conditions show an overall recovery rate of above 80%.

We believe the benefits of EVM have only been partially discovered, and more work must be carried out in this field. One of the essential steps is the spread of the obtained knowledge. Therefore, the ethno-veterinary science and practice group at the TDU provides opportunities for certificate, diploma, and PhD programs in EVM. We also provide training programs to other countries, including, but not limited to, Ethiopia, the Netherlands, and Uganda.

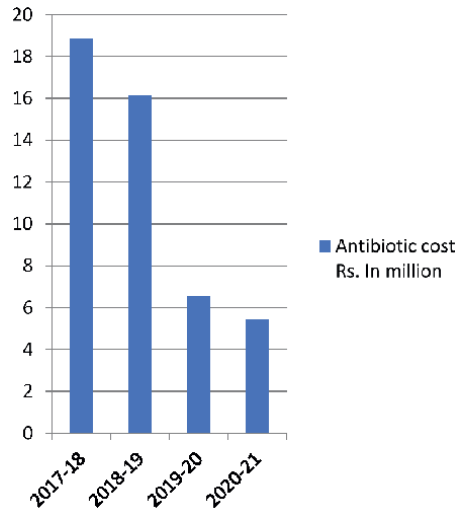


Figure 2.
Reduction of cost on antibiotics from Rs. 18.86 m (2017–2018) to Rs. 5.44 M (2020–2021), NDDB data (saved 13.42 million rupees from 2017 to 2021).

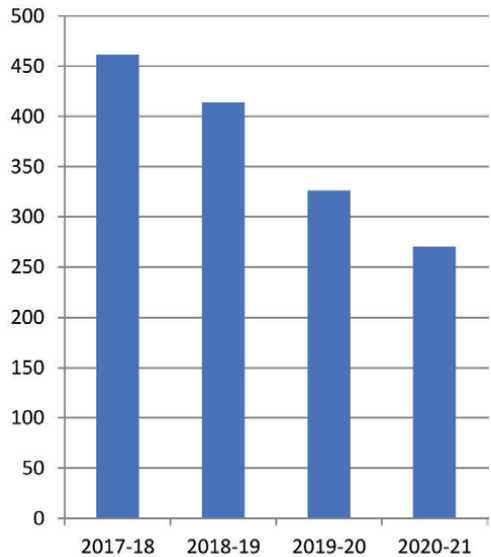


Figure 3.
Reduction of cost on other veterinary drugs from Rs. 48.12 M (2017–2018) to Rs. 34.31 M (2020–2021), NDDB data (saved 13.81 rupees from 2017 to 2021).

4. Conclusion

The use of the validated EVM-based herbal formulations as an alternative to chemical veterinary drugs has resulted in a significant reduction in antimicrobial residues in milk. The EVM can reduce the dependence on antimicrobials and other

chemical veterinary drugs in veterinary health, coupled with the production of safer and cost-effective dairy products, concurrently improving environmental sustainability. Rationalizing the use of antimicrobials through alternate approaches is a pragmatic proposition in developing countries, wherein the resources of stakeholders are limited. Transferring this EVM knowledge to the farmer would empower them to manage many ailments that dent their already meager income at minimal costs.

Conflict of interest

The authors declare no conflict of interest.

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Section 2

Theriogenology

Abortive Infectious Diseases in the N'Dama of Guinea

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Abstract

Abortive infectious diseases, in certain situations, cause serious damage to traditional livestock farming and hamper the economic development of livestock farmers. These diseases include Brucellosis, genital infections, Rift Valley Fever (RVF) and vaginal trichomoniasis. Their impact in rural areas remains poorly understood in Guinea. Several epidemiological surveys have been conducted, but in isolated cases and without covering the entire Guinean territory. Overall, the abortion rate on farms is lower than that in rural areas of Guinea. It varies depending on the implementation of biosecurity measures. Furthermore, to limit the risk of contamination in rural areas, artificial insemination is used less frequently. Bovine medicine encompasses the teaching and practice of health improvement, prevention, diagnosis and treatment of diseases in cattle. To reconcile animal health and the socioeconomic aspects of farms, interaction between livestock farmers and animal health professionals is imperative. A compilation of these studies could lead to the implementation of effective prophylactic measures.

Keywords: diseases, reproduction, abortion, N'Dama, Guinea

1. Introduction

The renewed interest in N'Dama cattle breeding is due to their hardiness and prolificacy, enabling rapid rebuilding of the herd after a heavy mortality [1, 2]. Improving N'Dama's productivity depends to a large extent on improving their health status, which in turn depends on a better understanding of their diseases. Climatic conditions offer opportunities for the occurrence of infectious diseases, such as genital diseases. These diseases, poorly diagnosed and treated, spread rapidly, leading to numerous cases of sterility and abortion. This is particularly difficult as many genital infections are not overly represented at the moment of their incidence. There are many different pathogens involved, such as: *Brucella*, *Campylobacter*, *Candida*, *Chlamydophila* and *Trichomonas* spp., and *Corynebacteria*, gonococci, herpes viruses, etc. [3, 4].

One of the main constraints affecting the productivity of Guinean livestock is the high number of abortions on most traditional farms, the causes of which are often not diagnosed. However, recent studies show that Brucellosis, genital tract infections, Rift Valley Fever (RVF) and vaginal trichomoniasis play an active role in the resurgence of these abortions [5].

A survey carried out by Sylla et al. [6] indicated a 6.9% prevalence of Brucellosis in various regions of Guinea. Most hygroma-carrying animals responded positively to all three serological tests (Wright's serum agglutination, buffered test and complement fixation). Another study by Fred et al. (2002) showed that the range of abortion on farms varies from 40% (21/50 farms) in Guinea to 70% (14/20 farms) in rural Gambia [7].

An initial study of small ruminants brucellosis in the Lower Guinean breeding areas of Dubréka and Kindia revealed immunoglobulin G (IgG) antibodies in 3% of animals (9/300 sera) in Dubréka, 7% of animals in Kindia and 3.6% of animals (1/271 sera) in Lower Guinea [8]. These immunoglobulins can be used to identify recent (immunoglobulin M (IgM)) and late (IgG) infections. Recent infections were observed in Dabola, and late infections in Boffa, Boké, Fria, Kankan, Kouroussa, Mandiana and Siguiro [9].

For Trichomoniasis in Nigeria, a prevalence of 8.75% in males and 6.23% in females was observed [10]. Recent epidemiological studies on abortifacient diseases carried out at the PROGEBE (Endemic Ruminant Livestock Management Project) and CAE (caprine arthritis-encephalitis) (Livestock Support Centers) sites in Boké and Beyla and their surroundings are the latest data available in Guinea [11].

2. About the N'Dama race of Guinea

N'Dama or N'Dama is a horned, humpless cattle breed from West Africa, renowned for its resistance and adaptability to agricultural environments. The cradle of the breed is in Fouta-Djalon (Guinea), from where it has spread to several countries: Angola, Burkina Faso, Côte d'Ivoire, Democratic Republic of the Congo (DRC), Ghana, Gabon, Cameroon, Mali, Senegal and Togo [12].

This expansion is largely due to its good butchering characteristics, its hardiness, resistance to trypanosomosis (trypanotolerance), transmitted by tsetse flies, and to piroplasmiasis transmitted by ticks. This makes it the only breed for the regions that can be bred without numerous health and husbandry problems [13–15].

However, due to their poor resistance to prolonged drought conditions, the expansion of N'Dama cattle is restricted to West and Central Africa [1, 16]. The N'Dama breed was introduced to the Caribbean in the nineteenth century, where similar infectious diseases were already endemic and where it was used as the basis for crosses with other breeds [17].

Population estimates by the Domestic Animal Diversity-Information System (DAD-IS) (2005) give approximately 7 million heads, while the Food and Agriculture Organization of the United Nations (FAO) mentions 9 million heads, distributed in the countries of West and Central Africa (**Figure 1**) [18].

2.1 Characteristics of N'Dama

The N'Dama is compact, short-legged and finely boned. The neck is thick and deep. The rear is broad and well-muscled. The back is level from the withers to the tail. The head is short and broad, with a straight profile and broad muzzle. The horns are around 60 cm long and lyre-shaped toward the top. However, dehorning at one month of age produces hornless adults. The typical coat is fawn with darker tips. The breed is medium-sized, with 100 cm at the withers for cows and 120 cm for bulls.

The N'Dama are renowned for their excellent butchering characteristics, producing tasty meat with no excess fat, and a carcass yield of over 50%.



Figure 1.
Distribution of the N'Dama in Africa [12].

A study by SATEC in 1973 and the Zootechnical Research Station in Kolda in Senegal in 1976 gave the following information:

- Bulls weigh between 280 and 320 kg and cows weigh between 219 and 237 kg. Age at first calving is 3–5 years. Calving interval is 16–20 months, with 80% of calvings occurring in the last 6 months of the year. The abortion rate varies from 10 to 20%, giving a calving rate of 56%. Calves are weaned at around 12 months and castrated at 3 years. The annual mortality rate is 12% overall, with 30% for calves under one year old, 15% for animals between one and two years old and 5% for adults. Leather production is used by craftsmen and industry [12].
- Dairy skills are fairly limited. Production reaches around 500 kg per lactation.

The N'Dama Large Livestock Farms Company in Central Africa (SOGENAC, formerly JVL) in the DRC currently has the largest herds of this breed, with a total of around 50,000 heads. It has been selecting the N'Dama breed since 1923 and has improved productivity, body conformation and adult weight without affecting hardiness (**Figures 2–5**) [12, 19].

A selection scheme is in place to improve the following criteria:

1. Hardiness: easy calving, tolerance to trypanosomiasis and resistance to piroplasmosis.
2. Female fertility: age at first calving, calving-to-calving interval, etc.
3. Dairy value of females: 120–210-day growth and weaning weight of calves, weight at 3 years for females and at 4 years for males.



Figure 2.
N'Dama cattle from Guinea in Boké [12, 19].



Figure 3.
N'Dama cows from Guinea in Beyla [12, 19].

Cows weigh between 260 and 430 kg and bulls weigh between 320 and 560 kg. Steers are generally marketed at the age of 4, weighing between 300 and 400 kg.

The heifers are put to the bull at 36 months, and the calving interval averages 14 months, giving a calving rate of over 80%. Calving is easy, and the unassisted birth rate is close to 100%. The overall annual mortality rate is around 3% [12].

2.2 Food

Strict selection, slash-and-burn management, mineral supplementation and veterinary care given for present-day N'Dama cattle have shown considerably improved results compared with traditional N'Dama.

In relation to the type of farming, the N'Dama cattle's diet varies from extensive farming, where the animals are left to graze, to intensive farming, where the animals are left to graze and provided additional feed.

Here are some general recommendations for feeding Guinea's N'Dama:

1. Fodder: The basis of their diet should be quality fodder, such as forage grasses, natural pastures, tree leaves and scrubs. Grazing must be well managed to avoid soil degradation and ensure a constant supply of feed.
2. Grains and concentrates: During periods of growth, lactation or work, it may be necessary to add concentrates (e.g., maize, sorghum, soya, etc.) to increase energy and protein content.



Figure 4.
Livestock market in Gaoual, N'Dama, Guinea [12, 19].



Figure 5.
N'Dama cattle from Guinea [12, 19].

3. Hay: In the dry season or when pasture is insufficient, good-quality hay should be fed to maintain health and productivity.
4. Minerals: Supplementation with minerals, particularly salt, calcium, phosphorus and other trace elements, is essential to prevent deficiencies.
5. Fresh water: Sufficient water should always be available, as it is vital for the animal's digestion, health and productivity.
6. Vitamins: Vitamin supplementation may be necessary, especially during periods of stress or dietary deficiency

It is important to adjust the diet according to age, physiological stage (growth, reproduction and lactation) and environmental conditions. Consultation with a veterinarian or a local animal nutrition specialist is recommended to establish a specific and effective feeding plan [20].

2.3 Importance of N'Dama cattle in rural areas

Here are some of its main uses in agriculture:

1. Draught work: The N'Dama is often used as a draught animal for agricultural work, such as plowing and transporting crops or tools, because of its robustness and resistance to fatigue.

Disease	Agent	Transmission	Symptoms/Signs	Diagnosis	Prevention/Control
Babesiosis (Piroplasmosis)	<i>Babesia</i> spp.	Ticks	Fever, abortions, anemia	Blood smear, PCR	Vector control (acaricides) Avoid pastures with transmission sources Vaccination
Bovine brucellosis	<i>Brucella abortus</i>	Contact with secretions through ingestion, environment	Abortions (usually in the 5th–7th month), infertility	Serology (Rose Bengal, ELISA), culture	Vaccination (S19, RB51), biosecurity, elimination of positives
Bovine chlamydiaophilosis	<i>Chlamydia phila abortus</i> and <i>C. pecorum</i> .	Contact with infected fetus, fetal adnexa, uterine or vaginal secretions, and milk from infected females	Abortions, metritis, infertility, irregular cycles	Stamp stain, PCR	Strict hygiene measures to prevent the spread of the disease, Antibiotic therapy (Oxytetracycline)
Bovine trichomoniasis	<i>Trichomonas foetus</i>	Venereal (natural mating)	Early abortions, return to heat	Smear, culture, PCR	Bull control, artificial insemination, hygiene
Genital campylobacteriosis	<i>Campylobacter fetus</i> subsp. <i>venerealis</i>	Venereal	Infertility, early abortions	Smear, culture, agglutination test	Bull health control, hygiene during insemination
Q fever	<i>Coxiella burnetii</i>	Inhalation of contaminated dust, contact with parturition fluids	Late abortions, but often subclinical in animals	ELISA and PCR serology: on placenta, milk or vaginal swabs	Manure management, collection and destruction of abortion products Vaccination
Rift Valley Fever	<i>Phlebovirus</i>	Mosquitoes (e.g., <i>Aedes</i> , <i>Culex</i> spp.)	Storm abortions, neonatal mortality	Serology, PCR	Mosquito control, vaccination (if available)

Table 1.
Abortive infectious diseases often encountered among the N'Dama in Guinea.

2. Meat production: It produces good-quality meat, which is highly prized by local consumers. Its carcass is often lean, and the breed is valued for the hardness of its meat.
3. Milk production: Although its milk production capacity is moderate, the N'Dama provides milk for local consumption with an appreciable butter content, particularly in rural areas where cattle rearing is essential for nutrition.
4. Genetic improvement: The breed is often used in selection programs to improve other local cattle breeds or to increase their resistance to disease and their adaptation to difficult conditions.
5. Contribution to soil fertility: The grazing management and dung produced by these cattle contribute to soil fertility in rural areas.

In short, the N'Dama plays a multidimensional role in agriculture, combining economic and sociocultural activities, while providing valuable resistance to the region's climatic and health challenges [21].

2.4 Abortive diseases in N'Dama cattle

A study carried out in Guinea as part of a research project funded by PROGEBE-Guinea in 2020 has highlighted several diseases linked to abortion in N'Dama cattle in Guinea [11].

Several pathologies can lead to abortions, premature births and lesions of the reproductive organs in cows. The abortion rates observed in some studies vary between 10 and 20%. Several references provide a solid basis for an idea of the abortifacient diseases affecting N'Dama cattle in Guinea [6, 22, 23].

Overall, these abortifacient diseases often encountered in N'Dama cattle are presented in **Table 1**. Generally speaking, the many studies carried out on abortifacient diseases in Guinea have shown that Brucellosis is in first place compared with other diseases. It varies according to the farming system, the geographical areas considered and the diagnostic methods used [3, 24, 25].

3. Bovine brucellosis

Brucellosis is an infectious disease common to many animal species and humans. It affects cattle, pigs, sheep, goats, horses, camels and dogs. It can also affect other ruminants, certain marine mammals and humans. It is caused by bacteria from different biovars belonging to the *Brucella* genus.

It is both a serious, notifiable zoonosis for humans (a disease transmitted from animals to humans through the handling of contaminated material or contact with contaminated animals) and a contagious disease in farm animals with a major economic impact (production losses and trade barriers). In animals, the signs are often abortion or reproductive failure. Generally, animals recover and succeed in giving birth to live offspring after an initial abortion, but the animals may continue to excrete the bacteria and thus transmit them. In animals, *Brucella* bacteria concentrate preferentially within the genital organs. Brucellosis is therefore generally spread venereally and during abortion or parturition; high concentrations of bacteria are

Age groups			2-5 years			6-9 years			10-13 years			14 years		
Sites	N	P	%	N	P	%	N	P	%	N	P	%		
Beyla	83	11	13.725	44	13	29.55	25	9	36	2	0	0		
Boké	38	3	7.89	25	0	0	18	0	0	5	0	0		
Dinguiraye	40	0	0	20	0	0	0	0	0	0	0	0		
Gaoual	24	3	12.50	36	4	11.11	6	0	0	0	0	0		
TOTAL	185	17	9.19 [5.60-14.54]	125	17	13.60 [8.35-21.16]	49	9	18.37 [9.24-32.50]	7	0	0 [0.00-43.91]		

Table 2.
Prevalence of Brucellosis in PROGEEB-guinea primary sites.

found in abortion products and fetal fluids from an infected animal. The bacteria can survive for several months outside the animal's body, in the outdoor environment, particularly in cold, damp conditions. These bacteria in the environment remain a source of infection for other animals, which become infected through close contact (by the respiratory or conjunctival route, or even by ingestion). The bacteria can also colonize the udder and contaminate the milk. Humans then become infected mainly through ingestion of raw milk or dairy products, but also through close contact (via the respiratory or conjunctival route), mainly in the workplace. Most *Brucella* species can cause human contamination.

Studies have reported the prevalence of bovine brucellosis in the N'Dama. Bovine brucellosis has been found throughout Guinea (**Table 2**). It is often expressed clinically by the presence of hygromas, abortions (often reported) and sterility in infected females.

The overall prevalence found in 2000 was 11.75% (43 cases out of 366 samples). *Brucella abortus* was frequently found. Positivity according to age was higher in young animals than in older animals [11]. It has also been noted that the prevalence of Brucellosis is spreading from the coast toward the interior of the country.

4. Bovine trichomoniasis

The causative agent of Trichomoniasis is *Tritrichomonas foetus*, a protozoan of the subkingdom Protozoa (now classified in the kingdom Protista). *T. foetus* is usually piriform in shape but is pleomorphic. The parasite is 5–25 µm in size, spindle-shaped with four flagella, which are whip-like projections, and an undulating or wavy membrane [26].

After copulation, *T. foetus* invades the cow's vagina and migrates to the cervix and uterus. Infection has little impact on conception and early embryonic development. However, the organism induces an inflammatory response that can lead to endometritis, cervicitis, vaginitis, and possibly salpingitis, all of which can result in embryonic and early fetal death. Fewer than 1% of cows become chronic carriers. Trichomoniasis occurs worldwide, wherever cattle are present.

The four epidemiological features typically associated with Trichomoniasis are the following:

1. The disease is transmitted via coitus (or by artificial insemination with infected semen).
2. Typically, infected bulls are subclinical carriers.
3. Females are infected at the time of breeding, and most of them mount an immune response and clear the infection after two or three estrus cycles.
4. Trichomoniasis results in decreased herd fertility and irregular calving distribution. Infection of a cow by an infected bull is extremely efficient, whereas bulls are not readily infected by cows. Trichomoniasis can be transmitted mechanically via artificial insemination; however, this method is not a major contributor to disease transmission.

The most common indicator that *T. foetus* has infected a herd, especially in naive populations, is that a large proportion of cows show clinical signs of returning

to estrus as a result of embryonic death; however, embryonic loss can occur after maternal recognition of pregnancy, resulting in long interestrus intervals. Conceptus losses early in the breeding season result in an overall decrease in pregnancy rates, especially in a restricted breeding season, and in an increase in late-season pregnancies. The prevalence of postcoital pyometra is increased in herds infected by *T. fetus*. Although the main effect of Trichomoniasis is conceptus losses early in the breeding season, late-gestation abortions can occur, and live calves can be born to infected cows. Typically, infected bulls do not show clinical signs of Trichomoniasis.

Results obtained by the research team on the above-mentioned project show that N'Dama cattle are free from the disease. Herds infected with *T. fetus* are cleaned up by culling (selling) males over 4 years old, and by postponing the breeding of infected females until they have cleared the infection. The use of artificial insemination has greatly reduced the prevalence of this infection, but it persists, particularly on extensive farms [11, 27].

5. Genital campylobacteriosis

Bovine genital campylobacteriosis is a venereal disease. The causative pathogen of this venereally transmitted disease is *Campylobacter fetus* subsp. *venerealis* (Cfv), one of the three subspecies of *C. fetus*. Cfv infection in cattle leads to infertility, early embryonic death and abortions, resulting in considerable economic losses. Bulls are important reservoirs of the causal agent. The Cfv infection in cattle is manifested by abortions, but its prevalence is more sporadic. The pathogen can be isolated from aborted bovine fetuses, demonstrating its clinical relevance in cattle. *Campylobacter* has a pronounced tropism for the genital tracts of both male and female cattle. The bacterium is mainly transmitted during natural breeding. The disease can also be spread by artificial insemination when the bull's semen is infected. While *C. fetus* is considered to be primarily an animal pathogen, as an opportunistic pathogen, this bacterium is occasionally observed in humans.

The infection is most often diagnosed in pregnant women or immunodeficient patients, and is often systemic, with various complications depending on its location. Although industrial vaccines are available and it is possible to use an autologous vaccine produced from the strain isolated on the farm, there is a general lack of scientific proof that vaccination is effective. In relation to this disease, the circulation of *Campylobacter* spp. strains in certain farms has been reported [28].

6. Rift valley fever

Rift Valley Fever is a viral zoonosis that mainly affects animals, but can also affect humans. In both animals and humans, the infection can cause severe signs and symptoms. It causes considerable economic losses through the death and abortion of animals in infected herds [29].

The RVF virus belongs to the *Phlebovirus* genus. It was first identified in 1931 during an investigation into an epidemic affecting sheep in Kenya's Rift Valley. Since then, outbreaks have been reported in sub-Saharan Africa. In 1977, an explosive outbreak occurred in Egypt, where the RVF virus was introduced through cattle trade along the Nile irrigation system. In 1997 and 1998, a major outbreak occurred in Kenya, Somalia and Tanzania, following an event linked to the El Niño phenomenon

and heavy flooding. In September 2000, trade in infected cattle from the Horn of Africa led to the spread of the RVF virus to Saudi Arabia and Yemen. This was the first time the disease had been observed outside the African continent, raising concerns about its possible spread to other parts of Asia and Europe [30].

The epidemiology of Rift Valley Fever is based on the relationship between the causal agent, the animal host and the vector in its biotope. The presence of rivers and lakes encourages the proliferation and sedentarization of vectors (*Aedes* spp.) carrying the infectious agent of this disease. Frequent contact between cattle and sheep and the vector is a key factor in the presence of the disease. At the sites visited, the absence of favorable edapho-climatic conditions and disease-carrying vectors seems to prevent the appearance of infections [31, 32].

7. Bovine chlamydophilosis

Chlamydophilosis, previously known as chlamydiosis, is an infectious disease caused by bacteria of the genus *Chlamydophila* and can affect N'Dama cattle. The disease is often subclinical, but can lead to reproductive problems, such as abortions, metritis, infertility and irregular cycles. Respiratory problems and mastitis may also be associated.

In N'Dama cattle, the disease is caused in particular by *Chlamydophila abortus* and *Chlamydia pecorum*.

The disease is transmitted mainly via feces, but also via fetuses, fetal appendages, uterine or vaginal secretions and the milk of infected females.

The disease is often subclinical, but can manifest itself as:

- a. Reproductive disorders: abortions, metritis, infertility, irregular cycles.
- b. Respiratory problems: Difficulty breathing, coughing.
- c. Mastitis: Inflammation of the mammary glands.

Diagnosis can be difficult, but it can be made by:

- 1. Stamp staining: Older method, less sensitive and specific.
- 2. PCR: Polymerase chain reaction (PCR), performed on different media (swab, placenta, abortus).

Prevention can include strict hygiene measures to avoid the spread of the disease. Treatment may involve the use of tetracyclines, but it is important to note that efficacy can vary, and the bacteria can persist in the body.

Few studies have reported chlamydophilosis in N'Dama cattle. This condition goes unnoticed but can have serious consequences for reproduction. Careful monitoring and early diagnosis are essential to limit economic and health losses.

8. Babesiosis (Piroplasmosis)

Babesiosis, or piroplasmosis, is a disease transmitted by ticks of the genus *Ixodes*, caused by protozoa (intraerythrocytic parasites) of the genus *Babesia*. It is one of the

most common infections, characterized in the acute phase by hemolytic anemia and fever, with hemoglobinuria and a rapid progression to death in the absence of treatment [33].

It is distributed worldwide and affects many mammal species. The natural mode of transmission is the bite of an infected *Ixodes ricinus* tick. Babesiosis is a rapidly progressive and potentially fatal disease.

The first sign is usually a high fever, with temperature reaching 41.5°C, which lasts for several days before other signs appear. This is followed by anorexia, ruminal hypotony to atony, risk of abortion and a high respiratory and heart rate.

In Guinea, the disease has been shown to circulate in cattle, particularly the N'Dama breed, in a number of localities. Numerous studies have revealed high prevalence in different areas, such as in the prefecture of Dabola, where the observed prevalence varied from 23.0 to 53.8% [34–36]. Similar studies were carried out in Dubréka with a prevalence of 57.4 [37], Mamou 58.4 [38] and Siguiri, 55.3% [39]. In case of suspected babesiosis, it is important to consult a veterinarian for advice on appropriate preventative measures in the area.

9. Q fever

Q fever is a bacterial zoonosis caused by *Coxiella burnetii*, an intracellular bacterium that is highly resistant in the environment. In cattle (as in sheep and goats), it is often subclinical, but it can cause reproductive problems.

There is no widely available vaccine. General prevention measures include abortion management (isolation, disinfection), safe disposal of placentas and fetuses, serological surveillance and raising awareness among livestock farmers about zoonotic risks.

A few serological surveys show moderate seroprevalence rates in certain pastoral areas [9]. For the first time in Guinea, a case of human Q fever has been identified and officially recorded. The role of livestock, small mammals and bats in the circulation of the pathogen has been established [23]. It has been shown that the main vectors in Guinea are ixodid ticks of the species *Amblyomma variegatum*, *Hyalomma truncatum* and *Rhipicephalus decoloratus* [23]. In another study, ticks were collected from cattle in six regions of Guinea. Laboratory analyses detected 7.2% (675/9620) of *C. burnetii* DNA in tick samples collected from cattle [40]. A study reported that Q fever is widespread throughout the country and represents the most frequently detected zoonosis with an average seroprevalence of 20.5% (95/463) in cattle [41]. The proportion of *C. burnetii*-positive sera in cattle in Guinea ranged from 3.2 to 18.7%, with an average of $8.0 \pm 0.6\%$ [42]. However, a more recent study indicates a relatively higher prevalence of 46.67% in cattle in the Mamou administrative region [22].

10. Teaching bovine medicine related to abortive infectious diseases in N'Dama

The field of bovine medicine encompasses teaching and practice in the health, prevention, diagnosis and treatment of disease in cattle. This will encompass the bovine abortive infectious diseases in N'Dama cattle.

Overall, bovine medicine teaching in Guinea includes the following areas:

Academic training: Bovine medicine is integrated into the general curriculum of veterinary disciplines, at the *Higher Institute of Veterinary Sciences and Medicine (ISSMV)* in Dalaba, Guinea, with modules on anatomy, animal nutrition, biosecurity, epidemiology, ethics and animal welfare, livestock management (breeding and management of animal populations), microbiology, parasitology, pathology, pharmacology, physiology, radiology, reproduction and obstetrics, research methodology and more.

In addition to this series of courses, laboratory techniques are essential to complete the diagnosis and treatment of animal diseases. In addition to the classic and universal basis of veterinary education, this exercise should also consider the specific characteristics of countries or geographical regions. This would apply to bovine abortive infectious diseases.

1. Practical placements: On-the-job training at institutes, livestock farms, slaughterhouses, research or similar institutions to enable learners to acquire practical skills. This should be introduced at group level and throughout the course, as, for example, at ISSMV in Dalaba. Practical placements, or workplace-integrated learning, will improve the understanding and practical competencies related to bovine abortive infectious diseases, but also other aspects of bovine medicine.
2. Conferences, seminars and workshops: It is essential to allow presentation of research results and innovation in the improvement of clinical and laboratory diagnostic methods, treatment and health control related to bovine abortive infectious diseases, but also other aspects of bovine medicine.

In terms of species-related approaches, bovine medicine often has to balance animal health with the economic aspects of farms. Interaction with breeders, farmers and other professionals is essential for effective management of bovine abortive infectious diseases, but also other aspects of bovine medicine.

11. Conclusion

Research findings on abortifacient infectious diseases among the N'Dama of Guinea vary according to the scope of the study and the area investigated. On the whole, Brucellosis is the most frequently found infectious disease. The seroprevalences observed in the herds in Guinea are within the overall seroprevalence range for bovine brucellosis in West Africa. Brucellosis is prevalent in Guinea and could hinder the development of livestock production. This means that Brucellosis and other infectious diseases must be strictly controlled, not only in animals intended for breeding, but also in livestock products such as fresh milk for consumption in Guinea. In-depth studies on pathologies linked to abortions and other reproductive problems in endemic ruminant livestock should be carried out with the aim of ensuring that livestock flourish in Guinea and throughout the geographical area occupied by the N'Dama breed of cattle. The training of veterinarians at professional and higher levels must be ongoing to enhance the value of this profession and make it a lever for strengthening food security in Guinea.

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Clinical Management of Premature Calves

Alper Erturk, Suleyman Serhat Iyigun and Mahmut Ok

Abstract

Premature birth in calves, which is defined as delivery between 230 and 260 days of gestation, is a major cause of neonatal morbidity and mortality in cattle industries. This chapter aims to provide a comprehensive overview of the clinical management of premature calves, focusing on emergency interventions, diagnostic criteria, daily care protocols and monitoring complications. Premature calves, due to the immaturity of multiple organ systems, typically present with respiratory distress syndrome, hypothermia, hypoglycemia, gastrointestinal dysfunction, and underdeveloped reflexes. Clinical, hematologic, and biochemical assessments, including blood gas analysis, play a vital role in evaluating prognosis and guiding treatment strategies. Emergency management includes airway clearance, oxygen therapy, thermal support, glucose infusion, and colostrum administration. Supportive treatments such as nebulized bronchodilators, corticosteroids, antimicrobials, and nutritional supplementation can enhance recovery outcomes. A structured flowchart-based approach has been developed to streamline critical early care. To illustrate a successful multidisciplinary management strategy resulting in full recovery within 72 hours, an example clinical case is presented. Early recognition, systematic monitoring, and treatment protocols are essential for improving survival rates in premature calves. This manuscript is intended to guide clinical veterinarians and veterinary students in the evidence-based management of neonatal bovine prematurity.

Keywords: preterm ruminants, immature lung function, oxygen therapy in newborn livestock, neonatal calf care, perinatal health in calves

1. Introduction

In cattle, parturition occurring before the 260th day of gestation is defined as preterm birth, while calves born alive between days 230 and 260 of gestation are classified as premature calves [1, 2]. Premature calf birth is a significant and commonly observed cause of calf mortality globally [3]. Several factors and different pathological mechanisms are involved in causing preterm calving. Premature births may be caused by infectious diseases (bacterial, fungal, protozoal, and viral), physical factors (hypothermia, hyperthermia, hypoxia, trauma), metabolic disorders (thyroid dysfunction and hepatic lipidosis), environmental factors (plant or synthetic toxins

and mycotoxins), deficiencies in protein, energy, vitamins, and minerals, hormonal influences (administration of corticosteroids, estrogens, and prostaglandins), live vaccines, and genetic factors (autosomal genes) [1, 4–6]. Inadequate placental maturation, which causes maternal caruncles to separate from fetal villi, is also implicated. In addition, inflammatory changes in the placenta can affect both maternal and fetal tissues, leading to placental degeneration and villous necrosis [2, 7]. Uterine infections occurring during the late stages of gestation, by increasing fetal cortisol levels, may also lead to premature births [8]. Due to the labor-intensive nature of the care and treatment of premature calves, both owners and veterinarians often struggle to provide adequate attention and care. The survival of premature calves is directly related to the interventions made, particularly in the first hours after birth and in the postnatal period. This chapter will provide a detailed description of the definition, clinical assessment, emergency and supportive treatment protocols, daily care practices, and management of potential complications in premature calves. The practical aspect of the subject will also be strengthened with clinical case management and procedural flow charts. The aim is to provide both clinical veterinarians and veterinary students with effective and practical information on the management of premature calves.

2. Clinical findings

Clinical findings such as low birth weight, poorly body condition score, weakness in muscles and tendons, short and soft hair, thin and elastic skin texture (**Figure 1**), teeth not fully separated from the gums (**Figure 2**), soft hooves (**Figure 3**), and weak or no suckling reflex may be observed in the premature calves. Due to incomplete organ development and the presence of hypoxia in premature calves, multiple problems related to the respiratory system, brain and locomotor system, cardiovascular system, gastrointestinal system, metabolism, and urinary system may lead to critical health complications [2, 4].

2.1 Respiratory system findings

The primary problem in premature calves is the insufficiency of surfactant secreted by type II pneumocytes in the lungs. Surfactant is a lipid-protein complex, consisting of 90% lipids and 10% proteins, that reduces surface tension in the alveoli,



Figure 1.
Short and soft hair, thin and elastic skin texture (original image by the authors).



Figure 2.
Teeth not fully separated from the gums (original image by the authors).



Figure 3.
Soft hooves (original image by the authors).

prevents lung collapse, and facilitates gas exchange [4, 9]. The composition of surfactant changes in parallel with fetal development, preparing the lungs for breathing after birth. Due to insufficient surfactant, premature calves develop atelectasis in the lungs after birth (on post-mortem examination, lungs appear dark red to purple in color, congested and firm in consistency, similar to the liver) (**Figure 4**), and respiratory distress syndrome (RDS) progresses, leading to death due to severe respiratory failure [10]. This condition begins about 10–15 min after birth and peaks in the first few hours of life [4]. In premature calves that develop respiratory distress syndrome, tachypnea, difficulty breathing, intermittent abdominal and wheezing respirations,



Figure 4.
The dark red, congested, and liver-like lung of a premature calf at necropsy (original image by the authors).

rales in the lungs and increased respiratory effort, observable in the nostrils, are common [11]. Due to the high negative pressure created to open the collapsed airways, the weak intercostal and flexible thoracic structure causes the sternum and ribs to move inward during inspiration (intercostal retraction). Cyanosis of the mucous membranes is common and clinical signs such as apnea are occasionally seen [2]. As a result of respiratory distress syndrome, hypoxia, interstitial inflammation, and pulmonary vasoconstriction are observed. Pulmonary arterial hypertension increases vascular permeability, leading to alveolar-interstitial edema [10, 12, 13]. A study using lung-specific epithelial and endothelial biomarkers showed that premature calves with RDS were more susceptible to developing pulmonary arterial hypertension compared to premature calves without RDS [13].

2.2 Brain and locomotor system findings

In premature calves, due to incomplete development of the relevant centers in the brain, general reflexes are weak [14]. In calves with weakness in tendons and incomplete bone ossification, weakness in the muscle tone prevents them from standing in the first days of life [4]. In addition, the hypoxia and hypoglycemia complex may lead to neurological signs in calves, such as lethargy, dullness, and opisthotonus (**Figure 5**) [15].

2.3 Cardiovascular system findings

Due to impaired myocardial function as a result of breathing difficulties and hypoxia, often there are tachycardia and arrhythmia. Weak peripheral pulses and a prolonged capillary refill time (CRT) of more than 2 seconds are commonly observed. Weak peripheral pulses, CRT greater than 2 seconds, and cold extremities are indicative of impaired peripheral circulation [16, 17]. A prolonged period of hypoxia in the body can lead to a decrease in cardiac output, hypoperfusion, and hypotension [18]. However, in a study comparing premature calves with RDS to term calves, blood pressure levels were found to be similar. In the same study, echocardiographic assessment in premature calves revealed the development of both systolic and diastolic dysfunction [19]. In another study, it was reported that serum levels of troponin I, troponin T, CK-MB, and lactate dehydrogenase (LDH) may be elevated in premature calves with RDS [17].



Figure 5.
Head position in a calf with hypoxia and hypoglycemia complex (original image by the authors).

2.4 Gastrointestinal system findings

In premature calves, the intestines are hypomotile, leading to a reduced absorption of immunoglobulins and nutrients. Excessive oral feeding can exacerbate hypomotility, leading to abdominal distension and potentially death (**Figure 6**) [20]. Insufficient digestion of colostrum and prolonged transit time, together with inadequate intestinal barrier defense, have been suggested to contribute to intestinal damage. In their study, Yıldız et al. found that excessive colostrum feeding in premature calves with RDS not only worsened the severity of RDS and increased the risk of mortality but also negatively affected intestinal microcirculation, leading to intestinal damage [20]. The authors recommended that premature calves should be fed small amounts of colostrum at frequent intervals. Gastroesophageal reflux may develop in 67% of premature calves, and this condition should be considered in the treatment planning [21]. In addition, defecation difficulties can occasionally be observed in premature calves, particularly in the passage of the first meconium [14].

2.5 Metabolic findings

Due to insufficient glycogen storage in their muscle tissue and liver and the lack of brown adipose tissue, premature calves are unable to regulate their body temperature. As a result, they are often hypothermic ($<35^{\circ}\text{C}$) [2]. Premature calves expend a significant amount of energy to maintain their body temperature within normal limits after birth, and due to inadequate glycogen stores, their blood glucose levels are typically below 1.7 mmol/L [10].

2.6 Urinary system findings

In premature calves, the immaturity of kidneys, the low number of nephrons, decreased creatinine clearance, increased reabsorption from immature tubules, and the decreased glomerular filtration rate may lead to increased serum urea and creatinine levels [22]. However, it should be noted that physiologically, creatinine can cross the placenta and reflect maternal kidney function within the first



Figure 6.
A premature calf with severe abdominal distension and death (original image by the authors).

24–48 hours of life. As the kidneys are sensitive to hypoxia, acute kidney injury can develop within 24 hours [23–25]. In a study assessing renal function using kidney-specific biomarkers, acute renal failure was detected in 38.5% of premature calves with RDS [22].

3. Laboratory findings

Blood gas measurements provide important information to the acid-base balance and oxygenation status [26]. If possible, RDS evaluation in premature calves should be performed with blood taken from the auricular arteries (**Figure 7**) [11]. There is a good correlation between the partial pressure of carbon dioxide (PaCO_2) in arterial and venous blood. However, there is no correlation between the partial pressure of oxygen (PaO_2) in venous blood and the PaO_2 in arterial blood. In calves with RDS, venous blood PaO_2 changes minimally [4]. Criteria for respiratory distress syndrome include respiratory acidosis, hypoxia ($\text{PaO}_2 < 60$ mmHg), hypercapnia ($\text{PaCO}_2 > 45$ mmHg), decrease in blood pH due to hyperlactatemia, tachypnea (respiratory rate > 45 breaths/min), decrease in oxygen saturation, abdominal paradoxical and/or wheezing breathing. Calves without these findings are referred to as premature calves that do not develop RDS [13, 14]. Even if hypercapnia is not observed on the first blood gas measurement, it may develop later due to tachypnea. Calves with RDS initially present with respiratory acidosis. The development of tissue hypoxia and redirection of blood flow to vital organs leads to impaired perfusion of peripheral tissues, resulting in increased lactate production by anaerobic metabolism, which subsequently causes mixed (respiratory and metabolic) acidosis [4].

Complete blood count measurements in premature calves can be used to evaluate the presence of infection and the red blood cell profile. In a study conducted on surviving and non-surviving premature calves, no significant differences were found in leukocyte, hematocrit, erythrocyte, and platelet values [27]. In premature calves whose biochemical parameters were evaluated, serum levels of total protein, albumin, cholesterol, triglycerides, calcium, and phosphorus were reported to be significantly low [28]. Additionally, in premature calves, abnormal coagulation profiles may also develop [29].



Figure 7. Blood sampling from the auricular artery of a premature calf using an insulin syringe. Note the bright red color of the arterial blood (original image by the authors).

4. Treatment

The treatment of premature calves begins with a prompt and accurate assessment immediately after birth. This process is of critical importance in determining the calf's vital functions and the need for emergency intervention, as well as the introduction of supportive and nursing care.

4.1 Emergency intervention protocols

Clearing the upper airways immediately after birth in premature calves is the first and essential step in facilitating effective respiration [2]. The mucus in the nasal cavity, oral cavity, and pharynx should be removed using a suction pump, and any fluid or mucus accumulated in the airway must be cleared. If there is no response to these interventions, hypothermic stimulation is the most commonly used method of resuscitation. To activate thermoreceptors in the skin and stimulate the breathing reflex, cold water can be poured on the ear, head, or whole body [4]. Rubbing the chest with a towel or bedding to stimulate the phrenic nerve, inserting materials pieces of straw into the nostrils, and performing acupuncture on specific points in the nasal region (nasal planum) can also stimulate the respiratory reflex in neonates, allowing the lungs to expand [30].

Although mechanical ventilation support is recommended for calves with respiratory arrest, due to the need for trained personnel and specialized equipment, among other disadvantages, its application is challenging [10]. Therefore, respiratory stimulants (such as doxapram, lobeline, etc.) may be administered [2]. Premature calves diagnosed with hypothermia should be immediately moved from the cold environment to a heated room and dried with a towel or hair dryer [2]. To facilitate breathing, premature calves should be placed in specially designed beds or placed in sternal recumbency with the chest elevated at a 30° angle. If the calf is placed in a lateral position, significant pressure may be exerted on the thorax, diaphragm, and lungs, potentially exacerbating hypoxia [31]. If a calf cannot maintain a sternal position, to prevent mechanical compression of the lung lobes under pressure, frequent turning should be performed [4].

Following these procedures, in premature calves, oxygen should be administered using an appropriate mask. Oxygen can be administered to calves at an initial flow rate of 5–6 L/min for 15 minutes, with a 10-minute interval recommended after each session to prevent the toxic effects of pure oxygen. This procedure is continued for approximately 3–4 hours, after which the oxygen flow rate is reduced to 3–4 L/min. Oxygen therapy increases the availability of oxygen in the tissues but does not affect ventilatory or atelectatic areas of the lung. Oxygen therapy aims to maintain saturation between 70 and 90%, and PaO₂ levels around 60–80 mmHg. Higher oxygen saturation can increase the concentrations of free oxygen radicals in the blood, potentially causing damage to the organism [4]. Oxygen used in premature calves must be humidified. Failure to provide adequate humidification can lead to problems such as reduced ciliary activity in the airways, thickening of secretions, inflammation of the lung epithelium, atelectasis, and pneumonia [18].

Hypoglycemic premature calves should be administered 100–250 mL of 5% dextrose intravenously. Following this, calves that have developed a sucking reflex *via* a bottle should be slowly given 200–300 mL of colostrum, which is vital for their life. Calves without a suckling reflex can be fed *via* an appropriately sized oral or nasogastric tube (**Figure 8**) until the suckling reflex develops.



Figure 8.
A premature calf being fed using a nasogastric tube (Original image by the authors).

4.2 Special treatments for the lungs

Lung-directed treatment consists of oxygen therapy and inhaler (nebulizer) treatment. Oxygen therapy eliminates hypoxemia, while inhaler treatment reduces lung inflammation, increases production of antioxidant enzymes and surfactant, relieves bronchial smooth muscle spasm, and reduces pulmonary edema [11, 32, 33]. For this purpose, surfactants, inhaler bronchodilators, and anti-inflammatory and anti-edematous agents may be used. Karapınar and Dabak administered a dose of 100 mg/kg bw of surfactant extract obtained from the lungs of young cattle by intratracheal injection to premature calves with a success [34]. However, commercially available surfactant products are expensive and, therefore, cannot be used in veterinary practice [4]. Studies have shown that bronchodilators, anti-inflammatory, and anti-edematous agents administered *via* nebulizer have provided economically favorable and successful outcomes in approximately 80% of cases [11, 14]. The use of medication *via* nebulizer provides non-invasive, localized, and rapid effects, while also significantly protecting against the systemic side effects of the drugs. The effectiveness of bronchodilators administered *via* inhaler begins within 5 min and reaches peak levels within 15–30 minutes, whereas oral administration takes 2–3 hours to reach peak levels [35].

β_2 adrenergic agonists and anticholinergics are used as inhaled bronchodilators. β_2 -agonist bronchodilators stimulate β_2 -adrenergic receptors on the surface of bronchial smooth muscle, resulting in bronchial smooth muscle relaxation. They also increase mucociliary activity and can prevent inflammation and edema formation within the lungs [36–38]. β_2 -Agonist bronchodilators are divided into two groups based on their duration of action: short-acting (3–8 hours) and long-acting. Short-acting bronchodilators, include commonly used metaproterenol, salbutamol, terbutaline, and fenoterol. Long-acting bronchodilators include commonly used salmeterol and formoterol. The most commonly used short-acting β_2 -agonist bronchodilator is salbutamol.

Nebulized corticosteroids increase surfactant production, reduce inflammation in the lungs, and decrease bronchial and pulmonary edema [39]. Inhaled forms of steroids such as fluticasone propionate, beclometasone dipropionate, triamcinolone, budesonide, and flunisolide are available [11, 14]. Among these, fluticasone propionate is most effective [40]. While it has been suggested that corticosteroids administered to calves can increase surfactant production, a study indicates that only prenatal administration of glucocorticoids may have an impact on surfactant production and lung maturation [4].

Diuretics are used to reduce the interstitial edema [41]. The most commonly used diuretic for this purpose is furosemide. In a study, it was reported that salbutamol (0.025 mg/kg every 6 hours), formoterol (total dose 12 µg every 12 hours), fluticasone (15 µg/kg every 12 hours), budesonide (total dose 400 µg every 12 hours) and furosemide (1 mg/kg every 12 hours) diluted with 2.5 mL of physiological serum and administered by inhalation for 5 min for 3 days achieved successful outcome in the treatment of premature calves with RDS [11, 14].

4.3 Daily care and supportive therapy

Premature calves placed should be hospitalized for 3–4 days in a heated room. During this period, their rectal temperature, respiratory rate, and heart rate should be monitored daily. The hospitalization area should be disinfected once daily. Oxygen therapy should be continued at regular intervals until oxygen saturation exceeds 80%. The amount of colostrum administered to calves should not exceed 500 mL per feeding, and 250–350 mL should be given every 6 hours. It should be noted that administering more than 500 mL of colostrum in a single feeding may lead to abdominal distension and respiratory distress, which can result in death [14, 20]. Supportive treatment may also include calcium, phosphorus, selenium, and vitamins A, B, C, D, and E. Calcium, phosphorus, and vitamins C and B can be administered once daily for 3 consecutive days, while selenium and vitamins A, D, and E may be administered intramuscularly as a single dose [2, 13, 14]. As previously noted, decreased levels of vitamin A, calcium, and phosphorus may be observed in premature calves [6, 28]. To address these deficiencies, administration of a combination containing vitamins A, D, and E, as well as a preparation including selenium and B vitamins, may be considered. Additionally, vitamin C can be used due to its potent antioxidant and immunomodulatory properties. The use of such vitamin and mineral supplements is consistent with the pathophysiological requirements of premature calves and is supported by previous studies [13, 14].

Gastric esophageal reflux may develop in premature calves, and therefore, ranitidine at a dose of 5 mg/kg twice daily for 3–5 days can be administered [2]. In calves that are unable to pass meconium, a rectal lavage (enema) should be performed and meconium elimination should be monitored (**Figure 9**).

Premature calves are at high risk for sepsis due to potential passive transfer insufficiency, and they should be closely monitored. Due to the insufficient development of the immune system in premature calves, to reduce the risk of sepsis and pneumonia, antimicrobial treatment is highly recommended. Dependent on the local regulations, broad spectrum antimicrobials should be used. The fourth-generation cefquinome (2 mg/kg, intramuscular, every 12 hours) and the third-generation cephalosporin, ceftriaxone (20 mg/kg, intramuscular, every 12 hours) may be recommended to treat infections caused by susceptible bacteria [42].

Calves that are unable to stand should have their hind legs massaged three to four times a day for 15–20 min at a time. If the umbilical cord of premature calves is not detached, it should be cut, leaving at least 5 cm on the body, and cleaned as soon as possible with a 2–7% iodine solution. The use of antimicrobial sprays or teat disinfectants increases the risk of infection because they do not dry the umbilical cord sufficiently. Umbilical cord care should be performed twice a day for a duration of 3–4 days. Despite all these daily care measures, premature calves should not be disturbed unless necessary [30].



Figure 9.
Meconium of a premature calf undergoing a rectal enema with paraffin oil (original image by the authors).

4.4 Clinical monitoring and complication management

Calf blood gas and haemogram results should be evaluated daily, with particular attention paid to monitoring blood pH, oxygen levels, oxygen saturation, lactate, and glucose levels. One study reported that in premature calves with RDS, where venous blood samples were taken, the risk of death was higher in calves with lactate levels >7.5 mmol/L and PaCO_2 levels >63.5 mmHg [27].

Hypothermia and hypoglycemia are important clinical findings in the monitoring of premature calves, and they should be monitored throughout the treatment process [14]. The oxygen saturation of hemoglobin can be monitored with a pulse oximeter (Figure 10), which is easy to use and simple [43].

For calves with mixed acidosis, 1.3% NaHCO_3 or lactated Ringer's solution should be preferred, depending on the severity of the acidosis. In the treatment of metabolic acidosis, excessive infusion of sodium bicarbonate can lead to hypernatremia, which can increase intracranial pressure and the risk of brain hemorrhage [18]. When administering intravenous sodium, it is essential to ensure that the calf is ventilated adequately and that there is no carbon dioxide retention. This is because HCO_3^- combines with H^+ in the blood, forming carbonic acid, which then dissociates into CO_2 and H_2O . If the resulting CO_2 cannot be eliminated, paradoxical acidosis may occur [26].

Heaters should not be placed within two meters of premature calves. The room should not be overheated. In premature calves with hyperthermia, respiratory function may deteriorate.



Figure 10.
Measurement of oxygen saturation from the ear by pulse oximetry (original image by the authors).

5. Example case

NOTE: Not all figures below are from the example case.

Case: A female Holstein calf, born spontaneously 2 hours ago, with an estimated gestational age of 230 days.

Physical findings: Body weight 18 kg and body condition score (2/5). The calf is unable to stand. The skin is thin and hairs are short and soft. The teeth are not fully separated from the gums. Hooves are soft. The suckling reflex is absent. The umbilical cord ruptured spontaneously at birth.

Clinical examination findings: Rectal temperature is 34.2°C (85 breaths/min), and abdominal respiration are observed. The heart rate is 160 beats per minute. Cyanosis is present on the mucous membranes.

Initial intervention (first 30 minutes): The hypothermic calf was transferred to a warm room, dried with a towel, and placed and maintained in sternal recumbency. Subsequently, arterial blood was collected from the auricular artery for blood gas analysis, and an appropriately sized intravenous catheter was placed in the auricular vein.

Blood gas and hemogram findings: Mixed acidosis, hypercapnia, hypoxemia, decreased oxygen saturation, hypoglycemia, hyperlactatemia, and a base deficit were noted in the calf (**Table 1**). Hemogram values were within the reference range.

Diagnosis: Respiratory distress syndrome was diagnosed in a premature calf.

Emergency treatment plan (first 4 hours): Oxygen therapy was initiated immediately using an oxygen mask at a flow rate of 5–6 L/min and continued uninterrupted for 3–4 hours (**Figure 11**). The calf was administered 350 mL of 5% dextrose at a rate of 30 mL/min for 2 hours. The amount of bicarbonate (mEq) required for the calf with mixed acidosis was calculated using the formula: body weight (kg) × base deficit (mEq/L) × 0.6 (L/kg). In total, 150 mL of 1.3% NaHCO₃ solution was added to 500 mL of isotonic 0.9% NaCl solution and administered at a rate of 5 mL/min. After the initial fluid treatment, the calf developed a sucking reflex and had 250 mL of colostrum administered orally.

daily care, supportive therapy, and clinical course: The calf's blood gas and hemogram findings were evaluated daily, and the oxygen and fluid therapy plan was revised accordingly. Oxygen therapy (3–4 L/min, 1 hour, repeated four times daily with 30-minute intervals) was administered together with salbutamol (0.025 mg/kg every 8 hours) and fluticasone (15 µg/kg every 12 hours), both diluted with 2.5 mL

Parameter	Time of initial admission (hour 0)	Pre-discharge (hour 72)
pH	<6.95	7.42
pCO ₂ (mmHg)	85.1	39.2
pO ₂ (mmHg)	16.8	65.2
sO ₂ (%)	21.4	91.85
Glucose (mmol/L)	1.1	4.3
Laktat (mmol/L)	19	1.6
BE (mmol/L)	−15	3
HCO ₃ (mmol/L)	8	20

Table 1.
Arterial blood gas results of the premature calf before and after treatment.



Figure 11.
Oxygen administration to the premature calf (original image by the authors).



Figure 12.
Premature calf treated with nebulizer (original image by the authors).

of saline solution and administered *via* nebulizer within 5 min (**Figure 12**). Oxygen therapy continued intermittently when oxygen saturation was below 80%.

After the body temperature had been gradually raised to 38.5°C, the temperature of the room was kept at a constant level. Umbilical cord care was performed twice daily, and 300 mL of colostrum was administered every 6–8 hours. Occasionally, colostrum was administered by oral/nasogastric tube because the calf lacked a suckling reflex or developed abdominal distension.

The calf's first meconium passage was monitored. As it was delayed and there were difficulties with the defecation, a rectal enema with liquid paraffin oil was administered to the calf. Antimicrobial prophylaxis and vitamin-mineral supplementation were initiated. The calf was unable to stand. Therefore, massage was applied to all limbs two to three times a day for 15–20 min per session. The calf was gradually assisted to stand. Initially, the calf had difficulty standing, but by 24 hours, it was able to stand for short periods, and by 48 hours, it was able to stand unassisted and not interrupted. By the 72 hours of hospitalization, the calf was able to lift itself and stand (**Figure 13**). Special attention was given to keeping the calf in a sternal position, especially during the first 24 hours (**Figure 14**).



Figure 13.
A discharged premature calf (original image by the authors).



Figure 14.
Premature calves maintained in sternal position (original image by the authors).

6. Conclusion and evaluation

With the provided treatment and daily care, the calf's suckling reflex improved, its body temperature stabilized, and it was able to stand and walk. Therefore, the calf was discharged after 72 hours hospitalization. The calf owner was advised to keep the calf separated from other calves for some time and to provide small amounts of colostrum at frequent intervals. This case was successfully managed with early diagnosis and multidisciplinary support.

7. Emergency intervention flowchart for premature calf

See **Figure 15**.

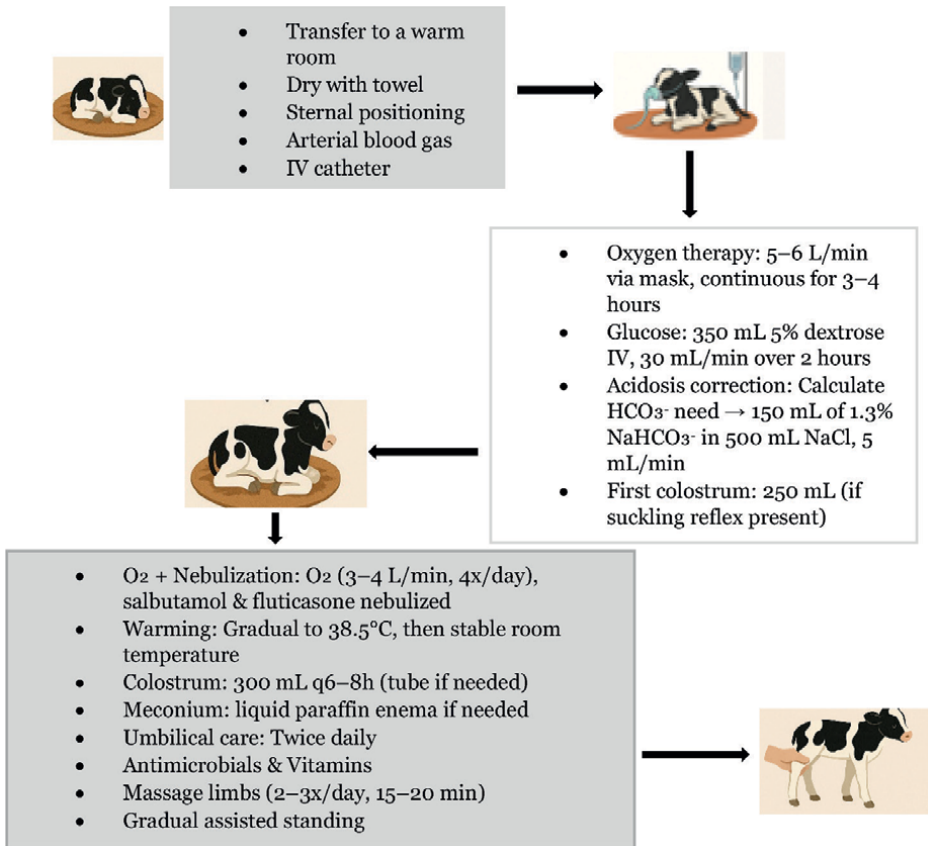


Figure 15.
Premature calf emergency flowchart.

Conflict of interest

The authors declare no conflict of interest.

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Strategic Therapeutic and Preventive Approaches to Controlling Leptospirosis in Dairy Herd with Reproductive Issues

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Abstract

Leptospirosis causes reproductive problems in cattle, which affects milk production. Studies are essential to demonstrate effective treatment and control measures that prevent the discharge of large volumes of milk and the indiscriminate use of antibiotics. Therefore, the objective of this study was to verify the reflexes of the adoption of an *in situ* strategic therapeutic and preventive conduct, in a dairy herd infected by *Leptospira* spp. and with a history of reproductive problems. For this research, the cows were divided into groups based on their serological status, as determined by the Microscopic Agglutination Test, and clinical signs. According to the needs of each group, treatment with streptomycin and vaccination were performed. After adoption of the treatment and vaccination strategies prepared for the herd, there was a reduction in the anti-*Leptospira* antibody titres, in the occurrence of abortions, of the repeated breeder cows, and a decrease in calving interval. The adopted methodology effectively controlled the outbreak of leptospirosis, avoiding reinfection and excessive milk discharge. Therefore, we can recommend this method as a practical tip for bovine veterinarians.

Keywords: *Leptospira*, dairy cattle, reproductive problems, treatment, vaccination

1. Introduction

Leptospirosis is associated with significant losses in productivity and reproduction in animals, including a drop in dairy productivity and the number of calves born per year, as well as an increase in calving interval. Additionally, this disease poses a significant public health problem [1–3].

Besides the concern with the indiscriminate use of antibiotics, the antibiotic therapy used to control leptospirosis in lactating animals is responsible for the discharge of large volumes of milk due to the presence of antibiotic residues, intensifying the economic impacts generated by the disease.

Most rural producers and professionals directly linked to milk production have doubts about how to combat disease outbreaks during lactation. Thus, it is necessary to study strategic therapeutic approaches that are effective in controlling the disease in herds, promoting the rational use of antibiotics, and generating less milk discharge.

Given the above, the objective of this study was to verify the response of the adoption of a strategic therapeutic and preventive conduct *in situ* in a dairy herd naturally infected with *Leptospira* spp. and with a history of reproductive problems.

2. Methods

This study was conducted in a dairy farm that uses the “Compost Barn” method and is composed of purebred Jersey cows with intensive milk production, localised in the region of Uberlândia, Minas Gerais, Brazil, at 18°54'16.02''S and 48°22'02.4'' W.

For the prevention of reproductive diseases, females were vaccinated for brucellosis, according to the National Program for the Control and Eradication of Brucellosis and Animal Tuberculosis (PNCEBT) of the Ministry of Agriculture, Livestock and Food Supply [4]. In addition, females and males older than 5 months were vaccinated annually for bovine viral diarrhoea (BVD) and bovine infectious rhinotracheitis (IBR). The herd had no recent history of leptospirosis vaccination.

Before the clinical suspicion, the serological diagnosis of leptospirosis was performed in cows of reproductive age, using the Microscopic Agglutination Test (MAT), and confirmed the presence of the disease in the herd. Then, a strategic

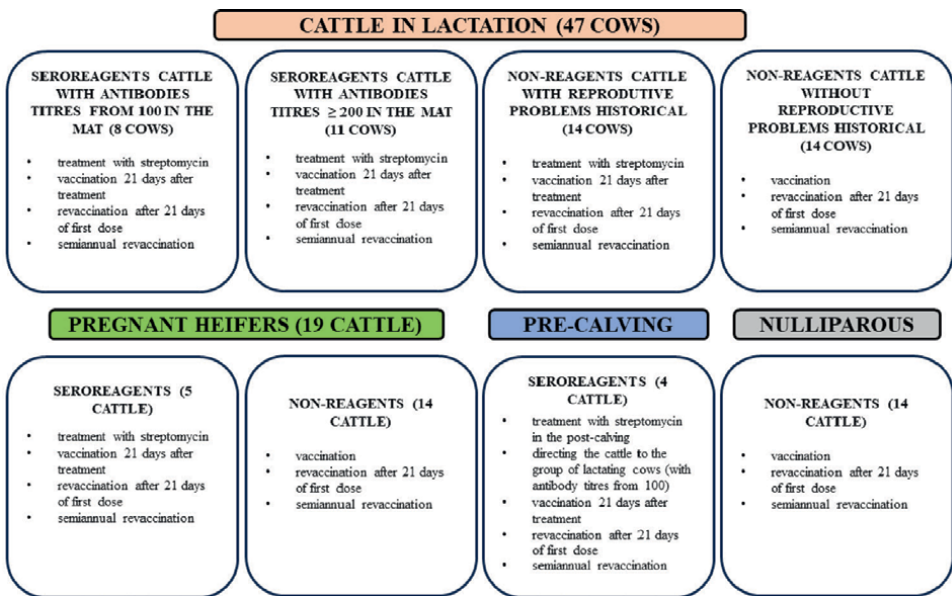


Figure 1.
Strategic division of dairy cattle herd to control the outbreak of leptospirosis.

therapeutic and preventive approach was developed (July 2016), in which the cows were divided into groups according to their serological status and presence of clinical signs (**Figure 1**).

Following the completion of this procedure in March 2017, after a period of high rainfall with peaks of 55–100 mm [5], a serological monitoring test was performed to analyse the influence of the therapeutic approach on the serology profile of the herd. To verify the effects on the reproductive indexes, research was carried out on the abortion cases, repeated breeder cows, and the calving interval from January 2015 to December 2017 (pre- and post-adoption of strategic preventive and therapeutic approaches).

2.1 Statistical analysis

The chi-square statistical test (X^2), at the level of 5%, was used to verify the existence of a significant statistical difference between the abortions and the cases of repeated breeder cows that occurred before and after the adoption of the strategic preventive and therapeutic approaches.

3. Results

In the MAT (pre-treatment), 31 reactive cows were detected for the Sejroe and Hebdomadis serogroups. In the post-treatment monitoring MAT, 22 cows were also identified as seropositive for serogroups Serjoe and Hebdomadis, in addition to the reactions to serogroups present in the bacterin used, such as Pomona, Icterohaemorrhagiae, and Canicola (**Table 1**).

Microscopic Agglutination Test (pre-treatment)—July 2016		
SEROGRUPS OF <i>Leptospira</i> spp.	NUMBER OF REAGENTS ANIMALS	%
Hebdomadis + Sejroe	24	77.4
Sejroe	6	19.4
Hebdomadis	1	3.2
TOTAL	31	100
Monitoring MAT (post-treatment)—March 2017		
SEROGRUPS OF <i>Leptospira</i> spp.	NUMBER OF REAGENTS ANIMALS	%
Hebdomadis + Sejroe	13	50.0
Hebdomadis	4	15.4
Sejroe	5	19.2
Icterohaemorrhagie	2	7.6
Hebdomadis + Sejroe + Pomona	1	3.9
Canicola	1	3.9
TOTAL	26	100

Table 1.
Frequency of serogroups of Leptospira spp. in MAT, in the dairy production property, in the region of Uberlândia, MG, Brazil.

Antibody titres ranged from 100 to 800 in the MAT of pre-treatment. In the monitoring test, there was a reduction of the titres, and a maximum titre of 100 was observed (Table 2).

Microscopic Agglutination Test (pre-treatment)						
SEROGROUPS	TITRES				TOTAL*	%TOTAL**
	100	200	400	800		
Sejroe	20	3	6	1	30	96.8 (30/31)
Hebdomadis	14	6	4	1	25	80.6 (25/31)
Monitoring MAT (post-treatment)						
SEROGROUPS	TITRES				TOTAL*	%TOTAL**
	100	200	400	800		
Sejroe	19	0	0	0	19	73.1 (19/26)
Hebdomadis	18	0	0	0	18	69.2 (18/26)
Icterohaemorrhagiae	2	0	0	0	2	7.7 (2/26)
Canicola	1	0	0	0	1	3.8 (1/26)
Pomona	1	0	0	0	1	3.8 (1/26)

**Total reactions by serogroup, disregarding cases of coagglutination.*
***Frequency of serogroups disregarding cases of coagglutination = Total reactions per serogroup/Total positive animals.*

Table 2.
Frequency of titres per serogroup for seroreagent cattle, from January 2015 to December 2017, in the dairy production property, in the region of Uberlândia, MG, Brazil.

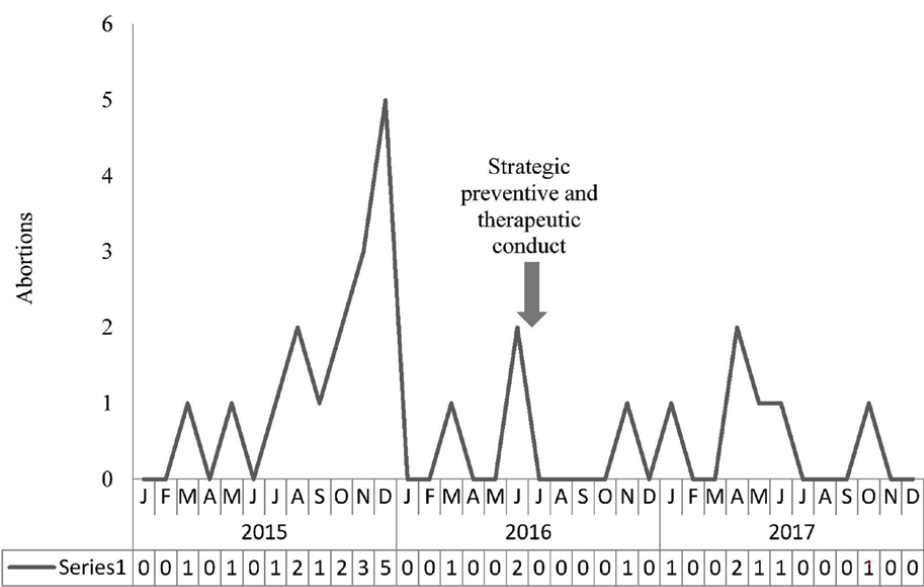


Figure 2.
Occurrence of abortions between January 2015 and December 2017, in the dairy production unit, in the region of Uberlândia, MG, Brazil.

A statistically significant reduction of 63.15% of the cases of abortion occurred after the adoption of the therapeutic and preventive approaches proposed in this research ($p < 0.05$) (**Figure 2**).

Despite the numerical reduction of cases of repeated breeder cows (42.8%), there was no significant difference ($p > 0.05$) after the adoption of strategic preventive and therapeutic approaches (**Figure 3**).

3.1 Strategic preventive and therapeutic conduct

About the calving interval, an increase was observed in the year 2016. It is essential to note that the therapeutic and preventive strategy was implemented in July 2016, and following its adoption, an 8.5% reduction in calving interval was observed in 2017 (**Figure 4**).

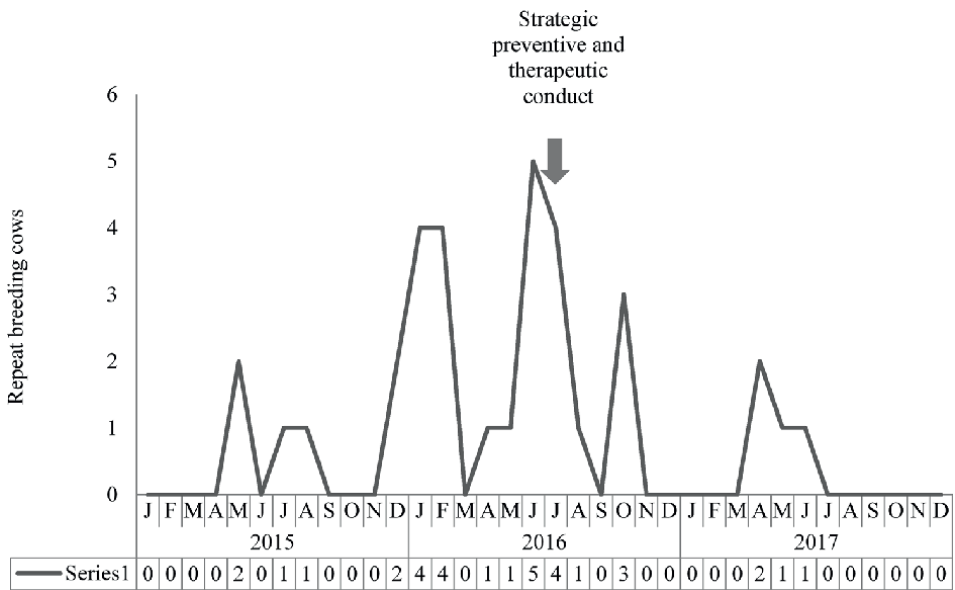


Figure 3.
Occurrence of repeated breeder cows between January 2015 and December 2017, on a dairy farm in the region of Uberlândia, MG, Brazil.

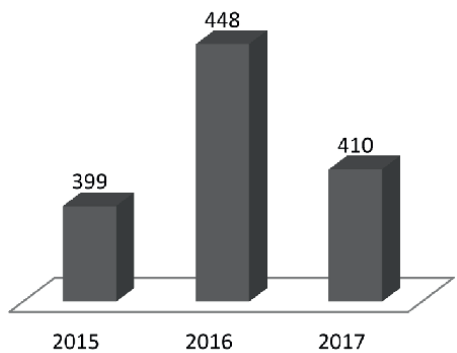


Figure 4.
Calving intervals, between January 2015 and December 2017, in a seropositive Jersey herd for leptospirosis, in the region of Uberlândia, MG, Brazil.

4. Discussion

The high prevalence of seroreactive lactating cows observed suggests that it is associated with deficient vaccination and the agglomeration of cattle during milking management. The use of the Compost Barn system improves the sanitary condition of the herd [6]. However, as cows are at a high stocking rate in the waiting corral, urinating in impermeable soil, may have favoured the maintenance and dissemination of the disease within the herd.

The reduction of anti-*Leptospira* spp. suggests that the separation of the cattle in groups, associated with the strategic vaccination of the herd and the treatment of the seroreagent cattle with streptomycin, avoided their reinfection. Moreover, as antibody titres have remained low, even after a rainy period, reinforces the effectiveness of the strategic treatment in controlling the disease in cattle. As leptospires are water-associated, it is understandable that there is a greater trend in the incidence of leptospirosis in bovine herds during high rainfall periods [7].

The reduction of 50.0% of the abortions, 70.4% of the repeated breeder cows, and the fall of 8.5% in the calving interval had positive effects on the reproductive herd indexes in 2017. Reproductive parameters in a seropositive bovine herd for leptospirosis are affected and found a calving interval ranged from 450 to 500 days [8]. This result agrees with the longer calving interval observed in the present study, which occurred in the year 2016, mainly due to the occurrence of repeated breeder cows observed in that year.

The efficacy of a standard control program for leptospirosis, which included streptomycin 25 mg/kg and semiannual vaccination against leptospirosis in the seroreactive herd, was not satisfactory, presenting 30% reproductive failures and positive serology with titres ranging from 200 to 1600. Therefore, the approach consisting of only treatment and vaccination was not able to control the disease in the herd. However, when the treatment of the cattle was combined with environmental control measures (replacing the water source), reproductive problems decreased to 3%, and antibody titres remained below 200 [9]. In agreement with the results obtained by these authors, the division of the cattle in groups, carried out in the present study, was a fundamental strategic measure for the control of reinfection. Additionally, the methodology employed in the present study avoided the discard of large volume of milk (of all 42 were treated in a total of 84 cattle), coupled with the indiscriminate use of antibiotics [9].

The water supplied to cattle on the dairy unit by 2016 was potentially contaminated, as other animals, including wild ones, had access to the watercourse used to for drinking. Thus, the use of water from an artesian well, supplemented with chlorine, was part of the strategic preventive measures proposed in the present study.

Because MAT has limitations related mainly to variable sensitivity and an inability to differentiate between acute and chronic infections [10], treating only seroreactive cattle cannot eliminate all bovine carriers of *Leptospira*. Another study verified that 50% of non-reactive bovines in the MAT were positive in the polymerase chain reaction (PCR) test in urine, and thus, to eliminate all cattle carriers of *Leptospira*, it would be necessary to treat the whole herd. The blitz treatment would cause significant economic and health damage to the producer, due to the discard of large milk volume and the probability of inducing bacterial resistance, even for other agents [11]. Additionally, the discarded milk would be environmental pollutant. Treating only a subset of cattle, decreased economic losses, antimicrobial use, and discarded milk [12] ultimately reduce the environmental impact.

Furthermore, the separation of cattle with different antibody titres was also necessary. High titres of antibodies anti-*Leptospira* in cattle indicate an active infection and, consequently, a potential source of infection for non-reactive animals. On the other hand, cattle with low titres may be associated with chronic disease and a renal carrier stage, or they may no longer be carriers but still have antibodies against the pathogen. Therefore, it was essential to prevent the transmission of the disease from cattle potential carriers of *Leptospira*, to non-carriers who had only been vaccinated and had not received treatment with streptomycin.

As the withholding period for streptomycin in milk is 4 days [13], milk discard occurred only in lactating cows that received treatment. Resistant bacteria in the gut and milk of dairy cattle can potentially transfer their resistance to pathogenic bacteria in dairy products and in the human gut microbiota, contributing to its spread in the environment [14]. As antibiotics use may result in the development of resistant strains, decreasing the number of treated cattle decreased the spread of antibiotic resistance. This is in line with the guidelines of antimicrobial stewardship.

In this context, data related to the therapeutic approach to be adopted in specific cases of the disease in dairy cattle herds are scarce. Therefore, it is essential to conduct further research related to therapeutic and preventive management that yields results while minimising adverse effects on farmers and the environment.

5. Conclusions

The adoption of strategic therapeutic and preventive measures developed in the present study reduced reproductive problems (abortion, repeat breeding, and calving interval), even during a rainy period, demonstrating that leptospirosis was controlled and there was no reinfection in the herd.

For these reasons, the following tips are highlighted for veterinarians and other professionals involved with the dairy industry: (1) Suspect and request diagnostic tests for leptospirosis when there are production and reproduction losses. (2) Separate cattle into subgroups. Treat reactive or clinical cows, even during lactation, discarding the milk to avoid antibiotic residues. (3) Vaccinate cows and heifers that are not reactive and are not clinical. (4) Vaccinate cows and heifers that underwent treatment and are cured. (5) Control the drinking water source and cleaning the water for facilities using chlorine. (6) Adopt preventive measures taken to control the rat population (not necessarily on this specific farm).

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Section 3

Educational and Extension Approaches

Bovine Tropical Theileriosis: An Update

Arvind Kumar Tripathi and Manu Jaiswal

Abstract

Tick-borne diseases (TBDs) cause major economic losses and affect many domestic animals, mainly cattle and sheep, in tropical and subtropical regions. Tropical theileriosis is a TBD caused by a protozoon called *Theileria annulata*, transmitted by several tick species of the genus *Hyalomma*. Clinical manifestations of theileriosis are expressed mainly as anorexia, febrile generalized lymphadenitis, and anemia, followed by lethargy, lacrimation, nasal discharge, and exophthalmia. Anemia is a feature point in tropical bovine theileriosis, and severity was positively related to parasitemia rates. Fatality due to infection is greatly dependent on the overproduction of cytokines, such as TNF- α , produced by the schizont-infected monocytes/macrophages and uninfected macrophages. Buparvaquone gave 86.66% clinical efficacy against *Theileria annulata*, but 97.1 and 95.2% efficacy against *Theileria parva*. In theileriosis, hemolysis occurs due to isoantibodies against RBCs. To prevent this isoantibody lysis, an immunosuppressive dose of a steroid such as dexamethasone at 2.2 mg/kg body weight could be used.

Keywords: anorexia, exophthalmia, TNF-A, buparvaquone, macrophages

1. Introduction

Tick-borne diseases (TBDs) pose a significant economic concern for livestock, primarily affecting cattle and sheep in tropical and subtropical regions worldwide. *Tropical theileriosis*, one of the most important TBD caused by a protozoan parasite known as *Theileria annulata*, is transmitted by several tick species of the genus *Hyalomma* [1]. Bovine theileriosis is considered a serious problem for livestock in tropical countries, affecting millions of animals, especially crossbreed and exotic cattle. In India, only *Theileria annulata* is responsible for the majority of theileriosis cases, as there are no reports of *T. parva* till now from the country [2]. *T. annulata*, a bovine-specific pathogen, causes tropical theileriosis in tropical and subtropical countries of Asia (India, China), Africa (Egypt, Sudan), the Middle East, and Europe (Portugal) [3, 4]. It leads to high mortality, decreased productivity, and draught power in infected animals and is hence considered a serious challenge to the livestock improvement program in these countries. In India, economic losses due to blood parasitic diseases in livestock cause the estimated losses of USD 498.7 million per annum, and among them bovine tropical theileriosis alone caused the loss of USD 384.3 million per annum [5].

2. Etio-pathogenesis

T. annulata sporozoites are transmitted by the tick *Hyalomma anatolicum* in the host and cause a lymphoproliferation in the adjacent lymph nodes, causing a disease similar to cancer [6, 7]. Due to the presence of an intracellular parasite, the biochemical signaling pathways of the affected cells are altered to ensure its survival within the host cell. The parasite then transforms lymphoblast and monoblast cells after infecting B-cells and other monocyte cells. After the transformation of the cells by the *Theileria* parasite, they act like cancer cells and consume a lot of glucose [8]. Anorexia is associated with other symptoms such as hyperthermia, lymph node enlargement, anemia, icterus, and hemoglobinuria during subsequent evolution of the disease in cows [9].

Hemolytic anemia syndrome is a specific clinical sign of tropical theileriosis. Icterus is seen in the early stages of tropical theileriosis, and hemoglobinuria appears toward the final phases of the disease. *T. annulata* merozoites cause hemolysis, so excretion of hemoglobin in urine happens [10]. Some non-specific symptoms are frequently reported, like hyperthermia and lymph node enlargement, because reinfection with a new genotype of the parasite causes relapse of tropical theileriosis or induces a transient superficial lymph node enlargement.

Exophthalmia is one of the emerging clinical manifestations of tropical theileriosis in cow calves, and it may be considered a diagnostic marker for this condition [11]. Cells infected with *T. annulata* produce high levels of inflammatory cytokines, especially TNF- α [12]. The cytokines are potent inducers of the majority of clinical symptoms of acute tropical theileriosis, such as pyrexia, anemia, anorexia, muscle wasting, and necrosis [13]. The activated lymphocytes secrete several cytokines, including TNF α , interleukin-1, and interferon-gamma (IFN- γ) [14]. The clinical manifestation of exophthalmia is hypothesized to be due to systemic overproduction of TNF- α or overproduction of TNF- α in the extraocular muscle by the parasitized lymphocytes, which may be accountable for the increase in intraocular pressure, resulting in the clinical manifestation of exophthalmia is also possible in diseased calves. *T. annulata* schizont-infected cells play a direct role in the failure of protective immune responses, as infected cells possess a powerful method of immune evasion [15]. However, the role of anti-inflammatory/immunosuppressive cytokines (IL-10 and TGF- β) in the pathology of tropical calf theileriosis and the host–parasite interplay is not well explored.

Due to an immune-mediated erythrophagocytosis, anemia occurs in the later stages of disease following parasitemia and is also responsible for the erythrocyte destruction [16]. The piroplasm-infected erythrocytes are removed by the macrophages in the organs of the reticulo-endothelial system, which also causes anemia [17].

In addition, TNF- α , a pro-inflammatory cytokine, has been implicated in mediating anemia in tropical theileriosis [13]. Erythrocytes may be destroyed by erythrophagocytosis, and oxygen radicals may also be involved in the pathogenesis of the anemia [18].

Schizont-infected cells disseminate through the lymphoid tissues into the pituitary and thyroid glands, causing injury [19]. The cattle suffering from theileriosis had significantly lower concentrations of zinc and selenium as compared to healthy controls, and additionally, cattle suffering from theileriosis exhibit lower concentrations of serum T3 and T4 [20, 21].

3. Diagnosis

In acute cases of theileriosis, diagnosis is mainly based upon clinical symptoms and microscopic examination of thin blood smears. However, only a small number of RBC infections cause difficulty in demonstrating the parasite in blood smears, so the affected cattle become long-standing carriers [22]. Such long-standing carriers act as the major contributors of infection through the ticks, and when these carrier cattle are transported to non-endemic areas, this causes a disease outbreak [23]. Hence, detection of piroplasms in carrier animals becomes more challenging. False-negative results and a low sensitivity of the blood smear method are also associated with the detection of carrier cattle [22].

Therefore, molecular diagnostic tests, such as polymerase chain reaction (PCR), have paved the way for more efficient diagnosis compared to conventional techniques [24]. Based on various studies performed on a wide range of parasites, it has been concluded that PCR is more sensitive than the conventional techniques [25].

For the effective detection of *Theileria* infections in carrier animals, several molecular diagnosis-based assays have also been developed with proven efficacy. A reverse line blotting (RLB) assay based on the amplification of the hyper-variable V4 region of the 18S rDNA gene of *Theileria* (and of closely-related *Babesia* parasites) and reverse hybridization of the products with species-specific oligonucleotide probes is the most sensitive test for detecting *T. annulata* [26]. Nevertheless, since RLB is a relatively cumbersome assay, it is not entirely suitable for use in the routine diagnosis of *Theileria* infections. Some PCR-based assays have also been described for detecting *T. annulata* [27].

4. Clinical pathology

Along with the destruction of the RBC, lower hemoglobin, PCV, and TEC values were observed in animals infected with *Theileria* due to the toxic metabolites of *Theileria* spp. [28]. Continuous loss of blood due to blood-sucking ticks is also a significant factor. Due to leukocytosis, higher values of total leukocyte counts occur [29]. The destruction of lymphocytes in lymphoid organs and their infiltration into various organs causes lymphocytopenia [30]. Neutrophilia observed in theileriosis-infected cattle due to the release of endogenous corticosteroid in acute disease, stress, or inflammation [31]. Due to macrocytic hypochromic anemia in *Theileria*-infected cattle, the values of MCV and MCH become higher. Total protein and albumin levels decrease due to the harmful effects of toxic metabolites of *Theileria*, which may be exacerbated by hepatic insufficiency [32]. Hypoglycemia observed may be due to the utilization of glucose by *Theileria* spp. present in the blood [33].

5. Therapeutic aspect

Early treatment is highly effective in eliminating both piroplasmic and lymphocytic stages of the protozoan parasites from blood and lymph nodes within 3–4 days post-treatment, resulting in clinical improvement and an eventual 100% cure rate [34]. However, late-stage treatment did eliminate the protozoal parasites from the

blood and lymph nodes; it failed to improve the clinical condition of the affected animals, possibly due to irreversible damage to the lungs. Gharbi et al. [35] reported that buparvaquone produces 86.66% clinical efficacy against *T. annulata*, but 97.1 and 95.2% efficacy against *T. parva* [36]. Bilgic et al. [36] reported that the late-treated cases failed to be controlled by the drug buparvaquone due to the production of pulmonary edema.

T. annulata hijacks the host immune response to invade and transform leukocytes [37, 38]. Knowledge of the pathogenic mechanisms underlying tropical theileriosis is scant, but there is evidence that *T. annulata* schizont-infected cells cause failure of a protective immune response.

The failure of the host to achieve a protective immune response in the initial stage of infection is due to nonspecific lymphocyte proliferation and disruption of antigen recognition effector mechanisms [39]. *T. annulata* schizont-infected cells possess a powerful method of host-immune evasion. Therefore, the use of adjunct therapeutic agents (like antioxidants and anti-theilerial agents) are helpful to neutralize immunological imbalances and eliminate the parasite from infected animals. Complex inter-relationships exist among certain micronutrients, immune function, and disease resistance in cattle. Many micronutrients have been shown to influence the immune response of animals. Trace minerals play an important role in the dairy cow's immune system [40], fertility [41], and growth [42]. Nockels et al. [43] reported that trace mineral retention ability was reduced in stressed calves. Inclusion of minerals in the diet does not ensure intake or absorption. During diseased conditions, dry matter intake is also decreased. Dietary mineral supplements may not be adequately absorbed due to interactions with other nutrients. Antagonists in drinking water, such as iron, can also harm trace mineral absorption from the digestive tract. In newborn calves, the therapeutic efficacy of conventional chemotherapeutics against bovine tropical theileriosis is not very encouraging. Therefore, trace minerals that cause immunomodulation in bovine tropical theileriosis could improve the therapeutic efficacy of anti-theilerial drugs [44].

A significant cause of hemolysis in theileriosis is the formation of isoantibodies against RBCs [45]. To prevent this isoantibody lysis, an immunosuppressive dose of steroid such as Dexamethasone @ 2.2 mg/kg.b.wt could be used. It also has stimulatory action on bone marrow to immediately release the reservoir RBC to circulation to help the animal pass that life-threatening phase of anemia. Usually, animals with low hemoglobin and low RBC die due to lung congestion followed by respiratory failure. There was a reduction in vascular permeability in the animals treated with dexamethasone that prevented them from developing pulmonary edema and sudden death [46].

6. Conclusions

Tropical theileriosis is a TBD caused by a protozoon called *T. annulata*, transmitted by several tick species of the genus *Hyalomma*. It causes significant economic loss to dairy farmers of tropical and subtropical countries. Clinical manifestations are expressed mainly as anorexia, febrile generalized lymphadenitis, anemia, lethargy, lacrimation, nasal discharge, and exophthalmia. Fatality due to infection is greatly dependent on the overproduction of cytokines, such as TNF- α , produced by the schizont-infected monocytes/macrophages and uninfected macrophages. Buparvaquone is considered the most efficacious drug against *T. annulata* infection.

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Chapter 6

Facilitating Clinical Reasoning in Bovine Medicine: A Case-Based Teaching and Learning Approach

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Abstract

Clinical reasoning is a core metacognitive competency in veterinary medicine, enabling practitioners to draw upon prior knowledge and experience to diagnose and manage clinical cases. Teaching and learning clinical reasoning are inherently complex, shaped by the multifactorial nature of veterinary presentations, the potential for cognitive biases, and the inevitability of uncertainty. This chapter presents a structured framework for veterinary education by situating clinical reasoning within an eight-stage cycle, ranging from consideration of the client-patient situation to reflection and new learning. Using a representative case of a “sick cow” syndrome, the cycle is illustrated in detail, emphasizing the process of data collection, synthesis, hypothesis generation, diagnosis, and decision-making. Common errors, including premature closure, anchoring, and failure to recognize comorbidity, are explored, with practical remediation strategies highlighted. To support educators, the Five Microskills model of clinical teaching is applied to demonstrate how uncertainty can be explicitly addressed through techniques such as transparent disclosure, probing for supportive evidence, safety netting, and debriefing. The critical role of timely, high-quality feedback is emphasized, both as a learning tool and as a means of mitigating the effects of bias. Furthermore, the chapter underscores the importance of communication competency within clinical reasoning, focusing on the effective use of open- and closed-ended questioning, while acknowledging the complementary roles of tone, pace, and body language. By integrating structured reasoning frameworks with deliberate teaching strategies and strong communication practices, the chapter provides practical guidance for enhancing the development of clinical reasoning skills in veterinary learners.

Keywords: deep learning, metacognition, anorexia, left-displaced abomasum, metritis

1. Introduction

Effective management of clinical cases requires veterinarians to develop well-refined clinical reasoning skills. Clinical reasoning is a metacognitive competency, as it entails applying knowledge from prior clinical experiences to the diagnosis and

treatment of current patients. As noted previously [1–3], the teaching and learning of clinical reasoning are inherently challenging due to the complexity of the process. Nonetheless, these challenges can be mitigated by employing a structured clinical reasoning framework, such as the staged process outlined in **Table 1**.

An essential consideration for both educators and learners is the potential influence of cognitive bias on clinical decision-making. To enhance learning outcomes, in addition to communication training and field exposure, the application of structured teaching models, such as the Five-Microskills model [4–6], should be considered. Moreover, although not examined in detail in this chapter, the timely provision of effective feedback represents a critical component in supporting learners' acquisition of clinical reasoning skills [3, 7–9]. As we have previously demonstrated [7], high-quality feedback also functions as an important remediation strategy for mitigating the effects of bias in veterinary clinical practice.

2. Case example

A “not well” animal—commonly described as sick, unwell, or “off color”—is a frequent presentation in livestock and other veterinary species. Consequently, such a case provides a suitable example for teaching and learning about the uncertainties inherent in clinical reasoning. In **Table 1** and throughout the text, italicized terms indicate verbal communication.

3. Elements of the clinical reasoning cycle

As outlined in the case description above, the clinical reasoning cycle comprises eight components. These are:

1. ***Consider the client and the patient's situation***, including the client's level of expertise and their understanding of the animal's condition. For example, “*What is the stage of production and has productivity been compromised?*”
2. ***Collect data to support the development of differential diagnoses***. Begin with the presenting problem, then expand the database through a health interview. Consider the climatic and housing environment, and husbandry, followed by a complete a clinical examination. Where appropriate, employ ancillary techniques and diagnostic tests to obtain additional information and reduce uncertainty.
3. ***Review the collected data in relation to the presenting problem***. Determine whether additional problems have been identified. Apply knowledge of pathophysiology to assess whether the presentation supports a straightforward diagnosis or suggests a more complex condition. Consider whether further data are required. Acknowledge the presence of uncertainty and develop the ability to recognize and articulate it.
4. ***Identify problems and case-related issues to progress toward diagnosis***. Interpret clinical findings in conjunction with the collected data to generate a coherent understanding of the case. For example, “*Can you make any inferences from this information, including possible prognosis?*”

Stage	Activity/ Element	Example of veterinary medical learner's Synthesis of information
1. Consider the client ± patient situation	NA	Mrs. Jane Do is an experienced dairy manager and has presented Buttercup, a 4-year-old Holstein cow, with the "Not well" syndrome. Buttercup has been "off food" for 2 days and is "going downhill". She calved 12 days ago with no assistance and has been "not in herself" since. She usually produces above 40 L/day. From calving she has produced around 20 L/day, but this morning's milking, she produced only 2 L.
2. Collect data	Presenting problem	Not well cow that calved 12 days ago without assistance and has been unwell since.
	Health interview	Buttercup is a home-bred cow. No previous health issues have been recorded for Buttercup. The current milking herd consists of approximately 750 milking and 50 dry cows, in addition to 170 pregnant heifers and 2 bulls. The entire population is purebred Holstein. Cows are milked twice daily through a rotary milking shed with 60 units. The entire population is on pure pasture (Ryegrass–Clover mixture) and grain in the milking parlor.
	Various examination steps	Environment and husbandry—no abnormalities detected (NAD). Buttercup's abnormal clinical examination findings General inspection: Moderate obtundancy. Poor rumen fill. Cardinal signs: Mild hyperthermia. Rumen atony. Tail end exam findings: Urine sample highly positive for ketones. Voluminous, smelly, muco-purulent, and red-brown vaginal discharge. Left side exam: "Pings", particularly audible at the costochondral junction of the ribs 10–12. Rumen sunken. Rectal examination: Enlarged, thickened and insufficiently involuted uterus, filled with fluid. Rumen sunken.
	Ancillary examination techniques/ tests	Pre-treatment collection of tail vein blood samples. Significant findings in blood biochemistry carried out in a portable analyzer: High beta-hydroxybutyrate. Increased fibrinogen. Mildly hypocalcemic and hypomagnesemic.

Stage	Activity/ Element	Example of veterinary medical learner's Synthesis of information
3. Analyze the data and 4. Identify problem/s ± Issue/s	Review data/ Problem representation	Mrs. Jane Do is an experienced dairy manager and has presented Buttercup, a 4-year-old Holstein cow that is obtunded, pyrexia, anorexic, acetoneic, and has left-sided "pings." Buttercup has copious muco-purulent vaginal discharge. The condition is acute, although it seems that there is some subacute problem since parturition 12 days ago.
	Review context	Mrs. Jane Do mentions that Buttercup is a good cow, and that she is willing to do her best to save Buttercup.
	Problem identification	Calved 12 days ago. Lower than expected milk production since calving, and a significant hypogalactia this morning. Moderate obtundancy. Pyrexia. Anorexia. Rumen atony. Smelly, muco-purulent, and red-brown vaginal discharge. Left-sided "pings." Delayed uterine involution and a thickened uterine wall. Acetonemia and acetouria. Mild hypocalcemia. Mild hypomagnesemia.
	Recall knowledge	The initial hypothesis may be confusing. Learners usually seek a single diagnosis that explains all signs and syndromes. In this case, there is comorbidity (i.e., left displaced abomasum, ketosis, and metritis).
	Interpretation	"Sick-looking Buttercup, the fever, and smelly vaginal discharge are highly suggestive of metritis." "The finding of left-sided 'pings' indicates left-displaced abomasum. Yet, at this moment, there is a level of uncertainty, and we would like to run a few more tests before we diagnose it." "The presence of ketone bodies in blood and urine indicates ketosis. It is, however, uncertain if the ketosis occurred first or if it is a sequel to the complete loss of appetite by Buttercup."
	Discrimination	"The pussy type of vaginal discharge is seen with serious infections of the genital tract. On rectal examination, a fluid-filled uterus was found. On vaginal examination, the vaginal wall was found intact. Hence, vaginal damage, as a source of the discharge, can be ruled out."
	Inferring	"Buttercup's metritis has led to loss of appetite, and this, in turn, has led to increased space in the abdomen for the abomasum to move on the left side. Returning the appetite will decrease this space, and the left displaced abomasum may disappear spontaneously."
	Matching	"Left displaced abomasum, ketosis, and metritis all occur soon after calving."
	Predicting	"As you called early, we will treat metritis and ketosis immediately. This treatment should return Buttercup's appetite, and, hopefully, the left-displaced abomasum will disappear spontaneously in the next few days. However, as we said before, there is a level of uncertainty, and the outcome is unpredictable."
	Relating	"At the moment, Buttercup's fever does not give us the option to operate on the left displaced abomasum."

Stage	Activity/ Element	Example of veterinary medical learner's Synthesis of information
5. Establish mutually agreed goals	NA	Mrs. Jane Do wants Buttercup to be saved. The best possible outcome would be Buttercup surviving and returning to milk. The client should be advised that Buttercup is unlikely to return to full milk production this season. All three presented morbidities are part of a transition period management failure and are unlikely to be sporadic problems. Rather, they are usually population-level problems. This may need to be discussed with the client, and preventative strategies may be proposed. Yet, the client is not the final decision-maker, and a follow-up may be required with the owner of the enterprise.
6. Take action	NA	In this encounter, most likely, anorexia has resulted in the left displaced abomasum. Anorexia is a result of metritis ± left displaced abomasum. Anorexia, coupled with compromised health and inadequate nutrition, has resulted in mild hypocalcemia and hypomagnesemia. Yet, a primary left displaced abomasum, due to the sudden introduction of grains in the diet, is also possible. Ketosis may be primary or secondary, resulting from the severe negative energy balance or as a sequel of the depressed diet intake due to left-displaced abomasum and/or metritis. However, ketosis may have occurred as a primary condition and may have increased the risk of left displaced abomasum and/or metritis. Therefore, the management plan should consider all three conditions. The initial decision should be which of the three conditions to treat first. The pyrexia should prevent surgical treatment of the left-displaced abomasum. Additionally, if the left-displaced abomasum has occurred as a sequel to anorexia, it may self-correct with the return of the appetite. Therefore, treatment of the left-displaced abomasum, for now, should be delayed. Metritis and ketosis can be treated concurrently. Hypocalcemia and hypomagnesemia should also self-correct with the return of appetite.
7. Evaluate the outcome	NA	Buttercup's ketosis and metritis should be treatable with clinical resolution in a few days. The client should be made aware that in 5–7 days, a recheck will be required. At the recheck, the position of the abomasum should be checked, and if not self-corrected, a surgical intervention may be indicated. The client should also be made aware to monitor for relapse of any of the conditions.
8. Reflection and new learning	NA	In this encounter, the knowledge of the pathophysiology and risk factors associated with metritis, and the role of the transition period feeding, should facilitate reflection and/or finding areas of new learning. When back at the clinic, reflective practice may be of assistance in discussing this encounter with the team. For example, <i>"I have this interesting case that I would like to hear your opinion on."</i>

Table 1.
Stages of clinical reasoning applicable to veterinary medical education and profession using a clinical encounter with a "sick cow" syndrome.

5. **Establish mutually agreed goals for the patient.** Clarify the client's expectations and assess their feasibility. Consider additional factors, such as whether the problem concerns a single animal or has potential implications at the herd level, and address these directly with the client.
6. **Take action/Implement appropriate actions.** Drawing on the collected data, prior knowledge, and mutually agreed goals, determine and initiate the most suitable course of action. Where multiple problems have been identified, consider whether treatments should be prioritized sequentially or undertaken simultaneously.
7. **Evaluate treatment outcome/s.** Assess whether the client's goals for the patient have been achieved and determine the effectiveness of the interventions undertaken.
8. **Engage in reflection and new learning.** Each case presents opportunities to strengthen clinical reasoning; however, meaningful learning requires deliberate reflection. Consider whether the diagnoses were accurate and whether the treatments were appropriate, given the problems identified and the data collected. Reflection is further enhanced through structured case discussions within the veterinary team.

4. Common difficulties and errors related to the example case

Each clinical encounter in bovine practice should begin with the evaluation of data from the initial presentation, followed by the formulation of an initial hypothesis. Subsequent information should then be collected in a hypothesis-driven manner through the health interview, environmental assessment, clinical examination of the individual or population, and additional diagnostic testing (**Figure 1**).

After data are collected, or at times even during data collection, a diagnosis may be reached and a management plan proposed. Early diagnosis and intervention can help minimize the economic impact of disease. Unfortunately, this process is vulnerable to a range of biases and challenges.

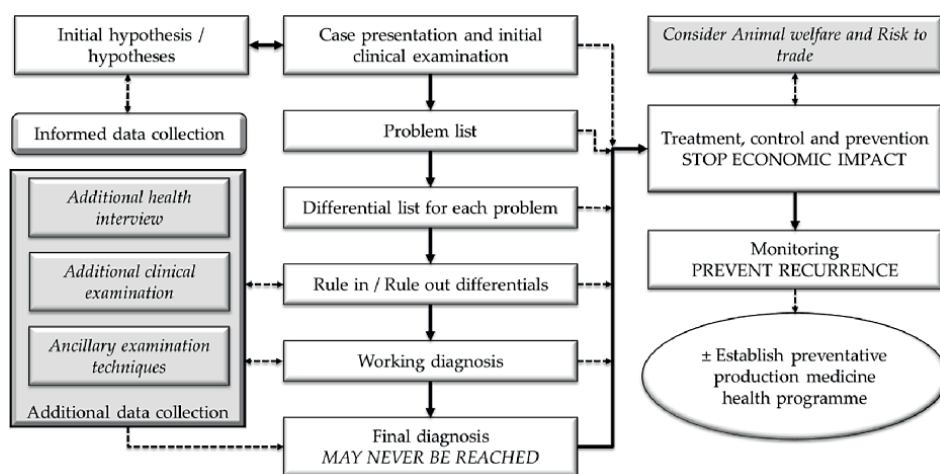


Figure 1.
Guideline to clinical problem management in livestock practice.

Common cognitive biases include affective and anchoring heuristics, in which experienced clinicians may become overconfident and rely excessively on pattern recognition rather than objective data. Such reliance often leads to premature closure, the most frequent diagnostic error. Premature closure results in inadequate further investigation, limited generation of alternative evidence-based hypotheses, and an incomplete differential diagnosis. Consequently, the management plan may be flawed, with important implications for clinical and economic outcomes (Table 2).

Stage of clinical reasoning cycle	Example difficulties	Example remediation strategies
1. Consider the client ± patient situation	Failure to identify primary complaint/s and spends too much time exploring minor points <i>Yes, I agree that Buttercup shows left-sided 'pings'. However, you also identified that Buttercup is pyrexia and has copious brown-red vaginal discharge. Can we link them somehow?</i>	Point the learner to the hypothesis-driven data collection <i>Let us discuss the need for a detailed examination of the neurologic system in Buttercup. Let us remind ourselves of the rules of data collection. The complete examination should be briefly carried out, and then, we should concentrate on collecting data that will actually assist us in rule-in and rule-out of potential diagnoses for the initially identified problems.</i>
2. Collect data	Premature closure and failure to explore data that could evoke additional hypotheses <i>I liked your initial hypothesis that Buttercup is probably suffering from metritis. Yet, not collecting sufficient data may have resulted in a decision too early, made without considering other possibilities.</i>	Point the learner to consider three differential diagnoses for the initial hypothesis <i>What three differential diagnoses would result in anorexia and pyrexia in freshly calved cow?</i>
3. Analyze the data	Inability to build an overall representation of the encounter <i>It seems that you are struggling with putting all this together. Can we assist you in some way?</i>	Point the learner to compare and contrast the discriminating features of tested hypotheses <i>Can we discuss the main signs of left-displaced abomasum?</i>
	Inability to generate prioritized list of differential diagnoses <i>Let us talk about the list of differential diagnoses you presented. We would need to consider all available data and make a decision, which potential differential diagnosis should be prioritized</i>	Ask the learner to justify the proposed list of hypotheses <i>Can we briefly discuss your thinking process and what discriminating features lead you to put ketosis as a primary differential diagnosis for Buttercup?</i>
4. Identify problems/issues	Lack of recognition of a particular issue/problem <i>What would be the usual findings in a cow calved 12 days ago with a healthy uterine discharge?</i>	Cognitive forcing strategies <i>Can we draw a mind map on the importance of taking a vaginal discharge sample from Buttercup for culture?</i>
5. Establish agreed goals	Proposed action and outcome are not the best representatives for the encounter <i>Mrs. Do is the decision-maker for Buttercup, but not for the entire herd, and you have concentrated on prevention, rather than Buttercup's treatment.</i>	Ask the learner to justify the proposed list of strategies <i>What made you propose treating Buttercup with a propylene glycol product?</i>

Stage of clinical reasoning cycle	Example difficulties	Example remediation strategies
6. Take action	Execution of the proposed management plan is rigid, stereotypical, and not tailored to the encounter, client, context, or patient <i>It is true that oxytetracycline products are preferred for treatment in dairy cows in the initial week post calving when the most likely uterine pathogens are Gram-negative bacteria and Buttercup is 12 days post calving. Let us discuss what are the most likely uterine pathogens at this stage and relate this to choice of the most suitable antimicrobial.</i>	Point the learner to tailored approaches to management <i>What would be an appropriate approach to Buttercup's treatment when we consider anorexia is the most likely reason for the left displaced abomasum in this case, and that metritis is the most likely cause of the anorexia?</i>
7. Evaluate the outcomes	Lack of follow-up plan <i>What would be the reason for calling Mrs. Do in a few days and asking how Buttercup is doing?</i>	Make the learner aware of the importance of the follow-up <i>Each clinical encounter should be used as a learning opportunity. Therefore, it would be very useful to know how Buttercup will react to our treatment.</i>
8. Identify new learning and review	Lack of reflection on thinking before, during and after the encounter <i>Can we think of reasons for having a discussion before, during and after the call out for Buttercup?</i>	Make the learner aware of the importance of reflective practice <i>Deep learning is facilitated by thinking of the process and steps taken during each clinical encounter. It will also assist us in developing basic approaches applicable to any encounter.</i>

Table 2.

Difficulties and their remediation, associated with the stage of the reasoning cycle.

5. Dealing with uncertainty in bovine clinical reasoning using the five microskills model of clinical teaching

As in all medical sciences, hypothesis testing in veterinary medicine, whether in the form of diagnosis or a management plan, is rarely associated with complete certainty [3, 10–15]. The degree of certainty or uncertainty depends on multiple factors, including but not limited to, those outlined in **Table 3** [11, 16–23].

Each clinical encounter may involve different types of uncertainty. In the example case, multiple uncertainties may arise, with common types summarized in **Box 1**. Accordingly, the teaching of clinical reasoning should include explicit recognition and acceptance of uncertainty. For instance, an instructor might emphasize that comorbidities frequently complicate diagnosis in veterinary practice, or that uncertainty is a normal and inevitable part of clinical work that must be identified and acknowledged. Such strategies foster a safe learning environment that encourages open discussion of uncertainty [14, 24–30].

Techniques such as transparent disclosure of uncertainty and safety netting can be applied within all five microskills. To further promote reflective practice, an essential component of deep learning, clinical reasoning should be reinforced with effective, timely feedback. Because discussions of uncertainty can be stressful for learners, they may, when appropriate, need to occur away from the client.

Type of uncertainty	Common factors	Examples
Inherent veterinary medicine characteristics	Available support	Access to support literature and software
		Ancillary testing equipment and tests
		Support peers, instructor, and team
	Character of the condition (e.g., chronology, severity)	Comorbidities
		Concurrent involvement of multiple organs/ systems
		Emergency (e.g., bloat or prolapsed uterus)
		Emerging condition
		Multifactorial epidemiology
		Variability in prevalence between the ages of livestock
	Clinical context	Distractors (e.g., economic constraints, fatigue, language barriers, limited time, and presence of support systems, such as equipment or tests)
		Stakes of the encounter (e.g., client expectations and pressure, economics, effects on animal welfare, and risk to trade)
	Clinical settings	Classroom (e.g., availability of teaching-assistance tools unavailable in field settings, fear of effects on grades, and peer pressure)
		Field work (e.g., lack of equipment and support)
		Hospital (e.g., difficult cases, risk of hospital-acquired infections)
	Imperfect diagnostic test characteristics	Effects of prevalence on morbidity on the test characteristics
		Lack of perfect sensitivity and specificity
	Limited veterinary medical knowledge	Continuous discoveries in veterinary medicine
	Observed signs and syndromes	Lack of a regular observation of livestock (e.g., in extensively managed livestock)
		Prevalence of the same or similar signs and syndromes in variable conditions
	Outcome unpredictability	Inherent variability in response (e.g., age and general health of the patient)
		Variability due to external factors (e.g., compliance with the selected strategy)
Personality	Client and practitioner	Cognitive capacity
		Decisiveness in decision-making
		Difference in perception of what is “normal” and what is ‘abnormal’
		Mental capacity
		Metacognitive capacity
		Personal beliefs and values
		Variability in tolerance of uncertainty

Type of uncertainty	Common factors	Examples
Veterinary professional-client relationship	Psychosocial aspects	Communication competencies
		Competing needs and priorities
		Cultural background
		Ethics and legislation
		Individual circumstances (e.g., attitude, beliefs, cognitive overload, sleep deprivation, and stress)
		Length of the veterinary professional-client relationship
		Organizational (workplace) culture
		Perception of loss of trust
		Presence of a support team
		Variability in expectations

Table 3.
Common uncertainties and common examples of interference with the clinical reasoning process.

Multifactorial etiology of the syndrome of “sick cow”.
Common risk factors related to the transition period.
The sequence of occurrence of the conditions.
Selection of the preferred management option, with the risk that the problem may be at a population level.
Stakes of the encounter (for Mrs. Jane Do).
The client is not the final decision-maker on the enterprise.

Box 1.
Veterinary medical uncertainties that are commonly related to the example case.

5.1 Get a commitment

Whenever uncertainty arises in the learner’s approach to a clinical encounter, the instructor should encourage open and constructive discussion. For example, “*Can we all talk about the level of certainty of the potential causes of the ‘sick cow’ syndrome in this encounter?*” or “*Before we discuss my initial thoughts on this ‘sick cow’ case, I would appreciate it if we could go through your initial thinking.*” Alternatively, the instructor may employ the technique of transparently disclosing their own uncertainty and encourage learners to adopt the same practice. For example, “*Indeed, at this moment, I am unsure of the next step, but it is important not to jump to conclusions before we have all the data available. What next steps could assist us in this encounter?*”

5.2 Probe for supportive evidence

The expression of uncertainty within this microskill provides an important opportunity for the instructor to stimulate learners’ self-reflection [24]. The instructor may use phrases such as:

- a. “*It would be good to hear in your own words what makes you feel uncertain about the role of the hypocalcemia in this case?*”; or

- b. *“What aspects of this encounter lead you to the conclusion that the left-displaced abomasum should be a management priority, and how can this be decided?”*

5.3 Teach general rules

The instructor should model effective approaches for managing uncertainty to guide learners in developing similar strategies [24, 25]. For example, *“In cases of comorbidity of left-displaced abomasum and ketosis, there is always a level of uncertainty on the order of occurrence of the disorders. Ketosis may lead to left-displaced abomasum but may also be a result of it.”* In this microskill, which addresses uncertainty, the instructor should prioritize explaining their reasoning rather than teaching general rules [24]. For example, *“In this encounter, most likely, the left-displaced abomasum is secondary to the ketosis and the low rumen volume. Yet, the order of occurrence, due to the intertwined relationship, is difficult to establish.”*

5.4 Reinforce what was done well

A learner's admission of uncertainty makes it more manageable [24, 31]. To support this process, the instructor should acknowledge and validate the learner's recognition and disclosure of uncertainty. For example, *“I think that you did well in recognizing that in this case, the ‘sick cow’ syndrome is intertwined with transition management risk factors. I also liked your approach to explaining the level of uncertainty about the role of transition to the client.”*

5.5 Correct mistakes

The technique of safety netting can be used effectively to correct learners' mistakes while also fostering their recognition and admission of uncertainty. For example, *“If Buttercup does not improve significantly over the next 2–3 days, do not hesitate to contact us. We may need to revisit the entire situation, and we may need to do some extra assessments, just to make sure we have not missed something.”*

5.6 Debrief

Debrief following each case, or at least on a daily basis, is an essential component of stimulating reflective practice and should explicitly address uncertainty [4, 10, 11, 16, 25, 32–36]. Debriefing should extend beyond the veterinary medical aspects of the encounter to also include non-medical and psychosocial dimensions. For example, *“Could we discuss the feelings of the client when she was told that we cannot resolve all the problems of Buttercup on this occasion? It would also be good to think of the discussion she needs to have with the owner regarding the transition cow management.”*

6. Communication strategies

The learning of veterinary clinical communication can be supported through the use of the Modified Calgary–Cambridge Model, as previously described [37, 38]. Communication competency relies on attentive listening [38–40] and the ability to communicate with clarity and assertiveness. Common challenges include linguistic

Stage of clinical reasoning cycle	Example sentences	
	Discussion (Open-ended)	Clarification (closed-ended)
1. Consider the client ± patient situation	<i>What do you think is going on with Buttercup?</i>	<i>Do you mean in kg of dry matter?</i>
2. Collect data	<i>I would appreciate it if in your own words you could explain what you have seen going on with Buttercup in the last few days.</i>	<i>Was that sudden or lingering on for some time?</i>
3. Analyze the data	<i>What we have to do now is to put it all together and see what we think is going on. Is that OK with you?</i>	<i>Do you want to see this in a diagram?</i>
4. Identify problems/ issues	<i>What do you think of the identified problems with Buttercup?</i>	<i>What do you mean by large?</i>
5. Establish agreed goals	<i>Now that we know what is going on with Buttercup, and we know what available options are, I would like to talk about creating a management plan for the next few days. Can you tell us what your preference in this case is?</i>	<i>Do you mean that you prefer products with no withholding period?</i>
6. Take action	<i>Let us go over it again; how will this work?</i>	<i>What is your preferred way?</i>
7. Evaluate the outcomes	<i>We agreed that Buttercup should be monitored in the next few days, and the milk yield will be the best indicator that she is getting better. What else do we need to discuss regarding Buttercup's monitoring?</i>	<i>Can she be separated in the TLC (TENDER_LOVING_CARE) group?</i>

Table 4.
Example open- and closed-ended inquiries related to the example case that can be utilized in various stages of the clinical reasoning cycle.

limitations, such as the inappropriate use of technical terminology [23, 37, 38]. Verbal communication is most effective when it prioritizes carefully constructed open-ended questions, supplemented by the tactful use of closed questions for clarification (Table 4) [38, 41]. In addition to the use of open- and closed-ended questions, verbal skills such as pace, tone, and voice are also critical. Furthermore, a substantial proportion of communication is conveyed through body language. A detailed discussion of these elements lies beyond the scope of this chapter.

7. Conclusions

In this chapter, we have examined the teaching and learning of clinical reasoning, using a representative clinical case to anchor key concepts. We highlighted the influence of uncertainty and bias on the formulation of differential diagnoses, both of which are commonly encountered by novice learners and instructors. To address these challenges, we emphasized the value of the clinical reasoning cycle in providing a structured framework for decision-making. While this framework does not eliminate all difficulties, we have outlined strategies for remediating problems associated with uncertainty and bias, including the application of the Five Microskills model.

Finally, to illustrate approaches for strengthening communication competencies in veterinary professional learners, an example dialog has been incorporated throughout the case discussions as done previously [42].

Conflict of interest

The authors declare no conflict of interest.

Glossary

Attentive listening	Giving full concentration and focus to the speaker, trying to actively receive and understand their message.
Bias (in clinical reasoning)	Preconceived notions and subconscious prejudices that veterinary medical professionals may hold toward clients or patients based on various attributes (e.g., client: age, disability, ethnicity, gender, gender orientation, race, or socioeconomic status; patient: age, breed, production type, reproductive status, sex, and species) and context (e.g., hygiene, production system, and season).
Clinical context	Relevant information and situation surrounding a clinical encounter that will influence the decision-making process.
Clinical encounter	Any physical or virtual contact with a veterinary patient and client (e.g., owner, employee of an enterprise) with a primary responsibility to carry out clinical assessment or activity.
Clinical settings	The specific location where the clinical encounter occurs (e.g., tutorial room for case discussion, or clinic or hospital for clinical case ambulatory practice).
Cognition	A mental activity or a process of acquiring knowledge and understanding.
Cognitive forcing strategies	A group of interventions that use mechanisms to disrupt the heuristic processing of information. It is part of the metacognitive approach.
Deep learning	Learner aims for mastery of essential academic content; thinking critically and solving complex problems; working collaboratively and communicating effectively; having an academic mindset; and being empowered through self-directed learning.

Effective feedback	A purposeful conversation between the clinical instructor and the veterinary professionals in learning to stimulate further development of clinical competencies and deep learning.
Hypothesis-driven data collection	A combination of data collection and clinical reasoning resulting in the early generation of hypotheses, and resultant data collection is used to rank competing differentials/ management approaches, resulting in limited but focused data collection. <i>Common synonym:</i> Serial-cue approach.
Metacognition	Critical awareness of one's thought processes and learning, and an understanding of the patterns of thinking and learning ("thinking about thinking").
Mind mapping	A diagram used to visually put medical information into a hierarchy linked to and arranged around a central concept/ hypothesis.
Reflection	The metacognitive process that may occur before, during or after an encounter that aims to develop a deeper understanding of the encounter, and self ± the team, to inform the ongoing and/or future actions, behaviors, and encounters.
Reflective practice	A metacognitive, learner-centered strategy that assists learners in making sense of the learned material and engaging in deep learning.
Safe learning environment	An environment in which a learner feels safe, relaxed, and willing to take risks in pursuing a goal; enhances self-esteem and encourages exploration. <i>Common synonym:</i> Proper learning environment.
Safety netting	A strategy used in medical and veterinary encounters to manage diagnostic uncertainty and ensure client and patient safety by providing information on potential risks and unknowns, empowering the client to actively participate in the client management.

The five microskills model of clinical teaching	An instructor-centered model of clinical teaching: (1) Get a commitment; (2) Probe for supporting evidence; (3) Teach general rules; (4) Reinforce what was done well; and (5) Correct mistakes. An additional stage is the “Debrief”.
Uncertainty	The subjective perception of an inability to provide an accurate explanation of the patient’s health problem or the client’s worries.

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Health Challenges for High-Producing Dairy Cows and Buffaloes

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Abstract

The intensification of dairy production to meet rising global demand has exposed high-producing dairy cows and buffaloes to a complex array of health challenges. These include infectious, locomotor, metabolic, and reproductive disorders, exacerbated by heat stress, nutritional deficiencies, suboptimal housing, and, sometimes, limited veterinary services. Buffaloes are notably affected by renal pathologies, including amyloidosis, interstitial nephritis, and nephrolithiasis, often linked to poor nutrition and undetected systemic dysfunctions. Fertility in high-yielding cows and buffaloes has declined due to negative energy balance, metabolic stress, and postpartum infections. Mastitis remains a leading cause of economic losses, reducing milk yield and reproductive efficiency, with the rising awareness of antimicrobial resistance complicating treatment. Body Condition Scoring (BCS) emerges as a critical tool in evaluating energy status, predicting disease risk, and guiding nutritional strategies, especially during the transition periods. Technological innovations, such as automated BCS systems, mid-infrared spectroscopy, and on-farm pathogen diagnostics, offer promising avenues for precision herd management. Effective health programs must emphasize context-specific interventions, early diagnosis, and prevention, and these must be included in teaching bovine medicine. Integrated approaches that combine advanced diagnostics, environmental control, genetics, and precision nutrition are essential for improving animal health, enhancing productivity, and ensuring sustainable dairy systems in resource-constrained environments.

Keywords: dairy cows, BCS, mastitis, transition phase, buffalo pathology

1. Introduction

The rapid intensification of dairy production, driven by the need to supply a growing global population, particularly in tropical and subtropical regions, has exposed high-yielding dairy cows and buffaloes to significant health challenges [1, 2]. Factors such as disease prevalence, heat stress, infrastructural deficiencies, limited feed

resources, and suboptimal animal husbandry and welfare compromise both animal health and productivity, necessitating comprehensive and sustainable management strategies. Environmental and nutritional stresses further contribute to the emergence of complex infectious, locomotor, metabolic, and reproductive disorders that are prevalent among high-producing cows and buffaloes. All disorders are multifactorial, and addressing a single cause in the management and prevention is futile.

Fertility in high milk-producing dairy cows has notably declined over recent decades, primarily due to the interplay between metabolic disorders, negative energy balance, and postpartum uterine infections [3, 4]. Key physiological disruptions, such as impaired delayed ovulation, early embryonic mortality, and estrus expression, have been linked to increased milk yield demands. Consequently, effective fertility management must integrate nutritional strategies aimed at minimizing energy deficits and enhancing estrus detection, ensuring better reproductive outcomes. Pascottini et al. [5] show that advances in genetics, nutrition, animal welfare and reproductive technologies have transformed bovine reproduction in Europe. Tools like sexed semen, embryo transfer, and AI-driven herd management have helped stop and sometimes reverse fertility decline [5]. This progress reflects a new, integrated approach aimed at not just maintaining, but improving dairy cow fertility.

Similarly, reproductive health in buffaloes is compromised by conditions such as ovarian cysts, uterine infections, and vaginal prolapse, which significantly lower fertility rates [6]. The overlap between infectious diseases, metabolic imbalance, and poor management underscores the need for integrated health programs focusing equally on prevention and early diagnosis.

In addition to reproductive issues, housing conditions have a profound effect on dairy cow welfare and health profiles. Pasture-based systems have been associated with lower incidences of lameness, mastitis, uterine disease, and mortality compared to continuously housed cows [7, 8]. However, the drive for intensification often necessitates continuous housing, which due to restricted movement and poor hygiene, unfortunately elevates the risk of locomotor and udder disorders, thus exacerbating existing metabolic and reproductive challenges.

These interconnected health issues become even more pronounced during the transition period, especially in the first 15 days in milk, a critical window characterized by profound anatomical, endocrine, and metabolic changes [9]. The metabolic stress during this phase not only predisposes bovine females to disorders such as displaced abomasum, fatty liver, hypocalcemia, and ketosis but also aggravates the vulnerability to infectious diseases, further compounding fertility problems and impairing milk yield.

Among the multifactorial health challenges, lameness remains a persistent issue, significantly impacting animal welfare, feed intake, fertility, mobility, and overall productivity. Lameness, largely resulting from orthopedic pathologies such as interdigital infections, sole ulcers, and white line disease, is exacerbated by inappropriate flooring, irregular hoof management practices, and management practices [10, 11]. Hence, improvements in housing environments and proactive hoof health management strategies are critical to mitigate its impact and sustain production efficiency.

Infectious diseases present additional major hurdles. Among many common and systemic infectious disorders, the bovine brucellosis remains a significant zoonotic and reproductive threat, especially in regions with insufficient biosecurity measures and veterinary services [11]. Furthermore, bovine tuberculosis in African buffaloes illustrates the role wildlife reservoirs play in sustaining infections that spill over into domestic populations [12]. Finally, skin nodular diseases also add to diagnostic complexities, often requiring sophisticated differential diagnosis to avoid mismanagement [8].

The quality and composition of milk are not immune to these systemic health challenges. Milk yield and composition are influenced by the environmental stresses, genetic factors, and health status of the bovine female, with mastitis being a predominant constraint to milk quality and profitability [4, 9]. Indeed, milk composition is not only related to health, and it can also be manipulated nutritionally. Nutritional strategies, such as lipid supplementation, have shown potential in modifying milk fatty acid profiles and improving milk nutritional value [2].

In tropical climates, where disease burdens and nutritional deficiencies are often compounded, dairy productivity faces additional obstacles. Hypocalcemia, lumpy skin disease, mastitis, and metabolic disorders like ketosis severely affect herd performance, necessitating context-specific intervention strategies [2].

Buffaloes, central to dairy production in South Asia, are similarly impacted by these challenges. Low productivity in buffaloes stems from environmental conditions, genetic limitations, high disease prevalence, and poor management practices, underscoring the urgent need for targeted genetic improvement programs and robust veterinary support services [13].

Ultimately, high-producing dairy cows and buffaloes face interlinked and multifactorial health challenges that span infectious domains, locomotor, metabolic, and reproductive. Sustainable dairy production demands a holistic and integrated approach, combining disease surveillance, environmental improvements, genetic selection, and precision management practices to safeguard animal health, productivity, and welfare. In the authors' opinion, the interlinks between environment, fertility, health, management, and nutrition are not covered well in local veterinary literature, and as such, bovine medical teaching is suboptimal. Therefore, we undertook a narrative review, aiming to cover the health challenges faced by high-producing dairy cows and buffaloes, focusing on disease susceptibility during the transition period, emphasizing the critical role of body condition score (BCS) in health outcomes, the predominance of mastitis in dairy cows, and the predisposition of buffaloes to renal diseases.

2. Materials and methods

A narrative literature review was conducted to collect, analyze, and synthesize relevant data. A significant number of peer-reviewed articles, clinical studies, and review papers were systematically selected from reputable academic databases, including PubMed, Scopus, ScienceDirect, and Google Scholar.

The inclusion criteria emphasized studies published in the past two decades that focused on body condition dynamics, dairy cows with high milk production, mastitis epidemiology, transition cow diseases, and renal pathology in buffaloes.

The literature search strategy incorporated combinations of keywords such as "close-up period," "high-producing dairy cows," "body condition score," "mastitis," "transition cow diseases," "buffalo renal disorders," and "pathology in dairy animals." Articles not relevant to the transition period or unrelated to the specified species (cows and buffaloes) were excluded.

Data extraction prioritized disease prevalence, management practices, metabolic parameters, and key health indicators, related to the transition period. Particular attention was paid to studies reporting clinical, immunological, and metabolic assessments around the time of calving.

The gathered data were subsequently synthesized and graphically illustrated to highlight key findings and trends. Figures and tables were developed to provide visual

representation of the impact of BCS on health, the incidence of mastitis in high-producing cows during the close-up period, and the specific renal pathologies identified in buffalo populations.

The approach allowed for a structured and evidence-based presentation of the critical pathological challenges impacting the health and productivity of high-performing dairy cows and buffaloes.

3. Body condition scoring in dairy cows

Body Condition Scoring (BCS) has long been recognized as a fundamental indicator of the nutritional and metabolic status of dairy cows. It serves as a noninvasive, cost-effective method for evaluating the amount of body fat and muscle reserves, offering critical insights into the nutritional management, overall health, productivity of dairy animals, and reproductive efficiency [14, 15]. This evaluation becomes particularly vital in high-producing cows, where intense metabolic demands make them more susceptible to energy deficits and associated health disorders [15].

Effective management of BCS in dairy cows is closely associated with enhanced herd profitability and sustainability. High-producing cows, in particular, due to the high energy demands of milk production, face greater metabolic challenges, often struggling to maintain optimal body reserves [15]. Cows with an optimal BCS at calving demonstrate faster recovery postpartum, higher milk yields, and improved reproductive performance, compared to over- or under-conditioned counterparts [16, 17]. Deviations from the optimal BCS range are linked to increased incidences of metabolic disorders, emphasizing the need for tailored management strategies, especially in high-yielding animals (**Figure 1**).

Maintaining an adequate BCS throughout lactation allows cows to better cope with these energy demands. Precise monitoring provides farmers with actionable data to adjust nutritional plans, thus preventing the onset of severe negative energy balance, particularly in cows at the peak of lactation. In an era where precision dairy management is becoming increasingly important, BCS has evolved from a simple observational tool to a strategic element embedded within broader herd health and productivity programs.

Albornoz et al. showed that 3D camera systems allow automated, highly accurate BCS measurement, replacing subjective manual scoring [18]. Real-time data integration into herd management helps detect changes early and adjust feeding or health strategies proactively. BCS thus becomes a key tool in precision farming, improving welfare, productivity, and sustainability.

A cow, as presented in **Figure 1**, typically indicates a severe negative energy balance. Therefore, the cow, to meet her energy needs, is utilizing body reserves. This condition is frequently observed in high-producing cows during early lactation and, to prevent metabolic disorders and support recovery, highlighting an urgent need for nutritional intervention.

The genetic background of dairy cows plays a significant role in determining variations in body condition score. Research by Pryce and Harris [14] showed that BCS is moderately heritable, with heritability estimates ranging between 0.23 and 0.32 during early lactation. Genetic selection programs increasingly aim to identify cows capable of maintaining stable BCS despite high milk output, addressing the metabolic vulnerabilities of modern, high-producing dairy herds [14]. Selecting for traits related to energy balance and body condition stability could mitigate some

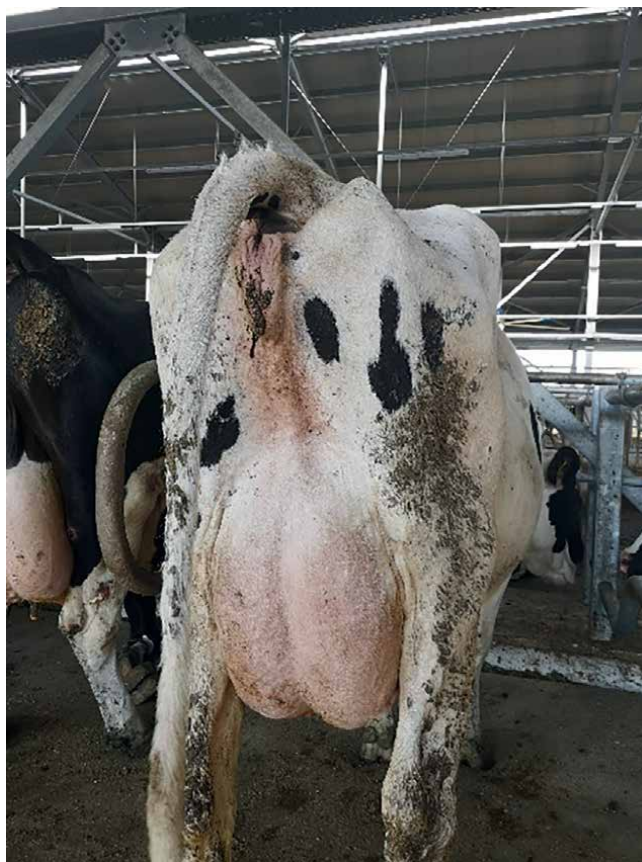


Figure 1.
Body condition score 2.0 (rear view) at calving, indicating a severe negative energy balance is associated with increased risk of fertility, health, productivity, and welfare challenges (Source: Dănuț Nicolae Enea).

negative consequences associated with intense milk production, supporting not only productivity but also animal health and welfare.

The onset of lactation induces profound anatomical, endocrine, and physiological adaptations in dairy cows, especially in high-yielding individuals. Postpartum, cows typically experience a negative energy balance as the energy required for milk production surpasses dietary intake [15]. High-producing cows are particularly at risk, mobilizing greater amounts of body fat and muscle reserves to sustain lactation, leading to steeper and earlier BCS losses [15, 19]. The nadir in BCS typically occurs between 40 and 100 days in milk, marking a critical window for metabolic diseases such as fatty liver and ketosis. Managing cows to calve at an optimal BCS between 3.0 and 3.5 mitigates these risks (**Figure 2**). Bedere et al. [16] further emphasized that cows calving at optimal BCS and minimizing postpartum losses experience enhanced fertility outcomes. However, for high-yielding cows, more aggressive nutritional support and closer monitoring are essential to prevent excessive depletion of body reserves during this vulnerable period [16].

A cow as presented in **Figure 2** typically indicates a good and balanced nutritional status. This condition (BCS of 3.0–3.5) is considered optimal for high-producing dairy cows at key physiological stages, providing a strong foundation for reproductive success and resilience to metabolic stress.



Figure 2.
Body condition score 3.0 (side view) is indicative of a good condition at calving (Source: Dănuț Nicolae Enea).

Traditional assessment methods for body condition scoring (BCS) rely on subjective visual inspection and palpation, processes inherently prone to observer bias and inconsistencies [20]. In modern dairy systems, particularly in high-producing herds where monitoring large numbers of cattle or buffaloes is challenging, these limitations necessitate more objective and efficient alternatives. Recent technological innovations have introduced automated body condition scoring (ABCS) systems based on three-dimensional imaging, computer vision, and deep learning algorithms. These systems offer continuous, noninvasive, and highly repeatable evaluations of BCS across the entire herd (**Table 1**).

Automated solutions not only standardize assessments, minimizing human error, but also enable real-time decision-making by providing daily updates on each animal's condition [21]. In current conditions, when we are facing a lack of qualified labor in the livestock sector, this becomes extremely important and advantageous for the farmer. In herds of high-producing cows, where energy reserves fluctuate rapidly during peak lactation, such precise monitoring is critical for early detection of negative energy balance and prompt nutritional interventions. Moreover, the integration of mid-infrared (MIR) milk spectroscopy with machine learning models represents a promising frontier. By analyzing milk composition data, particularly fat and protein estimations, it is possible to predict body condition changes, even before they become

Criteria	Manual vs Automated scoring
Subjectivity	High (manual)/Low (automated)
Labor intensity	High (manual)/Low (automated)
Frequency of monitoring	Low (manual)/High (automated)
Consistency	Variable (manual)/High (automated)

Table 1.
Comparison of manual versus automated body condition scoring systems (ABCS) (Source: Bewley and Schutz [20]).

visually detectable [21]. This approach holds particular value in high-yielding cows, whose subtle early-stage BCS variations could indicate impending metabolic stress or fertility issues. As wearable sensors, automated weighing systems, and precision feeding technologies continue to evolve, the capacity to integrate BCS data into holistic herd management platforms will enhance individualized cow care and overall herd sustainability. Investing in automated BCS monitoring is thus not only a step towards improving accuracy but also a fundamental necessity in optimizing management practices for high-productive cows. **Table 1** compares manual and ABCS methods [20]. Manual scoring, though widely practiced, is inherently subjective and labor-intensive, limiting its frequency and consistency. In contrast, ABCS, employing technologies such as 3D imaging and computer vision, offers frequent, objective, and highly consistent BCS evaluations. The adoption of ABCS monitoring is particularly beneficial in large-scale dairy operations and high-producing herds, where timely and accurate assessments are critical for prompt management of metabolic and reproductive health.

The relationship between body condition and reproductive performance is well-established and particularly pronounced in high-producing cows, as highlighted by Mulliniks et al., who demonstrated that body condition score (BCS) at calving strongly influences reproductive outcomes in young postpartum cows [22] and by Bezdiček et al., who further emphasized the biotechnological and physiological mechanisms linking body reserves to fertility [23]. Energy demands associated with intense lactation can lead to significant postpartum body condition loss, delaying the resumption of ovarian cyclicity and negatively impacting fertility [16, 17]. Cows that are unable to recover body reserves promptly after calving are at an increased risk of greater likelihood, lower conception rates, and prolonged anestrus of being culled for reproductive failure. Pfeifer et al., in 2023 [24], highlighted the importance of the timing of BCS nadir, noting that cows experiencing controlled early postpartum BCS loss, with a subsequent rapid recovery, tended to have better reproductive outcomes. In high-yielding cows, managing this delicate balance between necessary energy mobilization for milk production and timely recovery is especially critical. Nutritional interventions tailored to support early lactation energy demands, combined with vigilant BCS monitoring, can significantly enhance reproductive performance in these cows. Beyond maternal reproductive health, BCS at calving also influences calf viability and early growth [25]. Poczynek et al. note that BCS at calving influences maternal recovery, fertility, and even calf survival and growth, highlighting the need for optimal body reserve management [25].

Over-conditioned cows (**Figure 3**) are often a result of imbalanced dry period feeding or improper transition management, and present with altered colostrum composition. Ylioja et al. found that higher BCS at calving alters colostrum microRNA profiles [26], while Soufleri et al. showed it also reduces colostrum quality and quantity [27]. These studies stress the need to maintain optimal BCS during the dry period to protect colostrum quality and calf health.

A cow, as presented in **Figure 3**, typically indicates a dairy cow with a body condition score of 4.0, indicative of over-conditioning. Such a condition suggests an excess in energy intake relative to requirements and, if persistent, could impair mobility and reproductive performance and increase the risk of metabolic disorders, particularly in high-producing dairy cows.

Ylioja et al. demonstrated that excessive maternal adiposity alters the microRNA profile of colostrum, specifically decreasing levels of miR-486, a molecule implicated in glucose metabolism and immune system development [26]. This biochemical



Figure 3. Body condition score 4.0 (side view) is indicative of an over-conditioned cow at calving (Source: Dănuț Nicolae Enea).

alteration could compromise neonatal immune competence, reducing passive transfer of immunity and predisposing calves to early-life diseases. Thus, achieving and maintaining an optimal BCS in high-producing cows not only supports maternal productivity and fertility but also establishes a solid foundation for the health and future performance of the next generation.

In addition to its impact on reproduction, body condition scoring serves as a key indicator of a cow's resilience to disease. The intense metabolic load during early lactation places additional stress on the immune system, particularly in high-producing cows, heightening vulnerability to infectious and metabolic diseases such as displaced abomasum, fatty liver, hypocalcemia, ketosis, lameness, mastitis, and metritis [15]. Galecio et al. provided important insights into how metabolic status, reflected by BCS, can influence the effectiveness of antimicrobial therapies [28]. Their research demonstrated that alterations in the serum composition associated with poor body condition modify the *in vitro* susceptibility of mastitis pathogens to antimicrobials. This finding suggests that high-producing cows suffering from more pronounced BCS losses may require tailored therapeutic strategies for effective disease management. Furthermore, Guccione et al. highlighted the importance of udder health programs during the dry period, demonstrating that antimicrobial dry buffalo therapy reduces the incidence of mastitis and contributes to more stable body condition during the transition to lactation [29]. By minimizing the systemic inflammatory burden, these programs indirectly support energy conservation, preserving body reserves essential for coping with the demands of early lactation. Ultimately, proactive BCS management, combined with comprehensive health monitoring, is critical for sustaining disease resilience in high-yielding dairy cows and buffaloes, improving not only individual cow longevity but also overall herd profitability.

Body condition scoring remains a central pillar of precision dairy management, offering insights that extend beyond mere nutritional status to encompass calf health, disease resistance, and reproduction. In high-producing cows, the stakes are even higher: their exceptional metabolic demands necessitate an integrated management approach that emphasizes dynamic BCS monitoring, early intervention, and

Days in milk (DIM)	Average BCS change
0 (Calving)	Peak BCS
40–100 DIM	Lowest BCS (nadir)
>100 DIM	Gradual recovery
Late lactation	Approaches pre-calving BCS

Table 2.
Changes in body condition score across lactation in dairy cows (Source: Truman et al. [17]).

continuous adaptation of feeding and health programs. Advances in automated monitoring technologies, molecular profiling, and precision farming tools have revolutionized the ability to track and manage body condition at the individual cow level. Nevertheless, human expertise remains essential in interpreting data and applying nuanced management strategies that recognize the unique physiological challenges of modern, high-yielding dairy cattle (**Table 2**).

Table 2 illustrates the typical progression of the BCS throughout the lactation cycle in dairy cows, as monitored by ABCS systems [17]. Immediately postpartum, cows exhibit their peak body reserves; however, due to the significant energy demands of early lactation, rapid mobilization of fat stores leads to a noticeable decline in BCS, reaching a nadir between 40 and 100 days in milk. Following this critical period, as lactation progresses, gradual recovery of BCS is observed. Maintaining awareness of these natural fluctuations is essential, especially in high-producing cows, where excessive early BCS loss could compromise health and fertility.

By adopting a multidisciplinary strategy that incorporates genetics, nutrition, technology, and proactive health management, dairy producers can optimize body condition trajectories, enhance animal welfare, and achieve sustainable productivity gains across their herds.

4. Transition period

The transition period in dairy cows spans 3 weeks before to 3 weeks after calving, involving major metabolic, hormonal, and nutritional changes. Cows face increased energy demands, often leading to negative energy balance and risks like ketosis, milk fever, and metritis. Proper nutrition (balanced energy, minerals, and additives), stress reduction, and careful health monitoring are essential.

High-producing dairy cows, due to their elevated metabolic demands, are particularly vulnerable during the close-up period. The impending onset of high milk production necessitates substantial glucose availability, promoting intense lipid mobilization from body fat reserves [30]. Consequently, these cows often experience a more pronounced negative energy balance (NEB), predisposing them to metabolic disorders such as fatty liver and ketosis. Additionally, the immune system becomes transiently suppressed during late gestation and early lactation, making high-producing cows more susceptible to infections such as mastitis and metritis [31]. Stressors related to dietary inconsistencies, environmental conditions, and social disruptions further exacerbate these vulnerabilities. Therefore, meticulous close-up management is essential to mitigate health risks and sustain performance in high-yielding dairy cows.

Nutritional management is fundamental to supporting metabolic changes in high-producing cows. Feeding strategies must prioritize the adaptation of the rumen to lactation diets while ensuring proper dietary cation-anion difference (DCAD) in the late gestation period to mitigate the risk of hypocalcemia [32]. Providing diets with adequate energy density while avoiding over-conditioning is essential to prevent postpartum metabolic disorders such as fatty liver syndrome and ketosis.

Environmental factors also significantly impact the transition of high-yielding cows. Inadequate feed bunk access, overcrowding, and poor ventilation during the close-up period contribute to impaired immune responses, increased stress, and reduced dry matter intake [33]. Dairy farms that deliver consistent feed management practices, ensure adequate space allocation, and implement cooling systems improve the likelihood of a smoother transition into lactation.

Managing heat stress is particularly crucial for high-producing dairy cows during the close-up period. Reference [31] demonstrated that heat-stressed cows experienced altered metabolic profiles, delayed estrus, prolonged open days, and reductions in postpartum milk yield of 2–3 kg/day, which are characterized by elevated nonesterified fatty acids (NEFA) and tumor necrosis factor-alpha (TNF- α), along with decreased insulin-like growth factor-I (IGF-I) levels. Implementation of heat abatement strategies, including the use of shade, fans, and sprinklers, is essential to support the health and productivity of high-producing dairy cows during this vulnerable phase.

Micronutrient management, especially regarding vitamin D3 supplementation, offers another avenue to enhance transition cow health. Reference [34] reported that vitamin D3 injection administered during the close-up period improved insulin sensitivity beta-hydroxybutyrate (BHBA) levels and decreased NEFA, and contributed to better energy balance postpartum (**Table 3**).

Table 3 summarizes the main physiological and metabolic effects of heat stress and vitamin D3 supplementation during the close-up period. For high-producing cows, where metabolic demands are heightened, such interventions can be critical in minimizing the risk of periparturient metabolic disorders.

The BCS management of dairy cows must not be overlooked. Cows entering the close-up period with excessively high BCS are at an increased risk of metabolic diseases and reduced dry matter intake [30]. Targeting a moderate BCS at calving (3.0–3.5) through controlled energy diets during the dry period enhances postpartum performance in high-producing cows.

Parameter	Heat stress effect	Vitamin D3 supplementation effect
Milk production	Decrease by 2–3 kg/day postpartum	Improved energy balance, supporting yield
Reproductive performance	Delayed estrus, increased days open	Enhanced metabolic health, indirect support
NEFA concentration	Increased (metabolic stress marker)	Decreased (better energy balance)
TNF- α levels	Increased (inflammatory response)	Reduced inflammation (improved status)
IGF-I levels	Decreased	No direct effect reported

Table 3.
Effects of heat stress and vitamin D3 supplementation during the close-up period in high-producing dairy cows (Source: Stefanska et al. [31]; Hassanabadi et al. [34]).

Close-up duration	Risk of postpartum diseases
<14 days	Increased
14–21 days	Optimal
>28 days	Increased

Table 4.
Association between days spent in the close-up group and incidence of postpartum diseases (Source: Cook et al. [37]).

Precision feeding during the close-up period is equally vital. Gheller et al. emphasized that discrepancies between formulated and actual diets provided to close-up cows led to poorer early lactation outcomes [35]. High precision in diet delivery reduces the incidence of transition-related diseases and supports optimal milk production potential.

Duration of stay in the close-up group also influences health and productivity outcomes. Cows spending approximately 14–21 days in the close-up group showed better transition success compared to those spending either less than 10 days or more than 28 days [36, 37], (**Table 4**). Precise grouping based on calving dates and physiological readiness is thus critical for high-producing dairy herds.

In conclusion, optimal close-up management for high-producing dairy cows requires a multifaceted approach involving environmental control, nutritional precision, strategic health interventions, and stress mitigation. These integrated strategies collectively enhance transition success, supporting both immediate postpartum productivity and long-term herd profitability.

5. Mastitis

Mastitis is an inflammation of the mammary gland, being a health problem that relatively frequently affects dairy cows and buffaloes; it can alter a quarter or the entire udder [38, 39].

Mastitis is widely acknowledged as one of the most economically impactful diseases in the dairy industry, significantly affecting milk yield, milk composition, reproductive performance, and animal welfare [1, 3, 7]. The transition towards high milk-producing dairy cows has inadvertently increased the risk of mastitis, highlighting a fundamental challenge in balancing productivity with animal health. The economic repercussions of mastitis are substantial, encompassing direct losses from reduced milk production, discarded milk, veterinary costs, and labor, as well as indirect losses associated with diminished reproductive performance and premature culling [1]. Subclinical mastitis, characterized by elevated somatic cell counts (SCC) without obvious clinical signs, represents a particularly insidious form of the syndrome as it often remains undetected while significantly impairing milk quality and yield [3]. Environmental factors, genetic predisposition, management practices, and pathogen exposure synergistically contribute to mastitis susceptibility [7]. Moreover, climate-related stressors prevalent in tropical and subtropical regions further exacerbate the challenges of mastitis control, particularly in high-yielding cows [1]. Given these complexities, an integrated approach that includes early diagnosis, enhanced biosecurity, improved housing systems, innovative therapeutic strategies, and selective breeding for mastitis resistance is crucial for sustainable mastitis management.

The etiology of mastitis is multifactorial, involving the interaction of pathogenic microorganisms, host immune defenses, and environmental conditions [40, 41]. Contagious pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Mycoplasma* spp. are transmitted primarily during milking, whereas environmental pathogens like *Escherichia coli*, *Klebsiella* spp., and *Streptococcus uberis* originate from contaminated bedding, soil, and water [42]. Host factors influencing mastitis susceptibility include genetic predisposition, integrity of the teat canal, overall immune status, parity, and stage of lactation [3]. Environmental influences such as bedding management, climate, hygiene practices, and ventilation also play critical roles [7]. Poorly maintained housing systems, characterized by inadequate sanitation, overcrowding, and suboptimal ventilation, predispose cows to environmental mastitis [7]. Additionally, heat stress, prevalent in tropical regions, impairs immune function and exacerbates mastitis risk [1]. Nutrition also modulates mastitis risk, with deficiencies in trace elements, like selenium, and vitamins, such as vitamin E, being associated with impaired neutrophil function and increased susceptibility [40].

The pathogenesis of mastitis involves the invasion of the mammary gland by pathogens, followed by an inflammatory response aimed at eliminating the infection. The success or failure of this response determines the occurrence and the severity of the syndrome [43, 44]. Upon breaching the teat canal's keratin barrier, and entry into the mammary tissues, pathogens interact with mammary epithelial cells and immune receptors, initiating a cascade of pro-inflammatory cytokine production, neutrophil recruitment, and activation of innate and adaptive immune responses [45].

A wide range of bacterial species are linked to mastitis in dairy cows, with varying pathogenicity and clinical impact. Classic pathogens such as *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Escherichia coli* remain the leading causes worldwide [40, 41, 46–49]. *S. aureus* is particularly adept at establishing chronic infections by adhering to mammary epithelial cells, invading intracellularly, and forming protective biofilms, enabling immune evasion and resistance to antimicrobial therapies [46]. *Strep. agalactiae* spreads rapidly within herds, driving the need for strict control programs [40]. In contrast, environmental Gram-negative bacteria like *E. coli* and *Klebsiella* spp. trigger strong inflammatory responses *via* lipopolysaccharide (LPS) recognition through Toll-like receptors [44, 47], which, while essential for pathogen clearance, can lead to excessive inflammation, tissue damage, fibrosis, and impaired milk production.

Mastitis induces significant changes in both systemic and local biochemical and hematological profiles. These changes are important not only for understanding disease mechanisms but also for diagnostic purposes. Hematologically, cows suffering from subclinical mastitis often exhibit a decrease in erythrocyte count, hemoglobin concentration, and packed cell volume (PCV), indicating systemic inflammation and associated anemia [42]. Leukocytosis, particularly neutrophilia, is commonly observed as a response to the infection.

Biochemically, mastitis affects the composition of milk. Elevated concentrations of sodium and chloride, coupled with reduced levels of potassium and calcium, are indicative of disrupted tight junctions between mammary epithelial cells [50, 51]. Moreover, mastitis reduces essential milk proteins such as α -lactalbumin and β -lactoglobulin, thereby impairing the nutritional and processing qualities of milk [51]. These biochemical alterations contribute significantly to the economic losses associated with mastitis.

Accurate and early diagnosis of mastitis is critical for effective disease management and prevention of economic losses. Traditional diagnostic methods include

physical examination for clinical signs and cow-side tests like the California Mastitis Test (CMT), which estimates the level of SCC [52]. Measurement of SCC remains the gold standard for detecting subclinical mastitis, with values exceeding 200,000 cells/mL typically indicating infection [53]. However, the identification of the causative pathogen is essential for targeted treatment and management. Bacteriological culture is the traditional method for pathogen identification, yet it is time-consuming. Newer molecular techniques, such as polymerase chain reaction (PCR) assays and MALDI-TOF mass spectrometry, have enhanced the speed and accuracy of diagnosis, facilitating the activity of dairy cows, especially in the context of decreasing human labor force [48, 49]. Genotyping of pathogens has provided deeper insights into mastitis epidemiology. Blum and Leitner showed that specific *Escherichia coli* strains associated with mastitis possess unique virulence factors, distinguishing them from environmental strains [54]. Moreover, understanding pathogen-specific virulence mechanisms, such as biofilm formation by *Staphylococcus aureus* [46], has important implications for developing effective treatment and prevention strategies.

Ali et al. reported the prevalence of *Staphylococcus aureus* at 60%, *Streptococcus agalactiae* at 20%, and *Escherichia coli* at 13.33% in subclinical mastitis cases among buffaloes (Table 5), a profile reflective of pathogen distribution in dairy cattle as well [55]. Emerging research also highlights the increasing significance of coagulase-negative staphylococci (CNS) in subclinical infections, particularly in primiparous cows and heifers [41].

Table 5 summarizes the prevalence of major bacterial pathogens isolated from cases of subclinical mastitis in buffaloes, along with their corresponding antibiotic sensitivity profiles. *Staphylococcus aureus* was the predominant pathogen, accounting for 60% of isolates, followed by *Streptococcus agalactiae* and *Escherichia coli*. High sensitivity rates were observed for ciprofloxacin and enrofloxacin, whereas traditional antibiotics such as penicillin showed lower efficacy. Although derived from buffalo populations, these findings provide valuable comparative insights, reflecting similar pathogen distributions and antimicrobial resistance patterns seen in bovine mastitis. The results reinforce the importance of ongoing surveillance and tailored antimicrobial therapy based on culture and sensitivity testing to combat antimicrobial resistance. Yet testing of culture and susceptibility at the individual cow level may not be beneficial. Petrovski et al. showed that mastitis pathogens vary in antimicrobial susceptibility, highlighting the need for herd monitoring [56]. Constable and Morin stressed basing treatment on culture and sensitivity tests to preserve drug efficacy and curb resistance [57].

The consequences of mastitis extend beyond milk production, significantly impacting the reproductive performance of dairy cows. Cows affected by clinical or subclinical mastitis during the early postpartum period exhibit prolonged calving

Pathogen	Prevalence (%)	Most effective antibiotics
<i>Staphylococcus aureus</i>	60.0%	Ciprofloxacin enrofloxacin
<i>Streptococcus agalactiae</i>	20.0%	Cefotaxime, cloxacillin
Other Streptococci	7.7%	Erythromycin
<i>Escherichia coli</i>	13.3%	Ciprofloxacin

Table 5.
Prevalence of bacterial pathogens and antimicrobial sensitivity in subclinical mastitis of buffaloes (Source: Ali et al. [55]).

intervals, increased services per conception, and higher rates of embryonic loss [58, 59]. Mastitis-induced systemic inflammation, characterized by elevated concentrations of cytokines such as TNF- α and IL-6, disrupts ovarian follicular development, interferes with luteal function, and impairs uterine health [3, 44]. Moreover, the physiological stress associated with mastitis negatively affects estrus expression and reduces fertility rates [3]. Dalanezi et al. demonstrated that cows experiencing clinical mastitis caused by Gram-negative bacteria within the first 60 days postpartum had significantly longer intervals from calving to conception compared to uninfected cows [58]. Thus, effective mastitis control is essential not only for preserving milk production but also for maintaining optimal reproductive performance and overall herd productivity.

The increasing prevalence of antimicrobial resistance (AMR) among mastitis-causing pathogens poses a serious challenge to effective disease management and public health [40, 48]. Concerns have been raised that misuse and overuse of antimicrobials, particularly for prophylactic purposes, *may* contribute to the emergence of resistant bacterial strains within dairy herds [49], although several studies report that decades of mastitis therapy have not significantly altered AMR profiles [60]. *Staphylococcus aureus* isolates are particularly notorious for exhibiting resistance to beta-lactam antibiotics (**Table 6**), including penicillin and ampicillin [62]. The biofilm-forming ability of *S. aureus* further complicates treatment by reducing antibiotic penetration and shielding bacteria from the host immune response [43]. Dagnaw et al. reported high rates of resistance among *S. aureus* isolates: 75% to penicillin, 67% to amoxicillin, 65% to ampicillin, and 40% to cefalexin [62]. These findings highlight the need for judicious antimicrobial use, guided by pathogen identification and susceptibility testing. These findings emphasize the necessity for continuous monitoring of resistance patterns and the prudent use of antimicrobials in mastitis therapy. The development of alternative therapeutic approaches, including the use of antimicrobial peptides, bacteriophage therapy, and herbal formulations, has been suggested as a way to combat AMR in mastitis pathogens [40]. Moreover, emerging alternatives to conventional antibiotics include the use of hydrogel-based delivery systems for antimicrobials, such as berberine hydrochloride-carboxymethyl chitosan hydrogel, which has shown efficacy against *Staphylococcus aureus* in mastitis models [43]. Sharun et al. highlighted the potential of phytotherapy, vaccination, and immunomodulatory therapies as adjuncts or alternatives to traditional antibiotic treatment [40]. Yet mastitis management should be concentrated on prevention, rather than treatment.

To address the dual challenges of effective mastitis control and antimicrobial stewardship, innovative therapeutic and management strategies have been developed. Selective dry cow therapy (SDCT), where only cows with a high risk of infection receive antimicrobial treatment at dry-off, has been demonstrated to significantly reduce antimicrobial use without compromising udder health [61, 63]. On-farm bacterial culture or PCR testing to guide mastitis treatment further enhances antimicrobial targeting and effectiveness (**Table 6**) [61].

Group	Cases treated (%)	Antibiotic usage (%)	Cure rate (%)
On-farm tested (culture/PCR)	70%	55%	78%
Standard treatment (no testing)	100%	100%	79%

Table 6.
Effect of on-farm testing on antibiotic usage in mastitis cases (Source: Griffioen et al. [61]).

Table 6 highlights the influence of on-farm diagnostic testing on antibiotic administration in mastitis cases. The data reveal that utilizing on-farm bacteriological culture or PCR methods led to a substantial reduction in antibiotic use (55%) compared to the standard protocol without testing, which treated 100% of cases. Despite the lower antibiotic exposure, the clinical cure rate remained comparable between the two groups, supporting the strategy of pathogen-directed therapy. This underscores the critical role of rapid diagnostic testing in promoting judicious antimicrobial use, preserving antibiotic efficacy, and aligning dairy management practices with principles of sustainable production and animal welfare.

Mastitis remains a multifaceted challenge for the dairy industry, with significant implications for milk production, reproductive efficiency, animal welfare, and economic sustainability. Its epidemiology is complex, involving interactions between pathogenic microorganisms, the host immune system, and environmental factors. Advancements in diagnostic technologies, understanding of pathogen biology, and development of innovative therapies offer new opportunities for mastitis control. Emphasis must be placed on prudent antimicrobial use, selective treatment strategies, and adoption of alternative therapeutic approaches to mitigate the threat of antimicrobial resistance [40, 43]. Future research should focus on the development of vaccines, immunomodulatory therapies, and novel diagnostics to further reduce mastitis incidence and promote sustainable dairy production systems. Additionally, as a priority, all efforts should be made to minimize the risk of mastitis incidence rather than relying on treatment. The prevention should be a multifaceted approach with optimized animal health, biosecurity (both bioexclusion and biocontainment), husbandry, and nutrition, paying particular attention to the hygiene and maintaining cows' standing in the perimilking periods.

6. Pathology in buffaloes

Renal pathology in buffaloes (*Bubalus bubalis*), particularly due to the often-subclinical progression of lesions and multifactorial etiology, represents a significant challenge to health and productivity. Recent histopathological investigations have highlighted the breadth of kidney-related diseases in buffaloes, ranging from nephrolithiasis-induced damage and tubulointerstitial nephritis to amyloidosis and hormonal dysregulation associated with chronic renal lesions. Yadav et al. reported that many buffalo kidneys had interstitial nephritis, glomerulonephritis, nephrosis, and amyloidosis [64], while Deshmukh et al. showed nephrolithiasis caused severe tubular damage and pyelonephritis [65]. These studies reveal buffalo renal disease ranges from inflammation to stone-related injury, demanding thorough diagnosis.

One major contributor to renal dysfunction is nephrolithiasis. A case-based investigation reported severe renal damage in two adult buffaloes associated with spontaneous kidney stone formation [65]. Postmortem findings revealed renal calculi, interstitial fibrosis, and glomerular atrophy, suggesting chronic obstruction-induced parenchymal loss. Histological evaluations indicated progressive fibrotic remodeling, accompanied by hyalinization and tubular necrosis. Nutritional mismanagement and excessive mineral intake were identified as contributing factors. This highlights the need for improved dietary control in buffalo herds.

In a broader cohort-based study, Shahabi et al. demonstrated significant hormonal alterations in buffaloes with histologically confirmed renal lesions [66]. Among 102 slaughtered animals, chronic tubulointerstitial nephritis was predominant. Serum

analysis revealed suppressed erythropoietin levels—explaining the concurrent anemia—and elevated parathormone concentrations, indicative of secondary renal hyperparathyroidism. These hormonal imbalances underscore the systemic impact of kidney dysfunction and its role in compromising buffalo productivity and overall health.

Amyloidosis, though less frequent, poses a considerable threat to buffalo health. A survey by Kumar et al. found amyloid deposition in 1.75% of kidney samples examined [67]. Affected organs displayed waxy consistency and histological evidence of glomerular and interstitial amyloid accumulation. Such deposition compromises glomerular filtration and induces chronic renal failure. The progressive nature of amyloidosis, confirmed by glomerular atrophy and proteinaceous casts, necessitates early diagnosis and appropriate herd-level management practices (nutrition management, environmental control, and regular veterinary monitoring). Renal amyloidosis in buffaloes, confirmed by glomerular atrophy and proteinaceous casts, requires herd-level strategies focused on early detection and management. Kumar et al. documented amyloid deposition in buffalo kidneys along with inflammatory lesions [67], while Salah et al. demonstrated elevated Serum Amyloid A levels in buffaloes with subclinical inflammatory disease [68]. Together, these findings support regular APP biomarker monitoring (particularly SAA) and prompt control of chronic inflammation to prevent progression of renal pathology in affected herds.

Further support for the prevalence of renal pathology is found in studies of lower urinary tract disorders. Yadav et al. identified lesions such as chronic cystitis, ureteritis, and urolithiasis in buffalo specimens [69]. Although primarily affecting the urinary tract, these conditions were often associated with renal histopathologies like interstitial nephritis and glomerulonephritis, particularly in chronic cases [69]. The transition from lower tract infections to kidney damage exemplifies the pathological continuum that occurs when urinary disorders remain unresolved or mismanaged.

Kidney disorders in buffaloes are multifactorial and often progressive. Lesions may arise from immune-mediated, infectious, metabolic, and obstructive processes, contributing to renal parenchymal degeneration and systemic dysfunction. Preventive strategies must include nutritional correction, responsible drug use, and early screening to preserve renal health and overall herd productivity (Table 7).

A recent comparative study has revealed that the incidence of kidney lesions detected at slaughterhouses is a significant indicator of the overall herd health status, with findings suggesting buffaloes and cattle exhibit similar susceptibilities [70].

Type of lesion	Main histopathological features	Possible causes	Reference
Nephrolithiasis	Tubular necrosis, glomerular atrophy, interstitial fibrosis	Nutritional imbalances, mineral excess	[65]
Tubulointerstitial nephritis	Interstitial inflammation, tubular necrosis, glomerular atrophy	Infections, toxins, drugs	[66]
Renal amyloidosis	Amyloid deposition in glomeruli and tubules, tubular atrophy	Metabolic disorders, chronic inflammatory diseases	[67]
Chronic cystitis	Mucosal thickening, epithelial hyperplasia, inflammatory infiltrates	Bacterial infections, mechanical irritation	[64]

Table 7.
Main types of urinary lesions in buffaloes and their possible causes.

7. Conclusions

The complex interplay between infectious disorders, locomotor, metabolic, and reproductive in high-producing dairy cows and buffaloes underscores the necessity for integrated herd health management strategies. Successful management must combine animal comfort, biosecurity, body condition monitoring, enhance animal welfare, environmental optimization, improve productivity, mitigate health risks, overall livestock management, precision nutrition, and proactive disease surveillance.

Advancements in diagnostic technologies, such as automated body condition scoring, MIR spectroscopy, and on-farm PCR testing, offer transformative potential in managing health challenges in high-yielding dairy herds. These tools enable early detection, individualized care, and judicious antimicrobial use, being a key to sustaining productivity while addressing the growing threat of antimicrobial resistance.

Buffaloes and dairy cows face species-specific and regionally amplified health burdens driven by environmental stressors, nutritional deficiencies, and inadequate veterinary support. Tailored disease prevention programs, genetic improvement, and region-specific interventions are critical to ensuring long-term viability and resilience of dairy systems in these climates.

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Section 4

Ancillary Diagnostics

Exploring Bovine Microbiome and Gut Parasites through Metagenomics: Educational Perspectives and Practical Strategies for Disease Resistance and Therapeutic Advancements

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Abstract

Cattle's alimentary system contains a varied microbiome that is necessary for digesting and immune function. Parasitic infections can upset this equilibrium, posing health and production issues. Metagenomics allows researchers to analyze microbial communities without culture, providing insights into the connections between intestinal parasites and the microbiota. This study looks into the influence of metagenomic research on bovine health, with a focus on disease resistance and therapy. Integrating metagenomics into veterinary and agricultural education enhances understanding of microbial ecology and viable animal management. Metagenomic research is indispensable for creating targeted antiparasitic medications, probiotic therapies, and nutritional recommendations to improve cow health and productivity. This study puts the latest research into practice, providing solutions for disease prevention and advancements in bovine health while also fostering equitable farming and the health of animals.

Keywords: community genomics, gut ecosystem, cattle pests, pathogen immunity, therapeutic breakthroughs

1. Introduction

The gut microbiome and parasite species significantly influence cow immunity, illness susceptibility, and digestion [1–4]. Metagenomics, which studies microbial diversity without culture, provides critical information regarding disease resistance and microbes' role in cattle health [5–8]. New studies have highlighted the gut microbiota's importance in immunological regulation, pathogen resistance, and food

metabolism [9]. Parasitic illnesses that alter this equilibrium can significantly reduce cow health and output [3, 10].

Combining metagenomic research into veterinary and agricultural curricula helps students understand microbial ecology and preventable diseases. Probiotics, feed modification, and the establishment of particular antiparasitic medicines are all important uses for enhancing livestock health. Metagenomics connects scientific research to practical cattle management, assisting disease prevention and advancements in bovine health.

This chapter will be used to assist veterinary medical and agricultural students and professionals in understanding metagenomics and its role in the health and productivity of cattle. Parasite metagenomics in cattle is discussed in detail.

1.1 Overview of bovine gut health

Cattle, like other ruminants, efficiently digest fibrous plant materials through microbial fermentation [11, 12]. The bovine gastrointestinal (GI) system is responsible for digestion, nutrient absorption, immune function, and overall health [13]. Understanding the composition and function of the bovine intestine is critical for improving animal health and performance [7].

1.1.1 Structure and function of the bovine digestive system

Cattle's stomach is divided into four chambers, the largest of which is the rumen [14]. It is specialized for the fermentation of fibrous plant material and contains a complex microbiome that ferments cellulose to create volatile fatty acids, which are cattle's principal energy source [14]. *The reticulum* functions alongside the rumen to capture larger particles and support the process of regurgitation necessary for further chewing during rumination [14]. *Omasum* filters particles to absorb nutrients and water needed for digestion and fermentation [14]. *The abomasum* functions as the true stomach, where enzymes break down proteins and other nutrients before they move to the intestines for absorption [14]. *The small intestine* assists digestion and is a major organ of nutrient absorption. *The large intestine* assists in water reabsorption and further fermentation of undigested material by microbes that, in ruminants, is of minor importance [14].

1.1.2 The role of the gut microbiome

The varied community of archaea, bacteria, fungi, protozoa, and viruses that make up the bovine gut microbiome aids in immunity, digestion, and metabolic control. The microbiome's primary roles include: (a) *Plant material fermentation*: Rumen microbes metabolize carbohydrates, proteins, fats, and other nutrients into volatile fatty acids (VFAs), thereby providing energy for the animal [15, 16]. (b) *Immunological modulation*: Helpful microorganisms aid in the management of immunological reactions and the prevention of infections [15, 17]. (c) *Nutrient synthesis*: Certain bacteria synthesize essential vitamins that facilitate metabolic processes, including vitamin K and B vitamins [15, 18]. (d) *Pathogen competition*: A robust microbiome diminishes disease risk by inhibiting the proliferation of harmful bacteria in the gut. In cattle, disturbances in microbial balance can lead to digestive issues and reduced productivity, often caused by antibiotic use, dietary changes, or diseases [15].

1.1.3 Common gut health challenges in cattle

Conditions that negatively impact bovine gut wellness: (a) *Contamination by parasites*: Gastrointestinal parasites, especially *Ostertagia ostertagi*, will instigate abomasal inflammation and malabsorption of nutrients, leading to weight loss. Other parasites and microbes, including *Cryptosporidium* spp., do this in cattle [19]. (b) *Improper diet*: Digestive upset, including acidosis and distension, may develop if there is a very quick alteration in the rations or too much concentrate [20]. (c) *Overuse of antibiotics*: Prolonged application of antibiotics may change the microbiome, resulting in lowering good bacteria and making the animals more susceptible to diseases [21]. (d) *Other environmental stressors*, high temperatures, poor housing, and stressful transportation, may also compromise immune and gut health [22].

1.1.4 Strategies for maintaining optimal gut health

Approaches applicable to livestock management for controlling digestive diseases and building a healthy gut community in cattle are: (a) *Balanced nutrition*: High fiber, essential nutrients, and probiotics foster microbial diversity along with the efficiency of digestion [7]. (b) *Pathogenic bacteria and viruses*: Their abundance is lowered by practicing routine deworming and adopting good hygiene. Additionally, supplementing food with good bacteria helps promote digestion and gut health [4]. (c) *Stress management*: Achieving gut integrity is possible by lowering environmental stresses and ensuring that the cattle have sufficient water [23].

1.2 Importance of studying gut microbiome and parasites

Knowledge of gut microbiome and worms helps in understanding and modifying digestion, health defense, immunity, and new treatments [24]. Good microbial population in the gut and worms matter for cattle health acutely and how diseases play out; linking them is key for progress in doctoring, vet care, and farming fields [25].

1.2.1 Role of the gut microbiome in health

The gut microbiome comprises archaea, bacteria, fungi, and viruses belonging to different communities which support physiological processes in the synthesis of vitamins and breaking down carbohydrates. The gut microbes also assist regulation of metabolism [26, 27]. (a) *Immune system modulation*: Beneficial gut microbiome helps strengthen the immune response to infections as well as inflammatory diseases [28]. (b) *Pathogen protection*: A good microbiome tilts the balance away from an ill gut microbiome and parasites, so disease does not occur. Imbalance can lead to greater vulnerability to infection, coupled with metabolic and digestive conditions [29].

1.2.2 Impact of gut parasites on health

Gut parasites, particularly protozoa and helminths, can greatly influence host health in the following ways: (a) *Nutrient competition*: Parasites consuming nutrients lead to malnutrition and deficiency in the host [30]. (b) *Illnesses caused by parasites*: Infections by parasites can upset normal microbial balance in the gut. They also cause inflammation that affects nutrient absorption and utilization [30]. (c) *Change in microbial diversity*: To improve their survival chances, many parasites change the

microbiome and thus affect the digestion and fermentation, as well as the immunity of the host [4].

Developing efficient parasite control methods and enhancing general gut health are made easier with an understanding of these connections [4]. *Interactions between microbiome and parasites*: The relationship between gut microbiome and parasites is intricate and mutually reinforcing. *The microbiota's effect on parasites*: While some gut microbiomes may help parasites survive, others may prevent them from colonizing [31]. *Parasite-induced microbial alterations*: Parasitic infections that change the variety of microorganisms can cause secondary infections and dysbiosis [31]. *Host immune response*: The microbiome promotes disease progression by altering immune responses to parasitic infections [31].

Investigating these linkages can help one better understand the causes of illness and potential treatment options. *Applications in veterinary and medical sciences*: Metagenomics has changed microbiome research by allowing for exact investigation of microbial populations and parasite interactions [32]. One of the most significant consequences of gut microbiome and parasite research is the discovery of microbial markers for early illness detection and intervention [33]. *Therapeutic innovations*: Developing probiotic- and microbiome-targeted therapies; and *Sustainable livestock management*: improving cow health through parasite control and microbiome-based feeding approaches [33] are possible when metagenomic knowledge is utilized.

1.3 Role of metagenomics in livestock research

Metagenomics has transformed the study of livestock microbial communities, providing insights into disease resistance, gut health, and productivity enhancement [32]. By directly examining genetic material from ambient samples, metagenomics allows researchers to explore microbial diversity without the need for culture, leading to considerable advances in veterinary and animal husbandry research [6].

1.3.1 Understanding metagenomics in livestock

The metagenomics technique involves sequencing and analyzing microbial DNA from livestock's stomach, skin, and respiratory system [6]. This approach helps identify both beneficial and harmful gut microbiomes, as well as their interactions with the host [6]. Key advantages include: (a) *Comprehensive profiling*: Discovery of unknown microbial species. (b) *Functional insights*: Understanding gut microbiome's roles in immunity, digestion, and illness prevention [9]. (c) *Non-culture-based analysis*: Bypasses traditional microbiology limitations by analyzing gut microbiome directly from samples, e.g., viable but non-culturable (VBNC) microbes [34].

1.3.2 Applications in livestock health and disease management

Metagenomics is vital for livestock health as it identifies emerging pathogens and changes in microbial communities linked to infections. It monitors antibiotic resistance, uncovers genes associated with this resistance, and helps develop probiotics. Additionally, it identifies beneficial microbes like yeast that support gut health and immunity, while also exploring how parasites affect microbial composition and disease susceptibility [6, 35].

1.3.3 Enhancing nutritional strategies through metagenomics

The makeup of the gut microbiota is strongly linked to animal nutrition [2]. Metagenomic research can assist in optimizing feed compositions by finding microorganisms that improve nutrition absorption and fiber breakdown [2].

1.3.4 Cutting down on methane emissions

Investigating methane-producing microbial activities may assist in the production of environmentally acceptable feeding techniques [36].

1.3.5 Personalized/livestock individual-based nutrition

Furthermore, metagenomics may allow for personalized nutrition. Personalized nutrition is the process of designing diets based on each individual's unique microbiome profile in order to enhance productivity and health [36].

1.3.6 Advancements in livestock genetics and breeding

Metagenomics enhances efforts for genetic improvement by uncovering microbial indicators for disease resistance and selecting calves with microbiomes that promote immunity [6]. Striving to improve reproductive health, metagenomics may assist by investigating how microbes influence fertility and reproductive success [6]. Understanding how microorganisms affect feed efficiency and weight growth is crucial for achieving peak development and performance [37–39]. Therefore, examining data from the bovine microbiome can help farmers and researchers understand more about microbial changes that indicate sickness risks before clinical signs appear.

1.4 Objectives and scope of the review

The fundamental goal of this review was to use metagenomic approaches to investigate the interactions between the microbiome and bovine intestinal parasites. The review aimed to: (1) examine the composition of the bovine gut microbiome to identify significant bacterial species and their functions in immunity, disease, resistance, and digestion; (2) investigate the impact of intestinal parasites on microbial ecosystems, examine how parasite infections impact host health and alter microbial diversity; (3) evaluate disease resistance mechanisms: Learn how the microbiome's makeup helps cattle fend off illnesses; (4) develop therapeutic strategies: Use metagenomic knowledge to develop probiotic-based remedies and antiparasitic measures that target the microbiome; and (5) Improve educational integration: Encourage the use of metagenomics in agriculture and veterinary sciences, aiming to improve livestock management techniques.

1.4.1 Scope of the review

The review covers a wide range of topics related to the health of the bovine gut, including metagenomic approaches that use sequencing technologies to evaluate microbial communities. (a) Pathogenic infections, their effects in identifying common intestinal parasites, and their impact on microbial balance; (b) Microbial diversity and functioning in assessing bacterial, fungal, and viral communities in

the bovine gut; (c) Disease prevention and treatment: Examining microbiome-based ways to improve cow health. (d) Applications for education and practice: These include studies into cattle management and veterinary education.

The project's goal was to bridge the gap between scientific research and practical implementations, promoting long-term cattle productivity and health.

2. Understanding bovine gut microbiome

2.1 Composition and diversity of gut microorganisms

Gut microbiota composition and diversity vary across animals, individuals, and habitats, influencing metabolic processes and disease risk [3, 40]. *Major microbial groups in the gut:* The gut microbiome is made up of numerous essential microbial phyla, each serving different physiological processes [41]. (a) *Firmicutes*: Helps break down complex carbohydrates and contributes to energy extraction and efficiency [42]. Bacteroidetes play a vital role in polysaccharide digestion and fiber metabolism [43]. (b) *Actinobacteria*: Essential for immune control and vitamin production [44]. (c) *Proteobacteria*: Cause a range of diseases, though they are frequently found in trace levels [45]. (d) *Verrucomicrobia*: Promotes mucosal integrity and intestinal health. (e) *Archaea*: Methanogens, involved in fermentation and production of methane. (f) *Fungi*: Contribute to fiber degradation. (g) *Protozoa*: Play a role in the degradation of fiber and complex carbohydrates.

2.2 Factors affecting microbial balance in bovine

For bovine species, microbial equilibrium is essential to their general well-being, output, and ability to reproduce. The microbial composition of the bovine body varies based on several factors, particularly in the reproductive and gastrointestinal systems [45]. Throughout life, the bovine microbiome changes, affecting disease resistance, digestion, and reproduction. Understanding these factors is critical for maintaining microbial balance and improving cow productivity and health. Continuous research is carried out looking for ways to change the microbiome and promote fertility and overall health [7].

(a) *Diet and nutrition in bovine*: Diet serves an essential part in determining the bovine microbiome. While unnecessary grain feeding may lead to acidosis, which perturbs microbial homeostasis, high-fiber diets encourage microbial selection. Proper diet ensures a stable microbial habitat [46]. (b) *Environmental conditions*: Microbial populations can shift as a result of exposure to environmental pressures. Environmental stresses such as pathogen exposure and poor housing conditions promote microbial imbalances [6]. (c) *Reproductive health*: The microbiota in the reproductive system determines bovine fertility [47]. Reproductive tract dysbiosis has been linked to infertility issues, particularly recurrent breeding syndrome (RBS). Microbial alterations during estrus, artificial insemination, and the postpartum period can all have an impact on reproductive success [45]. (d) *Medical interventions*: While antibiotics are necessary for treating infections, their use has a significant impact on microbial diversity. Antibiotic usage can cause resistance and chronic dysbiosis [48]. (e) *Genetic and physiological factors*: An individual's microbial composition is impacted by both genetic and physiological factors, such as age and immune system performance.

2.3 Methods for analyzing the gut microbiome of bovine species

A wide range of techniques is used to examine and characterize the microbial populations of the bovine gut, from traditional culture-based methods to cutting-edge molecular and bioinformatics approaches.

(a) *Culture-based techniques*: Conventional methods involve growing bacteria from cow intestinal samples on selective media. While beneficial for identifying specific microbes, since many gut bacteria are anaerobic and difficult to cultivate, these techniques generally fall short [49]. (b) *16S rRNA gene sequencing*: This technique classifies bacterial populations according to genetic sequences, suggesting microbial assortment [50]. (c) *Metagenomics*: This method evaluates the complete genetic content in bovine gut samples, revealing information about microbial connections and functions [51]. (d) *Shotgun sequencing*: A high-throughput technology that sequences all DNA fragments in a sample, allowing for a thorough investigation of microbial metabolic genes [52]. (e) *Metatranscriptomics*: Study the RNA transcripts to better understand microbial gene expression and response to environmental and nutritional changes [53]. (f) *Proteomics and metabolomics* studies microbial proteins and metabolites, emphasizing their importance in cow health and nutrition [54]. (g) *Bioinformatics and computational analysis* uses sequencing data to identify microbial species, predict metabolic pathways, and evaluate relationships, all of which are critical for creating medicines and improving cattle health [55].

3. Bovine gut parasites: Types and impacts

3.1 Classification of common gut parasites in cattle

Cattle's health, growth, and productivity are heavily influenced by gut parasites, including nematodes, trematodes, cestodes, and protozoa. Awareness of these parasites is essential for cattle health and productivity [56]. To reduce the effects of intestinal parasites in cattle, commonly used strategies include (a) regular deworming using suitable anthelmintics, (b) appropriate hygiene and sanitation in areas used for feeding and keeping cattle, (c) rotational grazing to lessen exposure to parasites, (d) ensuring sources of water are uncontaminated, (e) monitoring affected cattle, (f) maintenance of refugia, (g) use of natural parasitocides, and (h) prompt treatment as required [56–59].

Nematodes, or roundworms, affect digestion and nutrition [60]. Examples of internal nematodes include: (a) *Ostertagia ostertagi*: deters abomasal function; (b) *Cooperia spp.*: causes weight loss and diarrhea; (c) *Haemonchus spp.*: leads to anemia by feeding on blood [61]; and (d) *Trichostrongylus species*: results in weakness and poor appetite [57].

Trematodes (Flukes): Flatworms that primarily affect the intestines and liver, including: (a) *Fasciola hepatica* (Liver fluke): reduces feed efficiency; (b) *Paramphistomum spp.* (Rumen flukes): causes diarrhea and dehydration [58].

Cestodes (Tapeworms): These parasites interfere with nutrient absorption. Notable cestodes include: (a) *Moniezia spp.*: inhibits growth; (b) *Taenia saginata* (Cattle tapeworm): transmitted to humans through undercooked meat [62]; (c) *Echinococcus spp.*: causes harmful hydatid cysts [59].

Protozoa: Microscopic organisms causing serious intestinal problems, including: (a) *Cryptosporidium spp.*: leads to diarrhea in calves, (b) *Eimeria spp.* (Coccidia): causes severe diarrhea and dehydration, and (c) *Giardia spp.*: disrupts digestion and nutrient absorption [63].

3.2 Effects of gut parasites on microbial communities in bovines

Bovine microbial ecosystems are heavily influenced by gut parasites. These interactions can lead to dysbiosis, altering the balance between beneficial and harmful bacteria [62]. (a) *Disruption of microbial diversity*: Parasitic diseases can reduce microbial diversity by promoting harmful bacteria at the expense of beneficial ones, as seen with helminth infections reducing microbial richness in species [64]. (b) *Alteration of metabolic pathways*: Gut parasites inhibit nutrient fermentation and absorption. For example, *Ostertagia ostertagi* affects the rumen microbiota, influencing the generation of important volatile fatty acids [65]. (c) *Immune regulation and inflammation*: Parasitic infections boost inflammatory cytokines, upsetting microbial equilibrium and genetic material in microorganisms [66]. (d) *Competition for nutrients*: Parasites compete with beneficial intestinal microorganisms for nutrients, potentially reducing key bacteria like *Ruminococcus* and *Prevotella*, which aid in fiber digestion [67–71]. (e) *Potential for secondary infections*: Cattle with dysbiosis from parasites may also be more vulnerable to other bacterial infections, worsening gastrointestinal issues [72].

4. Metagenomics as a tool for understanding gut health

4.1 Principles and methodology of metagenomics

Microbial communities can be investigated directly in environmental samples using metagenomics, a powerful genomic approach that eliminates the need for culturing. It provides insights into ecological linkages, functional potential, and microbial diversity [73, 74]. (a) *Culture-independent analysis*: By directly studying genetic material from samples, metagenomics overcomes the limitations of classical microbiology, which relies on culturing bacteria [75]. (b) *Whole-Community genomic profiling*: By analyzing the collective genomes of microbial communities, relationships and functions can be studied [76]. (c) *High-throughput sequencing*: Advancements in sequencing technology, such as next-generation sequencing (NGS), enable rapid and cost-effective microbial DNA study. (d) *Bioinformatics and computational analysis*: Metagenomic data requires advanced computational approaches for sequence assembly, annotation, and functional prediction [55].

4.1.1 Methodology of metagenomics

Metagenomics methodology includes the following steps: (a) *Sample collection and DNA extraction*: Microbial DNA is extracted from environmental components (soil, water, and gut microbiota) using particular protocols [74]. (b) *Library preparation and sequencing*: DNA is fragmented and prepared for sequencing using platforms such as Oxford Nanopore, PacBio, and Illumina [77]. (c) *Sequence assembly and binning*: Following the assembly of raw sequencing reads into contigs, sequences are classified into taxonomic groups using binning algorithms [78]. Functional annotation and pathway analysis use bioinformatics tools to identify genes, metabolic pathways, and potential roles in microbial communities [79]. (d) *Statistical and comparative analysis*: Metagenomic data is analyzed using statistical techniques to compare microbial diversity in different ecosystems [80].

5. Educational perspectives: Integrating metagenomics in veterinary sciences

5.1 Importance of microbiome research in veterinary education

The knowledge that the microbiome considerably aids cattle's immune systems and physiological homeostasis has been generated. Understanding these microbial populations can aid in disease management, nutrition, and veterinary therapy [26, 27].

5.1.1 The microbiome's role in animal health

The gut microbiota regulates the (a) immune system, (b) absorbs nutrients, and (c) aids digestion. As already discussed, it promotes the creation of VFAs, which are required for metabolic health, the breakdown of carbohydrates, and vitamin synthesis. A balanced microbiome also helps to prevent infections by modulating immune responses and competing with pathogenic bacteria [81]. Dysbiosis, or microbiome asymmetry, has been linked to a number of illnesses, including digestive issues and metabolic malfunctions. Changes in gut flora may be associated with chronic renal disease and inflammatory disorders. Identifying these changes enables veterinarians to create personalized strategies that restore microbial balance and improve animal health [82].

5.1.2 Advances in veterinary diagnostics and treatment

Technologies such as metagenomics and next-generation sequencing have transformed microbiome research, enabling detailed characterization of microbial communities. This enables veterinarians to more effectively detect diseases and personalize treatments to each animal's microbiota. Diet has a significant impact on the microbiota, influencing microbial diversity, digestion, and overall health. With a better understanding of these interactions, veterinarians may recommend diets that foster a healthy microbiome, improving animal welfare and production [2].

5.2 Training students in metagenomic techniques

Metagenomic training is important because it allows students to investigate microbial communities, interpret sequencing data, and apply results in biotechnology, agriculture, and medicine, thereby offering insights into microbial diversity and ecosystem interactions. Proficiency in metagenomic techniques is critical for ecological surveillance, therapeutic applications, and research into the microbiome [6, 7]. This review highlights key components of metagenomic training, including sequencing technology, experimental design, bioinformatics analysis, and practical applications.

Experimental design and sample preparation: Students should understand how to design studies using relevant sample types (soil, water, gut microbiome) and microbial DNA extraction procedures. The training should cover DNA extraction techniques, quality control for high-quality sequencing data, and library preparation for next-generation sequencing (NGS).

5.2.1 Sequencing technologies

Knowing the sequencing platforms is crucial. Training should include shotgun metagenomics for community analysis, amplicon sequencing (e.g., 16S rRNA) for taxonomic profiling, and third-generation sequencing (e.g., Nanopore, PacBio) for long-read sequencing. By understanding these domains, students will be well-prepared to progress the discipline of metagenomics [39].

5.2.2 Bioinformatics analysis and data interpretation

To process and analyze metagenomic data, students must be proficient in bioinformatics tools such as FastQC for quality control, QIIME2 and Kraken for taxonomic classification, KEGG and MetaCyc for functional annotation, and Statistical Analysis for Microbial Diversity and Comparative Studies [39].

5.2.3 Practical applications and hands-on training

Practical experience is required for proficiency in metagenomic methods [83]. Authors of this chapter held the opinion that veterinary curricula should include workshops on sequencing methods and bioinformatics pipelines, case studies involving microbiome research and environmental metagenomics, and collaborative initiatives using metagenomic approaches in real-world contexts.

5.3 Case studies and practical applications in academics

To bridge the gap between theoretical knowledge and practical experience, case studies and practical applications are critical components of academic learning. They promote critical thinking, problem-solving skills, and facilitate the creation of metacognitive ability to apply concepts across other disciplines [84].

5.3.1 Practical applications in education

Practical applications include hands-on activities that promote learning. The authors' opinion is that the following activities may contribute to teaching and learning metagenomics: (a) *Internships and fieldwork*: give students industrial experience and professional skills. Laboratory experiments are necessary for scientific and engineering students to test theories. (b) *Peer review and collaborative learning*: encourages students to provide constructive feedback and improve their work [85].

5.4 Challenges in veterinary education related to the introduction of teaching and learning metagenomics

The current veterinary education has a number of challenges related to the introduction of metagenomics in the curricula: (a) *Curriculum adaptation*: Traditional courses struggle to accommodate emerging concepts like precision medicine and artificial intelligence. (b) *Faculty shortages*: A lack of skilled educators has an impact on the quality of instruction. (c) *Economic constraints*: Rising tuition and insufficient funding make advanced education less accessible. (d) *Scientific collaboration*: Many universities lack the necessary infrastructure and networks to handle contemporary digital learning tools. (e) *Inclusive partnership*: Addressing concerns such as zoonotic

illnesses necessitates international collaboration, although many programs are still concentrated exclusively on animal health [85].

6. Practical strategies for livestock management

6.1 Implementing metagenomic insights in cattle farming

Applying metagenomic perspectives in cattle farming could enable (a) targeted treatments for disease control, (b) feed effectiveness enhancement, and (c) conservation of the environment. To optimize cow husbandry [86], farmers and veterinarians, by sequencing and studying the genetic content of bacteria in the cattle gut microbiome, can make data-driven decisions. Yet, this opportunity is not widely utilized in practice.

6.2 Applications of metagenomics in cattle farming

Improving gut health and digestion can be achieved by characterizing the rumen microbiome and identifying phyla responsible for fiber degradation and VFA synthesis may aid the development of appropriate diets. Farmers may optimize feed usage, improve the health of their cattle, and increase output results by putting precision nutrition ideas into practice [87]. The key nutritional strategies leading to improved gut health are discussed below in a separate heading.

Precision nutrition strategy: Feed formulations that are tailored to rumen microbial profiles have the potential to improve nutrient absorption while reducing releases of methane [88].

Probiotic selection: By using metagenomic data to improve gut health and avert dysbiosis, farmers, nutritionists, and veterinarians can introduce helpful bacteria [6].

Disease prevention and diagnostics—early pathogen detection: Figuring out switches in the number of microbial phyla may indicate the beginning of diseases like mastitis or bovine respiratory disease [89].

Antimicrobial resistance monitoring: Metagenomic oversight assists in monitoring the development of resistant bacterial strains. The knowledge of the prevalence of these strains hopefully will encourage substitute methods for treatment. Comprehending how cattle's immune responses are modulated by their microbiota allows for unique veterinary care [6].

Improving productivity and reproduction—microbial influence on development rates: To improve weight gain and feeding efficiency, assessing microbiota's effects on metabolism enables tailored management strategies. *Environmental and sustainability benefits—methane emission reduction:* Modifying the composition of the rumen microbiota helps lower methane-producing archaea, thereby reducing greenhouse gas emissions. *Waste management:* Understanding manure microbiota supports better composting and nutrient recycling strategies [6].

6.3 Key precision nutrition strategies for gut health optimization

Fiber management for rumen stability—balanced fiber intake: Optimal ratios of structural (indigestible) and fermentable fiber ensure proper rumen fermentation and prevent disorders like acidosis. *Effective fiber sources:* Including high-quality forages (alfalfa, clover, silage) and strategic fiber supplementation may improve microbial diversity.

Probiotic and prebiotic supplementation—*probiotics*: Live beneficial microbes (e.g., *Lactobacillus*, *Saccharomyces cerevisiae*) enhance gut resilience and microbial homeostasis in ruminants. *Prebiotics*: Compounds such as oligosaccharides, beta-glucans, and mannan selectively promote beneficial microbial phyla [46].

Precision feeding with functional ingredients—*yeast culture*: Enhances microbial fermentation, improving VFA production and fiber degradation. *Essential fatty acids*: Omega-3 and conjugated linoleic acid modulate inflammation and support gut barrier integrity. *Phytogenic compounds*: Bioactive plant extracts (e.g., tannins, flavonoids) regulate microbial populations and reduce pathogenic load [6].

Protein optimization for microbial balance—*rumen-bypass proteins*: Prevent excessive ammonia release, reducing nitrogen overload that can disrupt microbial equilibrium. *Amino acid precision feeding*: Supplements such as methionine and lysine enhance microbial metabolism and immune function [3, 10]. Additional complication in the amino acid feeding is rumen microbial fermentation. Hence, these should be rumen-protected.

Mineral and vitamin—*tailoring*—*zinc and copper*: Essential cofactors for microbial enzyme function and gut epithelial integrity. *Vitamins A, D, and E*: Support cellular repair mechanisms and immune defense [90].

Mitigating gut inflammation—*dietary antioxidants*: Polyphenols, carotenoids, and selenium reduce oxidative stress. *Controlled starch intake*: Excessive starch fermentation can trigger metabolic disorders; precision nutrition optimizes carbohydrate sources.

Technological advances in precision nutrition: Emerging technologies enhance precision feeding programs: (a) *Metagenomic sequencing*: Identifies microbiome profiles for tailored dietary adjustments. (b) *AI and predictive analytics*: Machine learning models optimize nutritional formulations. (c) *Automated feed monitoring systems*: Smart feeders adjust dietary intake in real time [88].

7. Conclusion

This review addresses cattle gut microbiome using metagenomics that can provide significant information about cattle's microbial ecosystems. This review discussed how internal parasites influence host health, illness resistance, and production. Veterinary professionals and students can use metagenomic approaches to study microbial biodiversity and detect prospective hazards, resulting in more effective bovine health management practices.

Metagenomics in veterinary medicine raises awareness of host-microbiome connections, offering students and professionals new approaches for illness detection and mitigation. Organized curriculum and hands-on research experiences allow students to apply what they have learned, perhaps changing the way they handle livestock and animal health.

By identifying beneficial bacteria that boost immunity and reduce parasite infections, scientists can develop probiotics and personalized nutrition plans to promote gut health. Early detection of infections allows farmers to take preventive measures, reducing disease outbreaks and increasing herd performance. Metagenomics offers transformative disease resistance and therapy solutions.

The use of metagenomic tools to research cow gut parasites and microbiomes has proven to be an effective technique for disease education and management. Collaboration among veterinarians, microbiologists, and agricultural professionals is

required for converting study findings into innovative solutions to revitalize animal production. Persistent financing for this research can improve animal welfare, effectiveness, and food security while lowering reliance on antimicrobial medications that are out of date.

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Conflict of interest

The authors declare no conflict of interest.

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Flow Cytometry Standardization and Validation for Immunohematology in Cattle

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Abstract

Flow cytometry has become the method of choice for studying the immune system and its response in mammals, including cattle. The development of a broad panel of precise monoclonal antibodies (MAb) to bovine leukocyte differentiation molecules now enables a detailed characterization of the immune response in cattle. However, before extending these studies, it is essential to ensure the consistency of laboratory results. Currently, indirect labeling is used in single and multiparameter analysis. This technique introduces a probability of experimental error due to the multiple steps involved in processing peripheral blood mononuclear cells (PBMC). The objective of the chapter is to describe the development and testing strategy for ensuring the reliability of dual-immunolabeling methods for PBMC. We detail the experiments used to define optimal secondary antibody dilutions; set up autofluorescence, nonspecific binding, and gating controls, implement gating strategies, and measure intra-laboratory consistency. Our results show that variation between paired samples was very low, confirming that reliable data can be obtained with careful sample preparation. We propose that incorporating statistical comparisons of repeated immunolabeling of the same samples using the same MAb can serve as an effective strategy for controlling experimental error in routine dual indirect flow cytometry. Defining controls and dilutions, measuring the variation between laboratory results, and controlling experimental error are key steps in the validation process.

Keywords: cattle, flow cytometry, immune response characterization, controls, assay validation

1. Introduction

The immune system is a highly integrated network of diverse cell types, numerous cytokines, and specific plasma proteins that act synergistically to eliminate infectious

agents, parasites, and harmful antigens. Once an invading organism is detected, this system responds immediately with innate immunity (nonspecific immunity), which lacks any form of memory, and in a similar manner and time frame to a repeated challenge. It also responds with acquired immunity (specific immunity or adaptive immunity), a more recent evolutionary development distinguished by its specificity for an invading organism and its ability to remember an encounter [1–3].

In immunology, it has been possible to generate knowledge about the functional characterization of the immune system thanks to two closely related breakthroughs. The first, conceived in the late 1960s and emerging in the early 1970s, consisted of a device that allowed the characterization of cells by fluorescence emission, called a flow cytometer. The second, developed in the mid-1970s and refined by the early 1980s, is the MAb, a reagent that significantly improved the precision of cytometric analyses [4].

In the mid-1980s, the field of bovine immunology directed its attention toward the functional characterization of cattle leukocyte populations, particularly the different T cell subpopulations and their receptors. Around 1990, research expanded to include cytokines, the signaling mediators of the immune response. Protein sequencing, MAb development for cytokine detection, and quantitative bioassays followed [5, 6]. In cattle, FC has been used: for counting total leukocytes in blood, in the study of neutrophil adhesion receptors, in the analysis of the immune response to pathogens, for evaluating vaccine effectiveness, and in the diagnosis of theileriosis [7]. In the analysis of immune responses to pathogens affecting *Bovidae* and humans, as well as in the evaluation of vaccine effectiveness, advances have been made possible by the use of proven MAb panels applied to tissue culture platforms, which enable the comparative study of the immune response to zoonotic mycobacterial pathogens and candidate vaccines [8, 9].

Despite these advancements, the gap between veterinary and biomedical flow cytometry remains substantial [10]. Veterinary flow cytometrists, whether working in diagnostics or research, face numerous challenges [11]. A major limitation in veterinary applications is the limited availability of antibody-fluorochrome conjugates [7]. To face this limitation, veterinary flow cytometrists can perform indirect immunostaining with secondary conjugated Ab of primary immunolabeled cells with MAb. This restricts the characterization of immune cell subpopulations and, as multiple samples per individual are needed to analyze different markers, increases processing time. These introduce the likelihood of experimental error, which requires rigorous control, because of the multiple processing steps.

To effectively mitigate these challenges, it is essential to perform rigorous validation of all assays conducted within the flow cytometry laboratory, regardless of whether the objective is clinical diagnostics or scientific research. The validation process must systematically address several critical considerations, including: reproducibility of flow cytometry experiments under consistent conditions, replicability of published data, reliability of generated data, flow cytometer calibration, robustness of the compensation strategy, the use of controls to define the target cell populations accurately, the statistical analyses applied, and the development and implementation of standard operating procedures and the establishment of best practices to select antibodies and controls, their titration protocols, and panel configurations [12].

The evaluation of laboratory performance is grounded in fundamental criteria, including accuracy, trueness, and precision. Accuracy refers to the closeness of agreement between a quantity value obtained by measurement and the true value of the measurand [12–14]. Trueness is the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value [12–14].

Precision describes the dispersion of replicate measurements, assessed intra-assay (“repeatability”) or inter-assay (interlaboratory: “reproducibility”). Precision is quantified using standard deviation and coefficient of variation (CV) [12–14].

This chapter describes the strategy we developed to achieve repeatability and standardization in flow cytometry experiments aimed at characterizing the immune response in cattle using indirect dual immunostaining.

2. Cattle PBMC indirect immunolabeling for flow cytometry

The indirect immunolabeling of the PBMC was performed to reveal the presence of CD4 and CD8 lymphocyte differentiation molecules expressed externally on the cell membrane. These molecules were primarily targeted using CACT118A and BAQ 111A MAb, ascites fluid mouse-produced immunoglobulins of the IgG1 and IgM isotypes (Monoclonal Antibody Center, Washington State University), respectively. Since the MAb were IgG1 and IgM mouse-produced, the antibodies needed for the secondary immunostaining were commercial goat anti-mouse antibodies. The anti-IgG1 antibody was phycoerythrin (PE)-linked, and the anti-IgM was fluorescein-linked (FITC). Fluorescence signals emitted by PBMC were acquired using a flow cytometer (BD FACS CANTO, Franklin Lakes, USA). The parameters analyzed were relative size (forward scatter or FSC), relative granularity (side scatter or SSC), and the relative intensity of the fluorescence emitted by the fluorochromes PE and FITC.

The immunolabeling protocol was provided by Dr. William Davis, the Director of the Monoclonal Antibody Center (Veterinary College, Washington State University, Pullman, USA). The protocol included controls for autofluorescence and nonspecific binding to secondary Ab.

2.1 Step-by-step protocol

2.1.1 Isolation of PBMC

Cattle PBMCs were isolated from noncoagulated blood by gradient centrifugation. After the morning milking session, Holstein dairy cows were jugular venipunctured, and the noncoagulant agent ethylenediaminetetraacetic acid (EDTA) was used. Restraint was made according to welfare guidelines using a nontraumatic halter.

Collected blood was diluted in phosphate buffered saline solution (PBS) (1:3) and layered onto a commercial density gradient (polysucrose and sodium diatrizoate, adjusted to 1077 g/mL). Diluted blood was centrifuged at 400 g for 30 minutes at room temperature with a swinging bucket rotor without a brake. PBMC were collected from the buffy coat, washed several times in PBS, and Trypan blue vital staining assessed viability. PBMC were resuspended in the first wash buffer solution at a 1×10^6 PBMC/mL concentration [15].

2.1.2 First wash buffer, second wash buffer, and fixation buffer

Three working solutions were used for the immunolabeling process: First wash buffer, second wash buffer, and fixation buffer. All solutions were prepared with sterile PBS adjusted to a final pH of 7.2–7.4, the most important proportional component.

The first wash buffer included 5 mL of a 20% sodium-azide solution in PBS, 50 mL of acid–citrate–dextrose anticoagulant solution, and 10 mL of horse serum to block nonspecific binding of secondary antibodies.

The second wash buffer had the same composition as the first, except that horse serum was excluded. The fixation buffer was prepared with 25 mL of 37% formaldehyde diluted in 475 mL PBS.

2.1.3 MAb preparation

For the primary immunolabeling, MAb were prepared in the first wash buffer at a working concentration of 15 µg/mL. Stock solutions were filtered through a 0.22 µm Millipore filter to remove particulates and kept at 4°C. MAb dose and storage conditions were those recommended by the Monoclonal Antibody Center, Veterinary College, Washington State University.

2.1.4 Secondary Ab solution

For the immunostaining, secondary antibodies anti-IgG PE-linked and anti-IgM FITC-linked were reconstituted with 1 mL and 0.75 mL of distilled water, respectively. Aliquots were stored at 4°C until used. The manufacturer recommended three dilution ratios: 1:50, 1:100, and 1:200. Optimal working dilutions were determined through titration experiments.

2.1.5 Dual color surface indirect immunolabeling protocol

To preserve cell viability and antibody integrity, throughout the entire procedure, all solutions, tubes, and the centrifuge were kept on ice at 4°C. Also, the tubes identifications with the antibody or solution placement were recorded on a grid sheet.

One hundred µL of PBMC were transferred into 1 mL volume conical-bottom polypropylene tubes. Using the grid sheet mentioned before as a guide, the primary MAb Anti-CD4 (CCAT118A) and MAb anti-CD8 (BAQ111A) were added and homogenized by gentle micropipetting.

Tubes were incubated for 15 minutes in the dark under refrigeration. After incubation, the tubes were centrifuged for 3 minutes at 2000 RPM at 4°C, and the supernatants were removed.

Then, three sequential washes were performed, adding 200 µL of the first wash solution to the pellets. The pellets were loosened by gentle micropipetting, and it was checked that PBMC were evenly dispersed.

After the last wash, the secondary antibody was added, and the pellets were resuspended by gentle micropipetting. Tubes were incubated for 15 minutes in the dark under refrigeration. After two washes were performed using the second wash buffer, following the specifications described for the primary immunostaining washes.

Secondary dual immunolabeled PBMC were fixed with 200 µL of the fixation solution. Samples were transferred to 12x75 mm polystyrene tubes with slotted-neck caps specifically designed for the flow cytometer.

Finally, the tubes were sealed with paraffin-coated cling film, wrapped in aluminum foil, and stored at 4°C until cytometer acquisition. The cells stored under the conditions described may be examined immediately or after a delay of up to 2 weeks.

3. Controls, secondary Ab titration, and gating strategies for secondary Ab titration

Critical aspects of establishing a standardized and reliable flow cytometry protocol were the implementation of appropriate controls and consistent gating strategies. These ensured the accurate identification of cell populations.

3.1 Controls and secondary Ab titration experiment

3.1.1 Experiment design

In this experiment, we evaluated the controls to perform the cytometer compensation, the controls that determined the absence of nonspecific binding during the immunostaining reaction [16], and the three dilutions of secondary antibodies recommended by the manufacturer.

CD4 and CD8 subpopulations were selected as controls because reference values obtained with MAb CACT118A and MAb BAQ111A were available for cattle in the literature [6]. CD4 and CD8 subpopulations also allowed easy distinction between fluorescent and nonfluorescent events due to their high quantity [17].

Following the protocol described before (title 2.1.1.), PBMC were isolated from noncoagulated blood. The blood was venipunctured from a healthy donor Holstein cow whose hematologic reference ranges were within averages reported for healthy cattle (data not shown) [18, 19].

Dilutions of the primary antibodies (MAb CACT118A and MAb BAQ111A) and of the secondary antibodies (anti-IgG1PE-linked and anti-IgM FITC-linked) were prepared separately and in cocktails.

One hundred μL of PBMC were dispensed into five polypropylene conical-bottomed tubes. The first tube was the autofluorescence control and did not receive immunostaining. This control distinguished true fluorescence signals from background noise. The second tube was the positive control, and was intended to fluoresce in the PE channel because the single immunostaining was with MAb CACT118A and detected CD4 T lymphocytes. The third tube was the positive control two because the single immunostaining was with MAb BAQ111A, which detected CD8 T lymphocytes and fluoresced in the FITC channel. The fourth tube was immunolabeled only with secondary antibodies, as it verified the nonspecific binding between the PBMC and the secondary antibody. This control was processed identically to test tubes, allowing the assessment of background fluorescence due solely to secondary antibody interaction. A significant signal in this control would have suggested the need for further blocking steps or dilution optimization. Finally, the fifth tube was the test tube and was intended to fluoresce in both fluorescence channels since it was primary and secondary immunolabeled with the MAb and secondary Ab in cocktails.

These sets of five tubes were repeated in triplicate since three concentrations of secondary antibodies were tested. One set of five tubes was labeled as the 1:50 ratio, the second set as the 1:100 ratio, and the third set as the 1:200 ratio.

3.1.2 Acquisition and gating strategies

Ten thousand PBMC were acquired using a BD FACSCanto™ (BD™, Franklin Lakes, USA) flow cytometer equipped with a 688 nm Argon laser, a 488 nm Helium laser, and a 633 nm red laser, and operated with the software BD FACSDiva™. The

instrument belonged to a national committee that performed HIV diagnosis, so it had regular maintenance and quality control, completing the optimization, calibration, and standardization steps [20]. The acquired data were processed and analyzed with the trial version of FCS Express 3 (De Novo Software, Los Angeles, USA).

Two histograms were created from the autofluorescence control PBMC to ensure that the fluorescence emitted by unstained cells was distinguishable from the positive signals emitted by the fluorochromes. One histogram was for the PE fluorescence channel, and another for the FITC channel (**Figure 1**). The fluorescence emission for both channels occurred in the abscissa section between 10^0 and 10^1 (the negativity zone for both fluorochromes), and no fluorescent events appeared in the 10^2 axes positivity zone. These confirmed that the fluorescence emitted by the unlabeled PBMC occurred outside the positivity zone for the fluorochromes.

The histogram for each fluorescence channel from each dilution ratio tube immunolabeled with the secondary Ab cocktail was created to verify the absence of nonspecific binding. Each histogram illustrated the dilution ratios of each secondary Ab and the lack of signal in the region of positivity for PE and FITC fluorescence channels (**Figure 2**) [21]. This indicated that the conjugated secondary antibody did not interact with the PBMC at any dilution ratios tested (1:50, 1:100, and 1:200).

The light gray patterns show the 1:200 dilution ratio, the black patterns the 1:100 dilution ratio, and the intense gray patterns the 1:50 dilution ratio.

The data acquired from the tubes labeled as positive control and test were also processed using histograms, which facilitated comparison between the positivity peaks obtained from the three dilution ratios of the secondary Ab using superpositions. Positivity gates were set up, and the resulting statistics determined the percentages of CD4 and CD8 subpopulations using the primary and secondary antibodies separately and in cocktails (a further description of this gating strategy will be provided below).

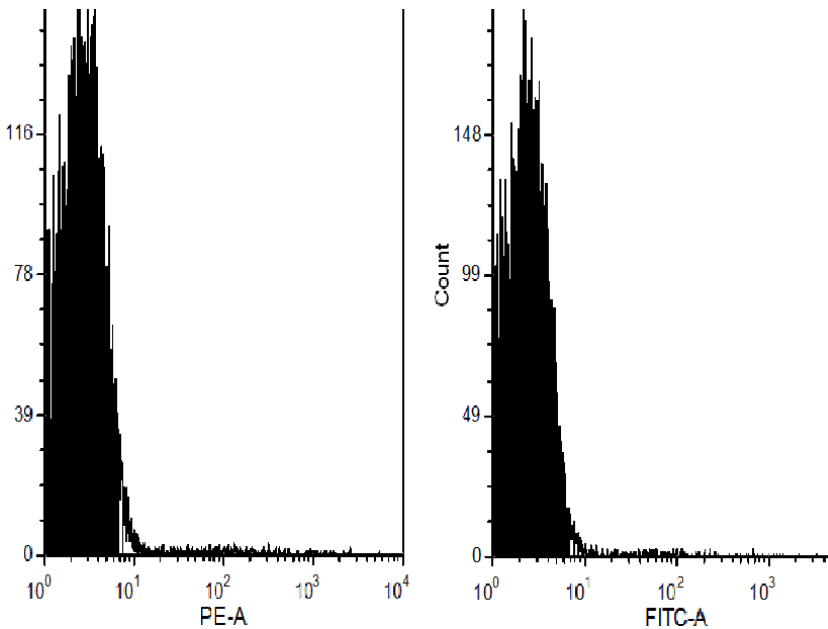


Figure 1.
PBMC autofluorescence in PE and FITC fluorescence channels was obtained from the control tube that contained non-immunolabeled cells.

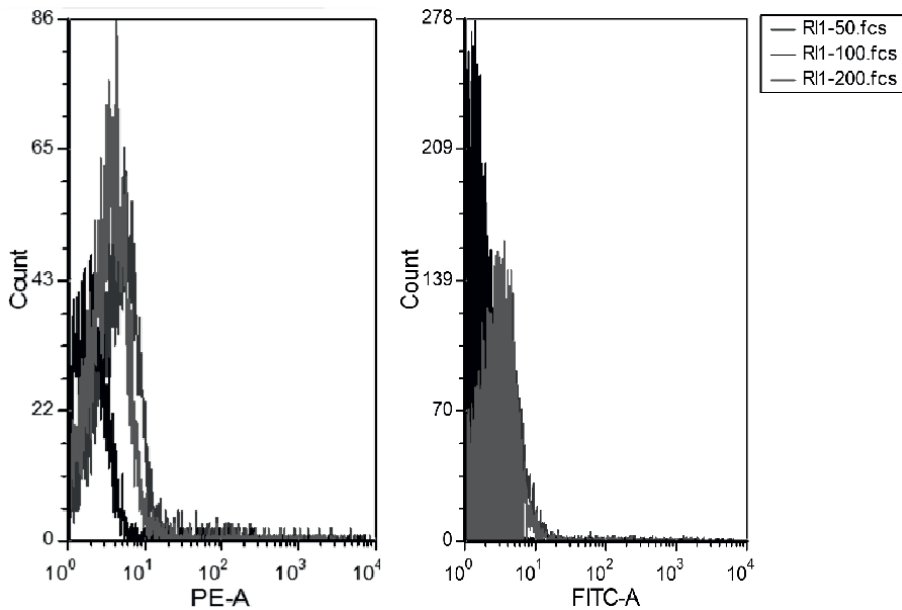


Figure 2.
Nonspecific reaction between secondary Ab and PBMC in PE and FITC fluorescence channels for the different dilution ratios.

Peaks were compared based on similarity in intensity, amplitude, and location. Positivity peaks for both fluorochromes were in the section of the abscissa encompassing 10^2 . The layers for the three dilution ratios of secondary Ab peaks tested separately and in cocktails were superimposed, indicating the same intensity and amplitude of emission (**Figures 3 and 4**).

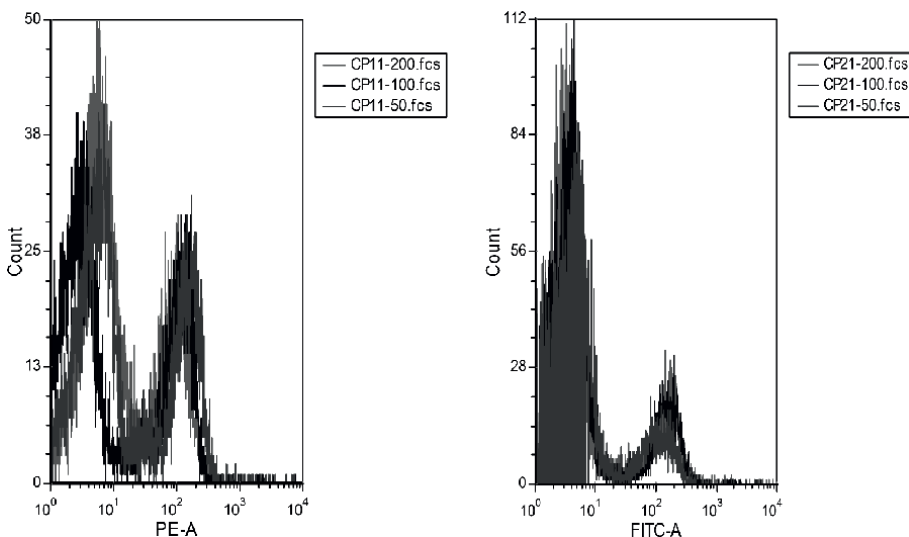


Figure 3.
Fluorescence emitted by PE-CD4 and FITC-CD8 immunolabeling with secondary antibodies separately. The light gray patterns show the 1:200 dilution ratio, the black patterns the 1:100 dilution ratio, and the intense gray patterns the 1:50 dilution ratio.

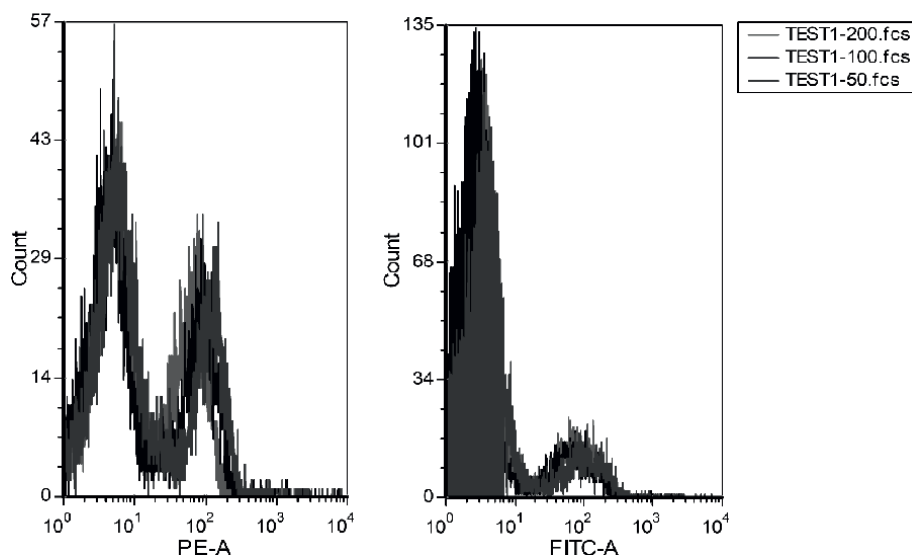


Figure 4. Fluorescence emitted by PE-CD4 and FITC-CD8 immunolabeling with secondary antibodies in a cocktail. The light gray patterns show the 1:200 dilution ratio, the black patterns the 1:100 dilution ratio, and the intense gray patterns the 1:50 dilution ratio.

Last, percentages of CD4 and CD8 cells were compared to literature reference values [6] and between dilutions [4]. The CD4 percentages were 31% using the 1:50 dilution ratio, 31% using the 1:100 dilution ratio, and 29% using the 1:200 dilution ratio when the Ab were applied separately, and 28, 30, and 33%, respectively, when the secondary Ab dilution ratios were used in a cocktail. CD8 percentages were 20% with the 1:50 dilution ratio, 27% with the 1:100 dilution ratio, and 28% with the 1:200 dilution ratio when the antibodies were used separately, and 21, 24, and 28%, respectively, when the secondary Ab were in a cocktail. Since the percentages of CD4 and CD8 cells were within the reported range, applying the dilution ratios either separately or in a cocktail, we decided to primary immunolabel with MAb in a cocktail, secondary immunostaining with Ab in a cocktail, and to use a 1:200 dilution ratio for the secondary Ab.

4. Intralab repeatability and consistency assessment

Through the statistical analysis of CD4 and CD8 subpopulations in duplicated samples, intralab repeatability was assessed. Following the protocol described before (title 2.1.1.), PBMC were isolated from noncoagulated blood venipunctured from six healthy donor Holstein cows. In these cows, average absolute red blood cell and platelet counts were within cattle reference ranges (data not shown). The average absolute leukocyte counts were higher than the reported ranges for healthy cattle, but according to the interpretation of leukogram patterns, leukocytosis and lymphocytosis were physiologic (data not shown) [18, 19].

The assay was designed by distributing 100 μ L of a 1×10^6 PBMC suspension in four 1 mL conical-bottom propylene tubes. To dual color indirectly immunolabel the cattle PBMC, we followed the protocol described in Section 2.1.3. These four tubes included the autofluorescence control, the PE positivity control corresponding to the CD4 subpopulation (primary immunolabeled with MAb CACT118A and secondary

Ab separately), the FITC positivity control corresponding to the CD8 subpopulation (primary immunolabeled with MAb BAQ111A and secondary Ab separately), and the test tube that detected CD4 and CD8 subpopulations so positivity was present in both fluorescence channels (immunolabeled with the MAbs mentioned earlier and secondary Abs, both in cocktail). In addition, the three tubes described were also used for compensation and calibration of the acquisition process, and the autofluorescence control was also set [20, 22, 23].

The acquisition was conducted as described in Section 3.1.2., and the data were processed using the trial version of FCS Express 3. For each duplicated sample, a first dot plot was created where the SSC angle of light was plotted on the ordinate axis and the FSC angle of light on the abscissa axis; the gate for the PBMC population was also set. The CD4 and CD8 lymphocyte subpopulations were displayed in the second dot plot through PE and FITC fluorescence standard quadrants, respectively, and the gate set in the first dot plot was applied. Third, both positivity gates were corroborated by histograms in each fluorescence channel. We confirmed the similarity in intensity, amplitude, and location of the peaks between the duplicated tubes because overlays from each fluorochrome from duplicates were superimposed. The gate defined in the first dot plot was applied, and the tables with the statistical information about the gating strategy were incorporated.

Figure 5 presents the gating strategy described before for a pair of tubes from the same sample. The first dot plot of SSC and FSC shows the gate corresponding to the PBMC population. The second dot plot, with standard quadrants, graphs the lymphocyte subpopulations. In one of the tubes, the fluorescence of CD4 T CACT118A immunolabeled cells is observed in PE, and within its duplicate, the fluorescence of CD4 T cells in the PE quadrant and CD8 T cells in the FITC quadrant is detailed. The histogram for the PE fluorescence channel is shown, where the positive peaks obtained from the tubes processed in duplicates were graphed. In the histogram, both peaks overlapped in the positivity zone (Section 10^2 of the abscissa axis) and

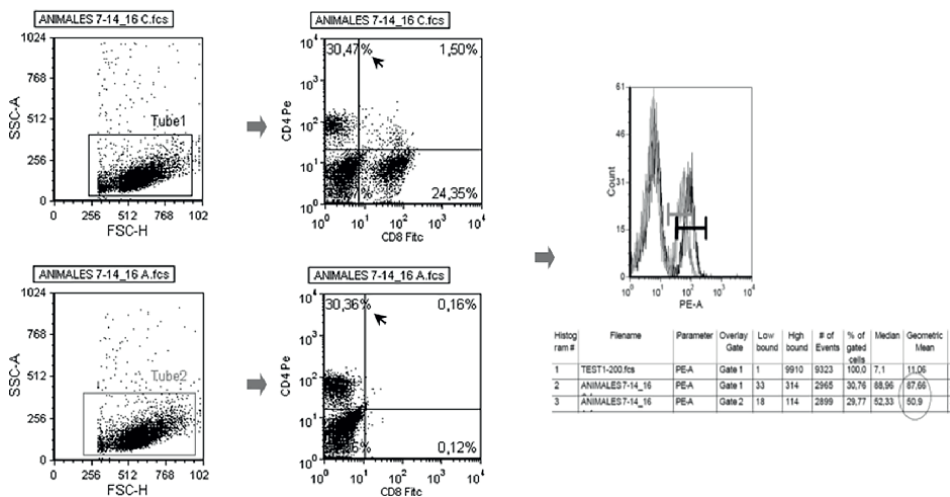


Figure 5. Gating strategy from a pair of tubes of the same PBMC sample with dot plots, dot plots with standard quadrants, histograms, and a table with statistics. SSC-A and FSC-H dot plots show PBMC gates in duplicated tubes of the same PBMC sample. One standard quadrant dot plot shows CD4 and CD8 T cells percentages, and the other shows CD4 T cells percentage. The histogram overlaps both positivity PE peaks for CD4 T cells, and the table shows the statistics of the strategy.

	PE-CD4				FITC-CD8				CV	
	Percent Tube 1	GMFI Tube 1	Percent Tube 2	GMFI Tube 2	CV	Percent Tube 1	GMFI Tube 1	Percent Tube 2		GMFI Tube 2
Sample 1	27.91	60.98	29.44	75.05	3.77	11.25	46.37	11.02	43.41	1.46
Sample 2	30.47	50.9	30.36	87.66	0.26	24.35	53.11	21.87	53.68	7.59
Sample 3	32.21	63.55	31.1	97.1	2.48	16.5	64.98	16.85	58.22	1.48
Sample 4	39.95	54.68	39.61	98.7	0.60	15.53	71.79	16.16	76.32	2.81
Sample 5	25.67	58.48	22.09	99.55	10.60	22.74	94.47	29.71	94.6	18.79
Sample 6	32.13	92.89	33.44	58.84	2.83	25.34	138.59	28.96	72.68	9.43

Table 1.
Percentages and MGIFs in paired samples and CV between CD4 and CD8 duplicates.

MAb	Samples	t-test to compare percentages		t-test to compare MGIFs	
		<i>t</i>	<i>p</i> -value	<i>t</i>	<i>p</i> -value
Anti-CD4 (CACT118A)	<i>n</i> = 6	0.50	0.6356	1.86	0.1215
Anti-CD8 (BAQ111A)	<i>n</i> = 6	1.09	0.3265	1.07	0.3327

Table 2.
Student t-test for paired samples to compare percentages and MGIFs.

presented similar amplitude and intensity. In the second dot plot, paired percentages are indicated by two black arrows and the median geometric mean fluorescence intensity (MGIF) by a gray circle.

For each paired sample, the percentages of each lymphocyte subpopulation and the geometric mean fluorescence intensity (GMFI) were obtained (**Table 1**). These values were statistically compared using the coefficient of variation (CV) and the Student t-test for paired samples (**Tables 1** and **2**).

Although the CV between duplicates exceeded 10% on two occasions, the Student t-test values, with their respective *p*-values above 0.05, when the percentages of CD4 and CD8 lymphocytes were compared pairwise, supported the finding of no significant differences between paired tubes. It is important to note that the International Council for Standardization of Haematology and International Clinical Cytometry Society consensus for intra-assay repeatability recommends a CV of less than 10% for abundant populations [12]. When the MGIF emitted by the samples from the paired tubes was compared using the paired t-test, the yielded *p*-values were higher than 0.05 (**Table 2**).

When in flow cytometry, dual indirect immunolabeling is routine, statistical comparison of results from repetitions of the same sample labeled with the same MAb would be strategic as an experimental error control method [24]. These findings support the reliability of the dual indirect immunolabeling protocol using anti-CD4 (IgG1 CACT118A) and anti-CD8 (IgM BAQ11A) monoclonal antibodies in combination with PE and FITC-conjugated secondary antibodies. The low intra-assay variability demonstrates that the most error-prone steps (washing, blocking, antibody incubation, and sample acquisition) are sufficiently controlled to allow consistent immune phenotyping of cattle PBMC. Furthermore, the statistical approach used here can serve as a model for future validation studies. It provides a framework for defining acceptable variability in experimental design, which is essential for comparative immunological research, vaccine trials, and diagnostic assay development in veterinary immunohematology.

5. Recommendations and future perspectives

The successful implementation of flow cytometry-based immunophenotyping in cattle highlights the importance of robust, validated protocols. However, additional considerations must be addressed. To minimize interlaboratory variability and ensure data comparability, it is essential to harmonize protocols and documentation, and reporting of each step of the experimental process, from PBMC isolation to data acquisition, should be thoroughly documented. Reports should include:

Details of antibody clones, fluorochromes, concentrations, and incubation conditions.

Instrument settings and calibration parameters.

Gates applied, and the rationale for gating strategies.

Statistical methods used for the analysis.

Establishing a standardized template for flow cytometry reporting studies in cattle will facilitate peer review, data sharing, and protocol refinement across laboratories. By adhering to rigorous standardization practices and investing in collaborative validation efforts, the field of bovine immunology can fully leverage the power of flow cytometry. This will enhance diagnostic precision, improve vaccine development strategies, and support evidence-based veterinary care.

6. Conclusions

Flow cytometry has revolutionized the study of immune responses in veterinary medicine, offering a highly sensitive and specific method to analyze individual cell populations. In cattle, advances in monoclonal antibody development and instrument capabilities have made it increasingly feasible to implement flow cytometric immunophenotyping in research and diagnostic settings.

This chapter detailed a validated protocol for indirect dual color immunolabeling of bovine PBMC, focusing on CD4 and CD8 T lymphocytes. We demonstrated that reliable data can be achieved with well-defined procedures, proper controls, and attention to critical technical variables. Statistical analysis confirmed low variability in repeated measures, indicating high assay precision and robustness.

However, achieving consistent results across laboratories requires broader adoption of standardized procedures, rigorous validation protocols, and harmonized reporting criteria. These practices in veterinary immunology will facilitate collaborative research, accelerate vaccine development, and improve disease-surveillance efforts.

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Optimization of Bovine Serum Albumin Purification by Thermocoagulation Using Response Surface Methodology

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Abstract

This study evaluated the thermocoagulation method for purifying bovine serum albumin (BSA) from bovine plasma. The use of BSA in the medical and veterinary fields, including bovine medicine, is common. Therefore, access to high purity BSA is clinically important. The process was optimized by varying key parameters: pH, temperature, caprylate concentration, and time. Experimental data were used to develop the statistical models through Response Surface Methodology and multiple linear regression. A second-order full factorial model was found to best describe BSA purity, which was mainly influenced by pH and temperature. Maximum purity (~100%) was achieved at [Cap] = 0.002 M, $T^\circ = 76^\circ\text{C}$, pH = 4.75, and $t = 130$ minutes. For BSA yield, a reduced second-order model was developed, in which [Cap] and pH were the most significant variables. Optimal yield (73.77%) was obtained at [Cap] = 0.02 M, $T^\circ = 60^\circ\text{C}$, pH = 7, and $t = 130$ minutes. Compromise solutions were also identified to balance purity and yield, depending on specific process goals. The use of the desirability function approach to find an optimal compromise solution was used. The use of statistical modeling enabled the prediction and adjustment of process conditions, enhancing efficiency and scalability. This approach confirms thermocoagulation as a practical, reproducible, and cost-effective method for BSA purification, especially useful when high purity is required and chromatographic resources are limited.

Keywords: animal reproduction, bovine plasma, heat shock, protein purification, purity, yield

1. Introduction

Proteins constitute a substantial fraction of the mass of all living organisms and are essential components of all tissues. Protein purification and isolation involve a series of processes aimed at extracting one or a few specific proteins from a complex mixture, typically derived from cell or tissue homogenates, whole organisms, or

microbial cultures. Protein purification can be an end in itself, such as in industrial-scale production, or serve scientific research purposes, including studies on activity, structure, structure-function relationships, and protein interactions.

During the first half of the twentieth century, protein purification methods were rudimentary compared to current standards. Developing an effective purification protocol for a particular protein could take years of work and requires large amounts of starting material [1]. It was not until 1926 that proteins were widely accepted as chemically well-defined substances, when James B. Sumner successfully crystallized urease from jack beans [1, 2]. However, even today, designing an efficient purification protocol for a specific protein remains a challenging and time-consuming task.

The development of protein purification techniques has been essential to many advances in biotechnology. Protein purification may involve a single-step precipitation process or require complex, large-scale procedures. Often, multiple purification stages are necessary to achieve the desired purity. Selecting the appropriate methodology is key to efficient purification [3]. The process typically involves separating proteins from nonprotein components and, finally, isolating the target protein from others; this last step being the most demanding. Each protein has a unique set of properties that can be exploited for its isolation. Separation stages usually take into account differences in size, physicochemical properties, affinity, and biological activity. Combining various techniques that leverage these features can enable successful purification. Each method should be assessed based on its capacity, resolution power, expected yield, and cost. For example, precipitation techniques offer high capacity but low resolution, whereas chromatographic methods provide high resolution but lower throughput. Early purification stages generally require high-capacity, low-cost methods, while later stages emphasize resolution [3].

A given protein, such as erythrocyte hemoglobin, may be abundantly present in tissue, while others, like the lac repressor in *Escherichia coli*, may occur in only a few molecules per cell. The same types of techniques are used for both, but the lower the initial concentration, the greater the effort needed to purify the target protein [1]. From the outset, purification methods must be designed with the intended quantity in mind. Some steps allow for easy scale-up, while others do not. The degree of purification required will depend on the protein's final application.

For research purposes, small-scale production with high purity is typically prioritized over yield. To minimize the risk of adverse effects, proteins intended for therapeutic use require much larger quantities and exceptionally high purity, often exceeding 99% [4]. In contrast, industrial uses usually prioritize high volume and low cost, even at lower purity levels. Product purity directly impacts process cost: The higher the purity, the more steps are required with lower yields and increased expense. Because protein is lost at each step, optimizing yield involves minimizing the number of purification stages. Accordingly, it is generally agreed that an economical process must reduce the number of separation steps and maximize the yield of each stage [5].

Mammalian blood is composed of two main fractions. The cellular fraction contains erythrocytes, leukocytes, and platelets. The other fraction is known as blood plasma. It is slightly alkaline, with a pH of approximately 7.4. It consists of about 90% water, 7% proteins, 1% inorganic salts, and the remaining 2% comprises various nonprotein organic compounds. The plasma contains a wide variety of proteins with diverse structures and functions. The most abundant plasma proteins are albumin (approximately 45%), immunoglobulin G (12%), and fibrinogen (4%). They all have a globular structure, which allows them to remain soluble in the polar environment of blood. Normal plasma protein concentrations in cattle are shown in **Table 1**.

Protein	Concentration (g/dL)
Total protein	6.0–8.0
Albumin	2.5–3.8
Globulins	3.0–3.5
Fibrinogen	0.1–0.6

Table 1.
Normal concentrations of plasma proteins in cattle [6].

Bovine serum albumin (BSA) is a major protein component of bovine plasma, accounting for approximately 50% of its total protein content [7, 8]. It is highly soluble in water, with an isoelectric point of 4.78, meaning that at neutral pH, BSA carries a net negative charge [8, 9]. BSA transports a wide range of endogenous and exogenous compounds, including fatty acids, metals (Ca, Cu, Ni, Mg, and Zn), amino acids, bilirubin, steroids, and drugs [8, 10, 11]. It also binds many substances reversibly, especially negatively charged molecules, enabling its transport functions [7, 8, 12, 13].

BSA is widely used in biochemistry, diagnostics, molecular biology, biotechnology, and other fields. It plays a role in animal reproduction protocols, diagnostic kits (e.g., for blood typing), immunoassay blocking solutions, *in vitro* embryo culture media, stabilization of protein solutions, total protein determination assays, and veterinary vaccine production (e.g., leptospirosis vaccines). Of particular note, due to its biocompatibility, biodegradability, and non-toxicity, is its application in nanotechnology as a raw material for the fabrication of nanoparticles used in drug delivery systems [14–16], as well as a common raw material for hydrogel preparation [17]. Advances in biotechnology have enabled the adsorption of BSA onto non-biological solid surfaces, making it useful for artificial implants and drug delivery systems [18]. In the food industry, BSA serves as a functional ingredient in stabilizing oil-in-water emulsions [19]. These broad applications underscore the importance of isolating and purifying BSA to a high degree of purity.

Human serum albumin has been isolated from other serum proteins using various methods. Since the 1940s, the Cohn fractionation method [20] has been widely used to separate plasma protein fractions. The Cohn method, also known as cold ethanol fractionation, remains the most extensively applied technique in the plasma fractionation industry. It relies on the differential solubility of albumin and other plasma proteins based on five variables: ethanol concentration, ionic strength, pH, protein concentration, and temperature. However, this method presents numerous challenges due to the use of reagents that can denature proteins. To avoid this, the process must be carried out at very low temperatures, with careful control of reagent dilution, order of addition, pH, ionic strength, and under vigorous and continuous agitation.

As an alternative to cold ethanol fractionation, chromatographic purification of plasma to isolate albumin was proposed [21]. In this method, clarified plasma is subjected to gel filtration and diafiltration, followed by ion exchange chromatography, often using one or more columns in series for final purification. The advantages of this approach include ease of automation, low cost at pilot scale, simple disinfection, and minimal damage to the target protein. Chromatographic methods also tend to yield higher recovery (typically 80–85%) and greater purity (around 98%) than cold ethanol precipitation (commonly 55–60% yield with 95% purity). Despite these advantages, chromatography-based purification of albumin has only recently gained

broader adoption, primarily due to the previous unavailability of large-scale chromatographic equipment to meet product demand. An alternative purification methodology is offered by membrane-based technologies, particularly ultrafiltration, which has emerged as a highly effective bioseparation process for purifying bioproducts such as therapeutic drugs, enzymes, hormones, and antibiotics [22, 23].

Currently, the most widely used technique for BSA production is thermocoagulation. This method, also known as heat shock [24, 25], is favored for its speed, simplicity, and reproducibility, and was developed as a scalable alternative for therapeutic albumin production. Previous work was based on that caprylate ions confer thermal stability to albumin [26]. The underlying principle of this method is the selective thermal denaturation of serum globulins and other plasma proteins without significantly affecting the serum albumin. At elevated temperatures, the bonds that maintain the native structure of proteins are broken, resulting in protein unfolding, loss of biological activity, and reduced solubility. To maximize both yield and purity, this is achieved by adding a defined concentration of caprylate ions to the solution, adjusting the pH to a critical range, and applying a narrow temperature window. The caprylate ions associate with albumin molecules, protecting them from heat-induced denaturation. This approach is suitable when albumin is the only protein of interest, as the heat irreversibly denatures all other proteins, rendering them unsuitable for further recovery in native form.

Caprylate ions are used at concentrations of 0.011–0.013 M, pH 5.0–5.2, and temperatures between 65°C and 71°C, with heating durations of up to 6 hours, resulting in albumin purity levels of 90–96% [24]. An alternative method was proposed using a low-molecular-weight aliphatic alcohol and a carboxylic acid salt [25]. Their treatment involved 15–33% alcohol and 0.1–0.6% aliphatic carboxylate, at temperatures ranging from 1°C to 30°C and pH values of 2–5. Protein denaturation and precipitation occurred between pH 4 and 5 at the same temperature range. However, this method yields relatively low albumin recovery (~55% of the theoretical value) when high purity is desired, and the high alcohol content (>15%) is difficult to remove, increasing purification costs. Another variation was proposed using a carboxylic acid salt to stabilize the albumin and treat it with a low-molecular-weight aliphatic alcohol [27]. The preferred alcohol was ethanol at concentrations of 8–12%, and the stabilizing agent was sodium caprylate at 0.004 M. The process was carried out at pH 6–7 and temperatures between 67°C and 69°C. Denatured proteins were precipitated, and the resulting albumin-containing solution was recovered at room temperature. The objective of this work is to analyze and model the purification process of BSA, focusing on purity and yield as key performance parameters, and to propose a compromise solution that optimizes both factors for applications in veterinary medicine.

2. Thermocoagulation process

In this study, this method was selected due to its speed, practicality, and reproducibility. Sodium caprylate was used as the thermal stabilizer for BSA. To determine the optimal conditions for the thermocoagulation process, a laboratory-scale study was carried out by varying caprylate concentration ($[Cap]$), pH, process time (t), and temperature (T°). A total of 210 mL of bovine plasma was used, with a concentration of 6.43% (w/v), equivalent to 5.14% (w/v) of total proteins. When required, the pH was adjusted using 0.5 N HCl, followed by the addition of 0.5 M sodium caprylate

to reach the desired final concentration. The solution was then slowly heated to the target temperature under continuous stirring to ensure uniform heat distribution. The timing for each experiment began once the desired temperature was reached.

The variables evaluated during the thermocoagulation process were as follows:

- *pH*: 4.5; 4.78; 5; 6; and 7
- *Temperature*: 60; 64; 68; 70; 72; and 76°C
- *[Caprylate]*: 0.002; 0.012; and 0.02 M
- *Process times*: 0; 10; 30; 50; 70; 90; 110; and 130 minutes

After the specified treatment time, the solution was rapidly cooled to room temperature to produce the heat shock effect. The resulting solution was adjusted to pH 4.2 using 0.5 N HCl. At this pH, denatured proteins formed a colloidal mass or coagulum, while albumin remained soluble. The denatured proteins were separated by centrifugation at 4000 rpm at 4°C. Optimal results were obtained after 45 minutes of centrifugation.

The resulting supernatant was collected and analyzed for total solids, purity, and BSA concentration. The purity and quantity of BSA in each sample were determined using SDS-PAGE gels, with commercial BSA (Sigma-Aldrich No. A-3059) and untreated plasma used as standards. The gels were analyzed using Gel-Pro Analyzer 4.0 software to quantify BSA content and assess purity in each sample. Based on the obtained data, modeling and statistical analyses were conducted to determine the optimal conditions for the thermocoagulation process.

3. Modeling and statistical analysis of the thermocoagulation process

The thermocoagulation process was modeled using multiple linear regression, specifically the least squares method, with Statistica 8.0 software. Based on this technique and using the experimental data of BSA purity and yield, four different models were fitted: first-order, first-order plus quadratic terms, first-order with interaction terms, and a full second-order model (including linear, quadratic, and interaction terms), with the predictor variables coded.

One advantage of coding the regressors is that, by transforming all variables to a common scale (approximately from -1 to 1), the magnitude of the coefficients can be compared to determine which variable has the greatest impact on the response. The coding was performed using the following general equation:

$$X_i = \frac{E_i - \bar{E}_i}{\frac{RE_i}{2}} \quad (1)$$

E_i : actual value of the variable

$\bar{E}_i$: mean of the actual variable

RE_i : range of the actual variable

The following variables were coded using this procedure:

$$X_1 = \frac{[Cap] - 0.011}{0.009} \quad (2)$$

$$X_2 = \frac{T^\circ - 68.33}{8} \quad (3)$$

$$X_3 = \frac{pH - 5.21}{1.5} \quad (4)$$

$$X_4 = \frac{t - 61.25}{65} \quad (5)$$

$[Cap]$: caprylate concentration

T° : temperature

t : time

A full second-order model was fitted for four coded predictor variables according to the following equation:

$$y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{44}x_4^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{34}x_3x_4 \quad (6)$$

In order to minimize the sum of squared errors, the model coefficients (b terms) were obtained using the least squares method:

$$\varepsilon_i = y_i - \hat{y}_i \quad (7)$$

ε_i : residuals

y_i : observed value

$\hat{y}_i$: value estimated by the model

To test the significance of the model and its individual coefficients, hypothesis tests were conducted. These tests required that the residuals ε_i be normally and independently distributed with a mean of zero and constant variance σ^2 . It was also assumed that the proposed model was correct. Model adequacy and the validity of the assumptions regarding the residuals were verified through graphical analysis of residuals, the coefficient of determination (R^2 and R^2_{adjusted}), and the CP statistic, a measure of the model's total mean squared error:

$$CP = \frac{SS_E(p)}{\sigma^2} - n + 2p \quad (8)$$

SS_E : sum of squared errors

n : number of experimental runs

p : number of model parameters

σ^2 : residual variance

The models were evaluated based on the CP statistic to identify the model with minimal bias. A CP value equal to p indicated negligible estimation bias, suggesting that the model was the best representation of the studied process [28]. The models were also assessed by their R^2 values, selecting the one with the highest value. If nonsignificant terms were found, they were sequentially removed, and the model was refitted and re-evaluated until a model with $CP = p$ was obtained.

The optimal levels of the process variables that maximized BSA purity and yield were determined using the desirability function approach [29], implemented in Statistica 8.0. Desirability and response profiles were constructed by fixing the remaining variables at their optimal values. This method was particularly useful for identifying the best compromise solution for multiple responses, but was also applicable to the optimization of a single response.

Once the model's adequacy and the optimal variable levels were confirmed, response surface plots (3D graphs) and contour plots (constant response curves) were generated. These plots allowed direct visualization of the optimum point, definition of a desirable operating region, and analysis of how the variables affected the studied responses.

4. Modeling and statistical analysis for BSA purity (% BSA)

Table 2 shows the CP values for the different fitted models. As observed, the full second-order model (including linear, quadratic, and two-factor interaction terms) was identified as the most appropriate, since it exhibited negligible bias with $CP = p$ (both equal to 15). All parameters were statistically significant with a $p < 0.05$. To assess the significance of the second-order regression model, an analysis of variance (ANOVA) was performed, confirming that the second-order model was appropriate for describing the process with respect to BSA purity.

To verify the adequacy of the model, a residual analysis was performed. The residuals followed approximately a normal distribution (linear behavior), indicating that the residuals were independent and had constant variance. Based on the values of R^2 , R^2_{adjusted} , CP, and residual analysis, the full second-order model was confirmed as suitable for describing BSA purity.

To determine which variables had the greatest impact on BSA purity, a Pareto chart of the regression coefficients (RC) was constructed (**Figure 1**). The most influential linear term was pH, followed by T° , with lesser influence from [Cap] and t . Among the

Model	p	SS_R	SS_E	MS	R^2	R^2_{Adjusted}	CP
Linear + quadratic + interactions	15	36,753.01	8642.30	24.41	0.8096	0.8021	15

p : number of parameters in the model.

SS_R : sum of squares of regression.

SS_E : sum of squares of error.

MS : mean square of the errors (variance).

CP: statistic calculated according to Eq. (8).

Table 2.
Model selection for BSA purity.

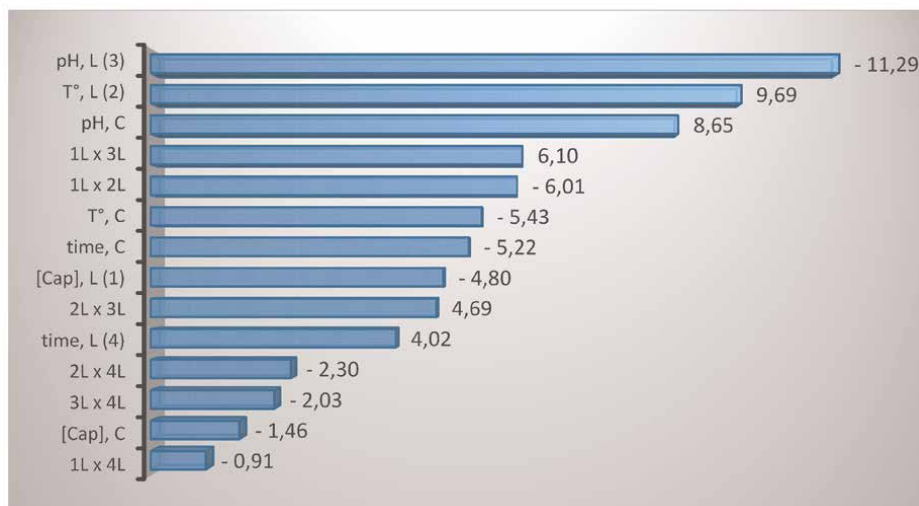


Figure 1.

Pareto chart of regression coefficients for the full second-order model of BSA purity.

quadratic terms, the most significant were pH and temperature, followed by time and [Cap]. For the interaction terms, the most important were [Cap] $\times$ pH and [Cap] $\times$ T°.

Based on the magnitude of the coefficients shown in **Figure 1**, the most influential variables for BSA purity were pH and temperature, with [Cap] playing a lesser but still relevant role, mainly through its interactions with pH and T°. Time had virtually no impact on % BSA. **Figure 2** presents the prediction profiles and desirability function values for % BSA. The graphs for each variable were constructed by fixing all other variables at their optimal values, where a response close to 100% was obtained.

According to this method, the optimal conditions to achieve maximum % BSA were: [Cap] = 0.002 M, T° = 76°C, pH = 4.75, and t = 130 minutes.

Given that pH and temperature were the most influential variables, to assess how they affected BSA purity, response surface (**Figure 3**) and contour plots (**Figure 4**) were created for these variables, with [Cap] and time fixed at their optimal levels. Since time was the least influential variable, it was fixed at 90 minutes, a sufficient period to reach maximum BSA purity, while minimizing energy consumption and operational costs. The [Cap] was fixed at 0.002 M, as calculated to be optimal. These plots allow for different types of analysis: examining how BSA purity varies when fixing temperature and changing pH; fixing pH and modifying temperature; or simultaneously varying both.

It was observed that increasing pH at low temperatures caused a more pronounced drop in BSA purity than at higher temperatures. At low pH values, BSA purity remained nearly constant, due to interaction effects. A BSA purity close to 100% could be achieved at any temperature, provided the pH was at its lowest. If a high pH was used, working at low temperature resulted in purities below 60%. In such cases, high temperatures were necessary to reach acceptable BSA purity levels. Thus, the model suggested that in general, increasing temperature and lowering pH improved BSA purity. **Figures 3** and **4** also show that the highest BSA purities were obtained when both pH and temperature were set at either high or low levels simultaneously. A wide range of pH and T° combinations could produce near-optimal BSA purity values, making it easier to find a compromise solution between purity and yield.

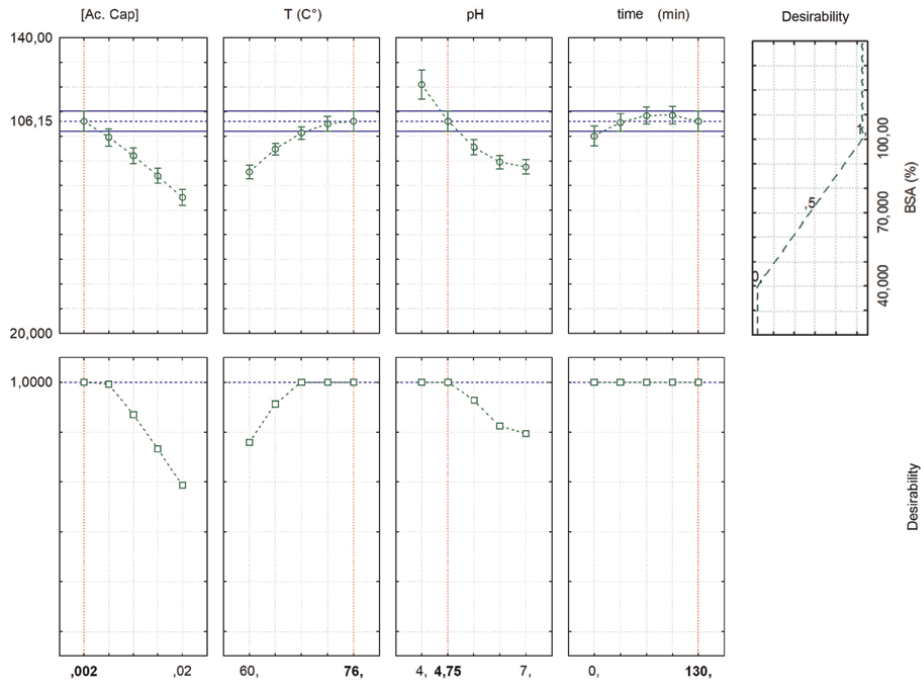


Figure 2.
Prediction profiles and desirability for BSA purity.

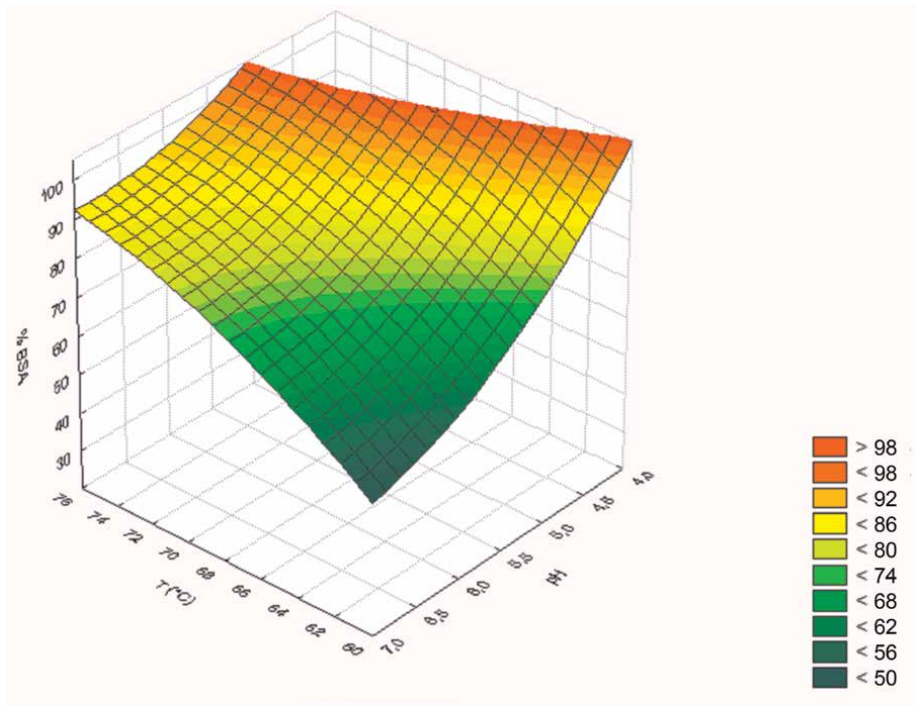


Figure 3.
Response surface for BSA purity with $[Cap] = 0.002\text{ M}$ and $t = 90\text{ minutes}$.

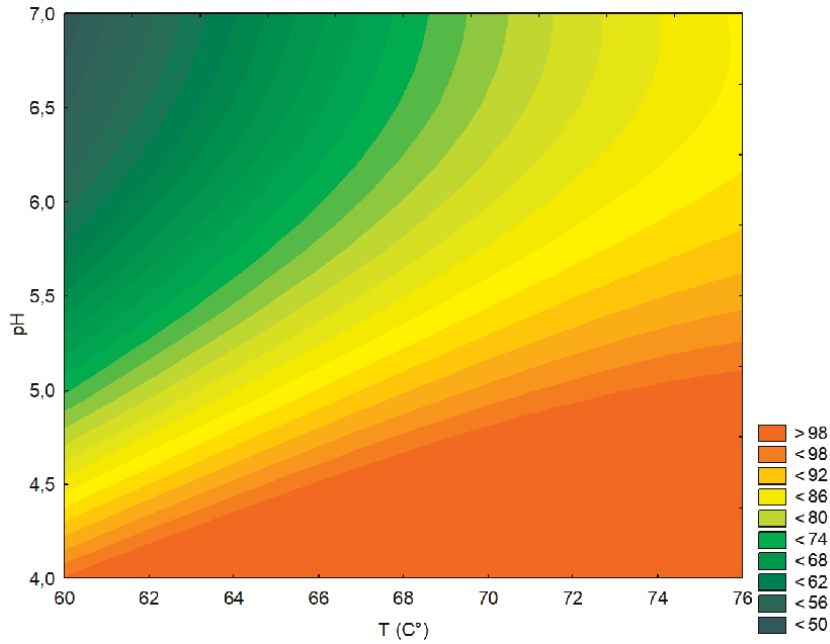


Figure 4.
Contour plot for BSA purity with $[Cap] = 0.002\text{ M}$ and $t = 90\text{ minutes}$.

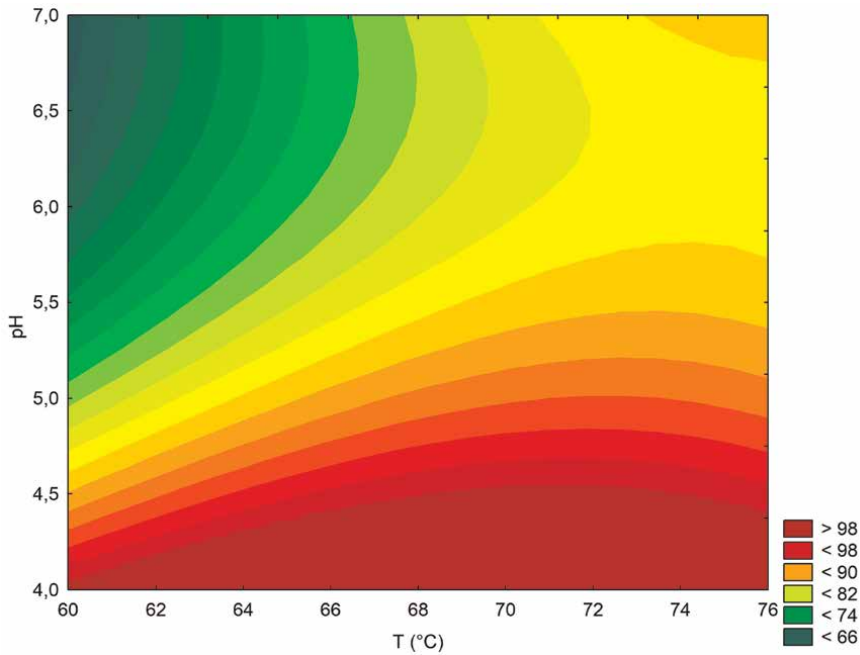


Figure 5.
Contour plot for BSA purity with $[Cap] = 0.012\text{ M}$ and $t = 90\text{ minutes}$.

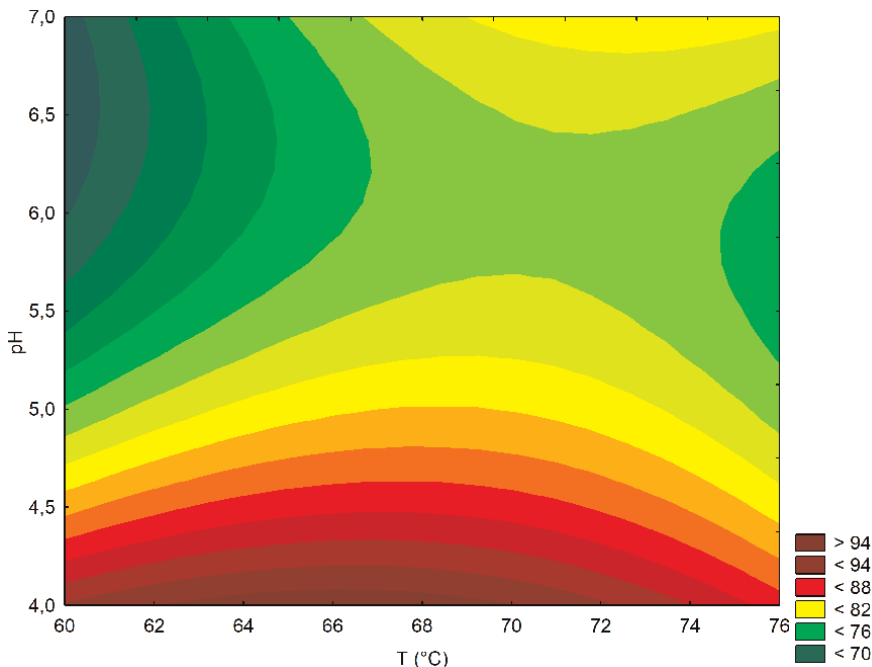


Figure 6.
Contour plot for BSA purity with $[Cap] = 0.02\text{ M}$ and $t = 90\text{ minutes}$.

Considering that the natural pH of plasma is approximately seven, and that in the next step the pH must be adjusted to 4.2, it was energetically favorable to operate under low pH and low temperature conditions, where BSA purity exceeded 98%. Although the linear effect of $[Cap]$ was not significant, its importance became evident through its interactions with pH and temperature. Therefore, contour plots of the response surface were constructed for the other tested $[Cap]$ concentrations (**Figures 5 and 6**).

At a $[Cap]$ of 0.012 M, the behavior was similar to that seen at 0.002 M. Again, an increase in T° , or a decrease in pH, generally improved % BSA. However, at high temperatures, increasing pH led to a drop in BSA purity, followed by a rise beyond pH 6.5. While an increase was noted, the maximum response values did not exceed 90%, unlike the 0.002 M case. Moreover, the area of >98% BSA purity was more limited, and only found in a smaller low-pH region. Thus, under these conditions, fewer combinations of pH and T° led to optimal % BSA, although many combinations still resulted in >90% purity—useful for compromise solutions between yield and purity.

At $[Cap] = 0.02\text{ M}$, increasing pH from 4.0 caused a decrease in % BSA, followed by a rise beyond pH 6.0, forming a saddle point or minimum. An increase in temperature also improved the response, but only up to $\sim 71^\circ\text{C}$. Beyond that point, % BSA began to decline. Under these conditions, regions of >98% purity were not obtained. A small area exceeding 88% BSA purity was identified, located in the same region where >98% BSA was observed at $[Cap] = 0.012\text{ M}$.

5. Modeling and statistical analysis for BSA yield

Table 3 shows the CP values for the two fitted models used to evaluate BSA yield. As with BSA purity, the full second-order model (including linear, quadratic, and

Model	<i>p</i>	<i>SS_R</i>	<i>SS_E</i>	<i>MS</i>	<i>R</i> ²	<i>R</i> ² _{Adjusted}	<i>CP</i>
Linear + quadratic + interactions	15	47,076.32	8084.86	24.80	0.8534	0.8471	15
Linear + quadratic + interactions – T° (C) – [Cap] × t – pH × t – pH(Q) – t (Q)	10	46,958.62	8202.56	24.78	0.8513	0.8473	10

Table 3.
Model selection for BSA yield.

two-factor interaction terms) proved to be the best, with negligible bias as the CP = *p* (both equal to 15). Since the model included nonsignificant terms, these were progressively removed. The model was refitted in each case, and CP was evaluated until a model with CP = *p* was obtained. The final selected model excluded the quadratic terms for temperature, pH, and time, as well as the interaction terms [Cap] × t and pH × t. This reduced model had CP and *p* = 10.

The statistical significance of the parameters for the best reduced second-order model showed that all model parameters were statistically significant with *p* < 0.05. ANOVA was performed to evaluate the significance of the best second-order regression model, confirming that it was suitable for describing BSA yield in the studied process. The residuals followed an approximately linear trend, indicating a normal distribution. Additionally, they showed no unusual patterns, indicating that the residuals were independent and had constant variance. Based on the values of *R*², *R*²_{adjusted}, CP, and residual plots, the reduced second-order model was deemed adequate for describing BSA yield.

A Pareto chart of the model coefficients was constructed (**Figure 7**) to identify the most influential variables on BSA yield. As shown in **Figure 7**, the linear terms with the greatest impact were [Cap] and pH. The time and T° were also statistically significant but had minimal effect on yield. The quadratic term of [Cap] was also highly impactful. Among the two-factor interactions, pH × T° had the greatest influence, followed by T° × t and pH × [Cap], while [Cap] × T° had the least impact. From

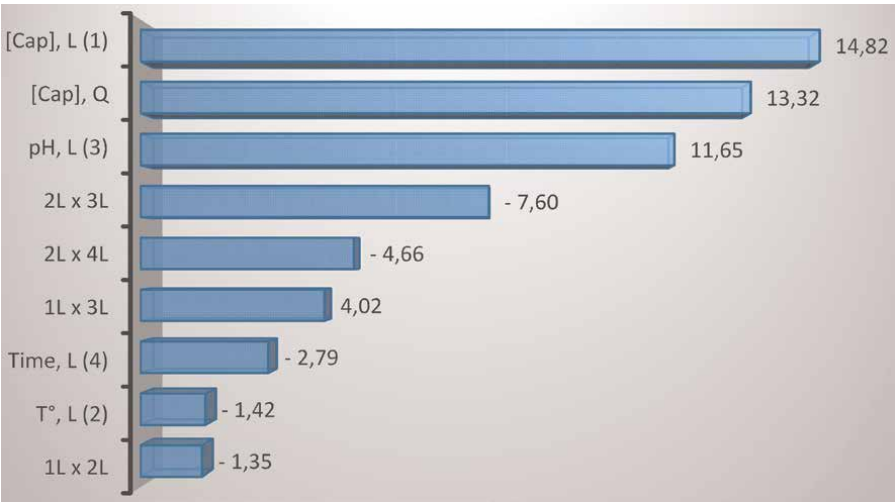


Figure 7.
Pareto chart of regression coefficients for the best second-order model for BSA yield.

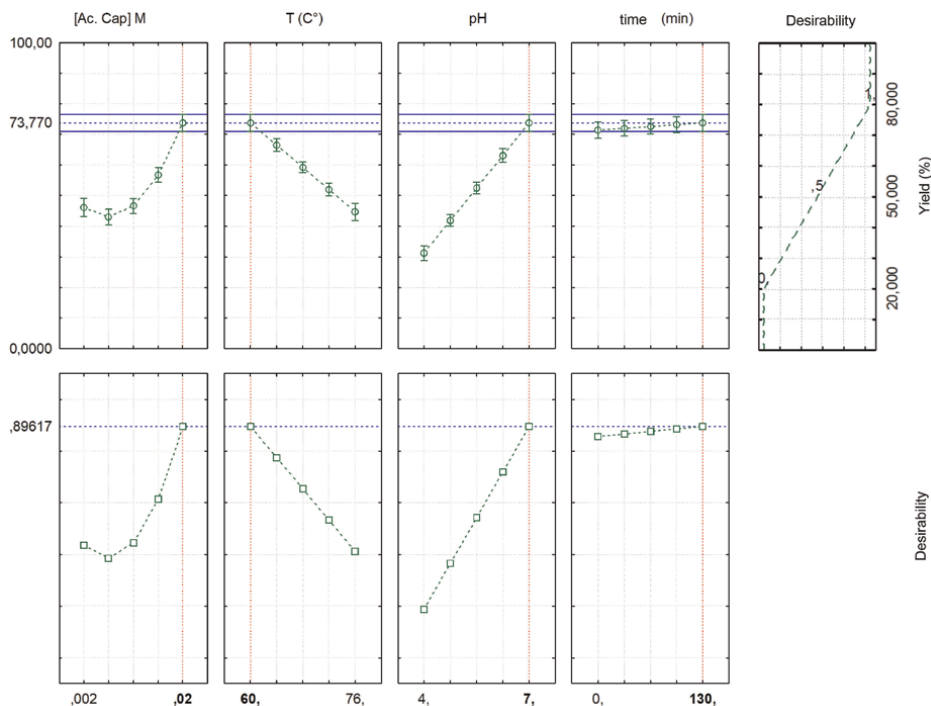


Figure 8.
 Prediction profiles for BSA yield and desirability.

the analysis of the model coefficients shown in **Figure 7**, it was evident that [Cap] and pH were the most important variables influencing BSA yield, while T° had a lesser effect. As with BSA purity, time had almost no influence.

Prediction profiles for BSA yield and desirability plots are presented in **Figure 8**. Each graph was generated by fixing the remaining variables at their optimal values. According to this method, the optimal conditions for maximizing BSA yield were: [Cap] = 0.02 M; T° = 60°C; pH = 7; and t = 130 minutes.

Considering that [Cap] and pH had the highest influence on yield, response surface (**Figure 9**) and contour plots (**Figure 10**) were constructed for these two variables, while temperature and time were fixed at their optimal levels. Since time had the least impact, it was fixed at 90 minutes as in the BSA purity analysis, following the same rationale. These graphs, presented in **Figures 9** and **10**, were analyzed in multiple ways. The effect of fixing [Cap] and changing pH, fixing pH and modifying [Cap], or simultaneously varying both variables was evaluated. It was observed that an increase in pH consistently resulted in higher BSA yield across all [Cap] values. At higher [Cap] levels, increasing pH had a stronger positive effect on yield than at lower [Cap] levels. Regardless of the caprylate concentration, operating at pH 7 always yielded the highest performance. Starting from approximately [Cap] = 0.008 M, both an increase and a decrease in [Cap] enhanced BSA yield at any pH level. Thus, from [Cap] $\geq$ 0.008 M, increasing either caprylate concentration or pH favored yield.

Figure 10 shows that the maximum BSA yields were obtained when both [Cap] and pH were at their highest levels. Although the region of absolute maximum yield was relatively small, there was a large area where yield exceeded 40%, which is

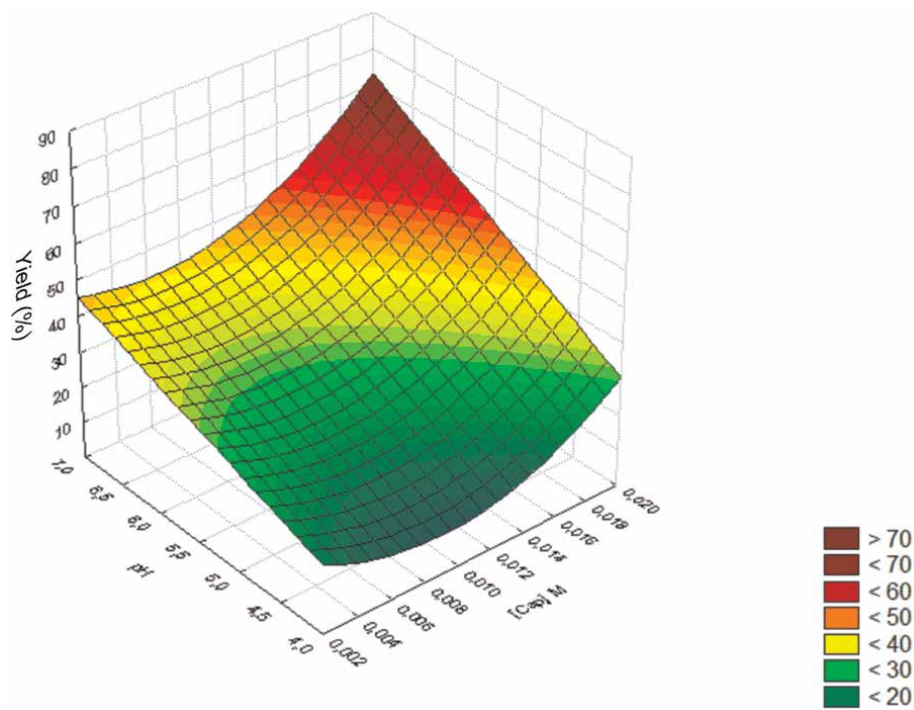


Figure 9.
Response surface for BSA yield with $T^\circ = 60^\circ\text{C}$ and $t = 90$ minutes.

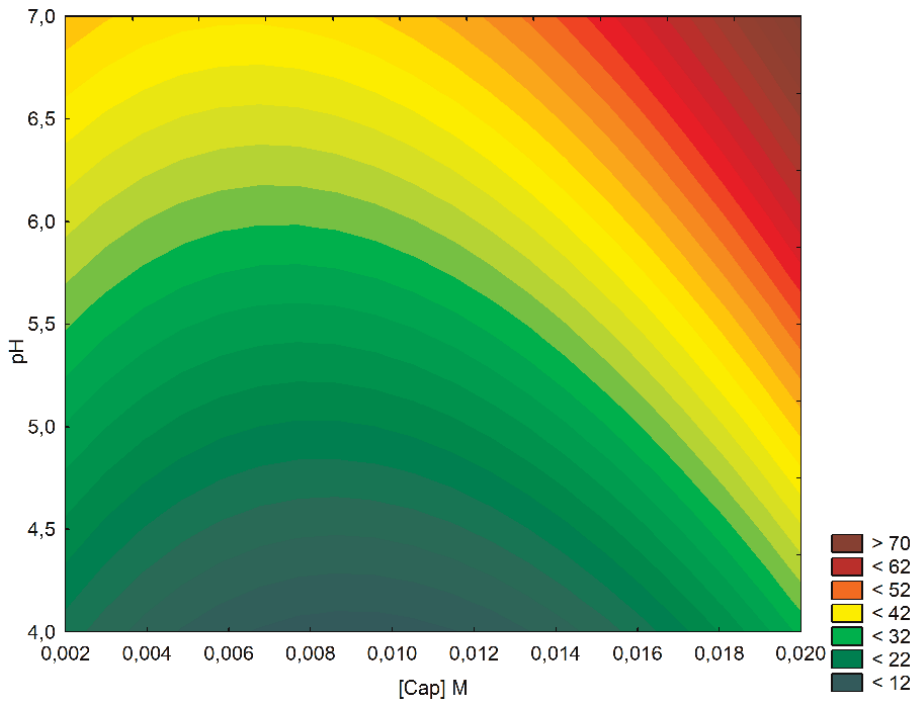


Figure 10.
Contour plot for BSA yield with $T^\circ = 60^\circ\text{C}$ and $t = 90$ minutes.

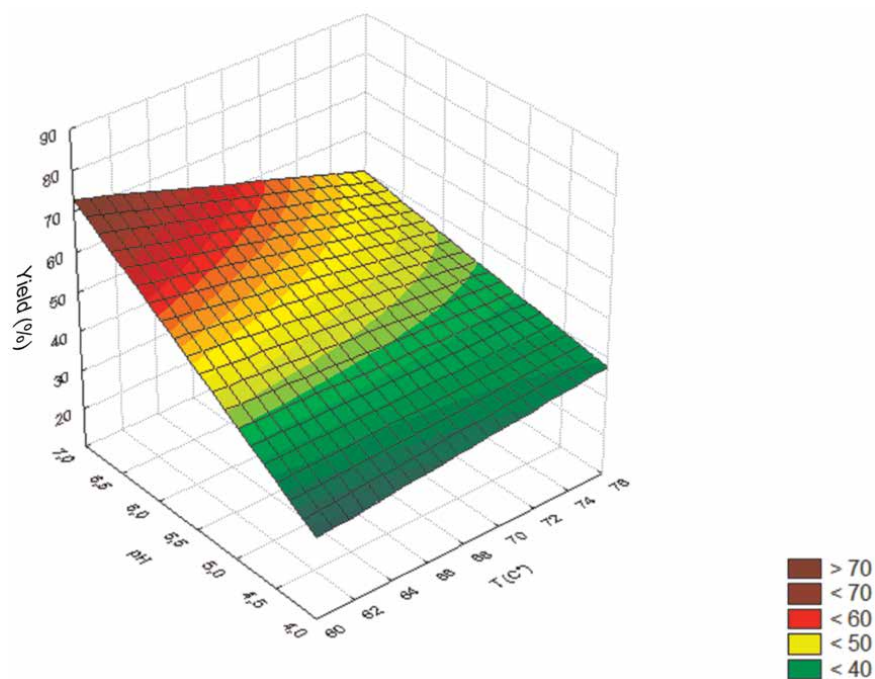


Figure 11.
Response surface for BSA yield with $[Cap] = 0.02\text{ M}$ and $t = 90\text{ minutes}$.

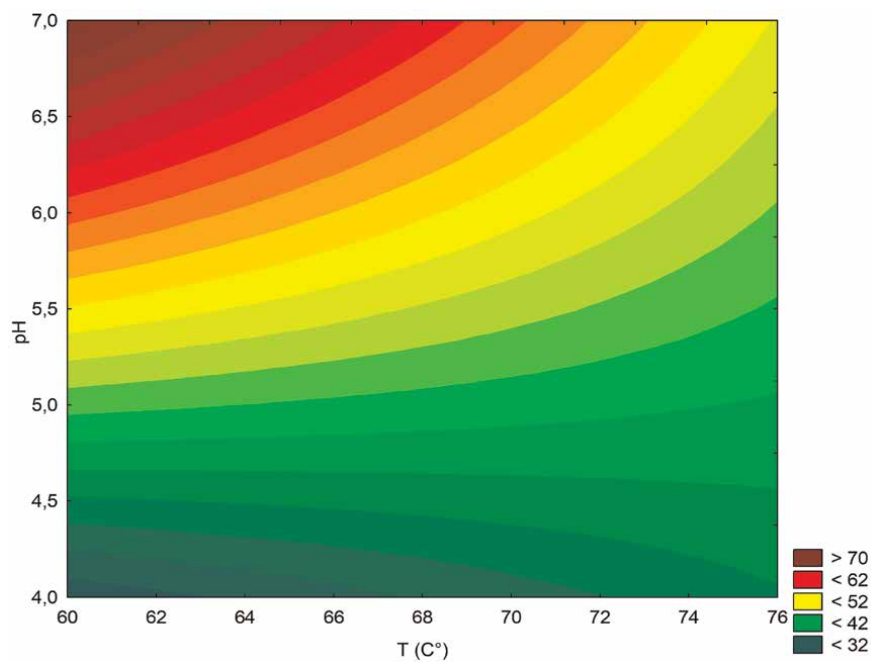


Figure 12.
Contour plot for BSA yield with $[Cap] = 0.02\text{ M}$ and $t = 90\text{ minutes}$.

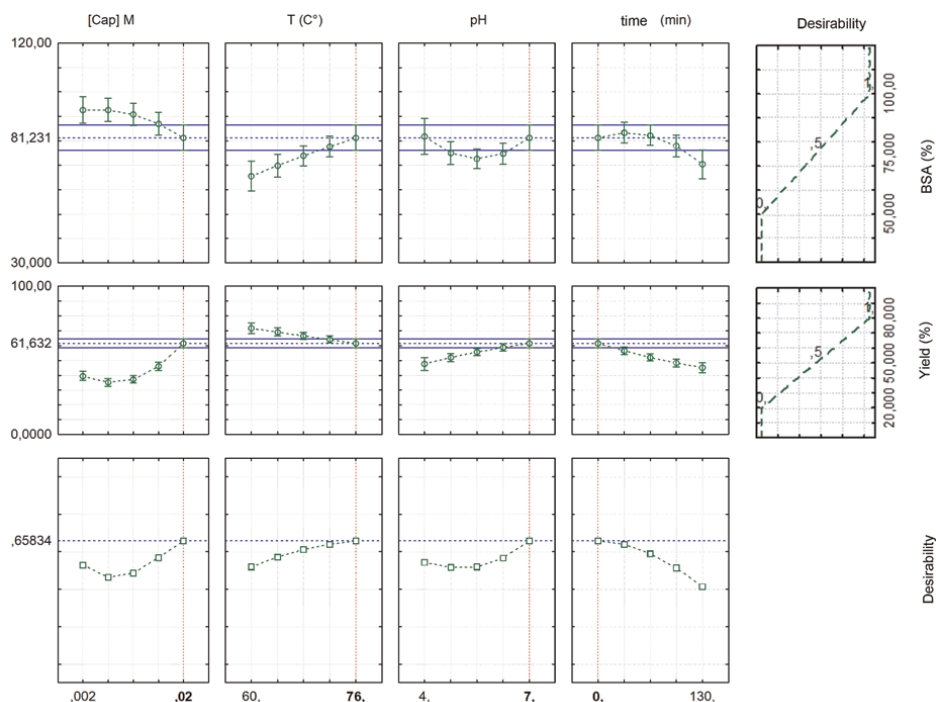


Figure 13.
Prediction profiles for estimated % BSA, yield, and desirability.

acceptable for protein purification processes. This facilitated the search for an optimal compromise between purity and yield. Since temperature also influenced BSA yield, additional surface and contour plots were created for temperature and pH, with [Cap] and time fixed at their optimal values (**Figures 11** and **12**). From the analysis of these figures, it was evident that increasing pH improved yield regardless of temperature. The increase was more significant at lower temperatures. Around pH 4.7 (close to BSA's isoelectric point), changes in temperature had no effect, and yield remained close to 40% across all temperatures. At lower pH values, raising the temperature slightly improved the yield; conversely, at higher pH values, increasing the temperature led to a decrease in yield. This decline was more pronounced at the highest pH values, though net yield remained higher at elevated pH levels.

Overall, it was concluded that higher pH and lower temperature favored BSA yield. The highest yield was achieved when the temperature was set at its lowest and the pH at its highest. As in the previous case, the absolute maximum was located in a small area, but a broader working region was found at higher pH values, where yields exceeded 40%.

6. Compromise solution for BSA purity and yield

Based on the analysis of the proposed models for BSA purity and yield, an attempt was made to identify the *best compromise solution* between both responses. For this purpose, several prediction profile graphs were generated for the estimated values of BSA percentage, yield, and overall desirability under the different sets of conditions.

Figure 13 shows the estimated profiles of BSA purity, yield, and desirability for each variable. The BSA purity range was set between 50% and 100%, while the yield range was set between 20% and 80%. The graphs for each variable were constructed by fixing the remaining variables at their respective optimal values. According to the software, the optimal conditions to achieve high purity and good yield were: $[Cap] = 0.02 \text{ M}$; $T^\circ = 76^\circ\text{C}$; $\text{pH} = 7$; and $t = 0 \text{ minute}$. Under these conditions, a BSA purity of 81.2% and a yield of 61.6% could be achieved.

To prioritize BSA purity over yield, the variables $[Cap] = 0.002 \text{ M}$ and $t = 90 \text{ minutes}$ were fixed (**Figure 14**). The prediction profiles for BSA purity, yield, and desirability were generated under these new conditions. The software again indicated $T^\circ = 76^\circ\text{C}$ and $\text{pH} = 7$ as the optimal conditions, consistent with the previous case. Under these parameters, a BSA purity of 97.4% was obtained, with a yield of 26.9%. This indicated that very high purity could be achieved, although the yield would be reduced to approximately half compared to the previous case.

Alternatively, different conditions were fixed to try to increase yield while still prioritizing purity. For this, the variables were set as follows: $[Cap] = 0.002 \text{ M}$; $T^\circ = 68^\circ\text{C}$; $\text{pH} = 5$; and $t = 90 \text{ minutes}$ (**Figure 15**). Under these settings, the prediction profiles of BSA purity, yield, and desirability were again generated. In this case, a lower pH and a lower temperature were used. These conditions yielded a BSA purity of 95.8% and a yield of 28.7%. While this represented a modest increase in yield, the purity decreased by 1.6%.

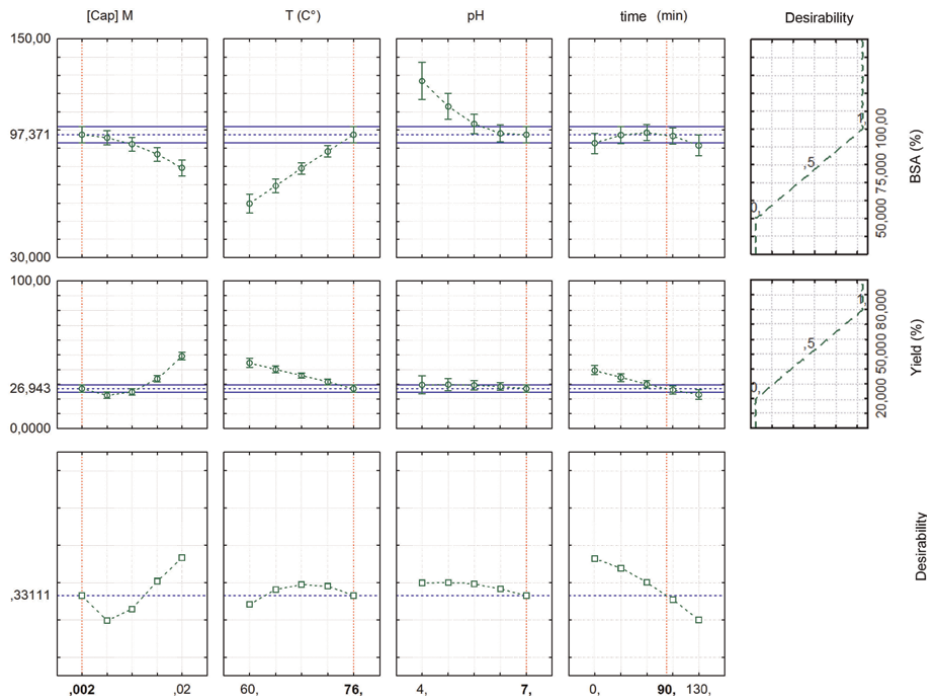


Figure 14.
Prediction profiles for estimated % BSA, yield, and desirability with $[Cap] = 0.002 \text{ M}$ and $t = 90 \text{ minutes}$.

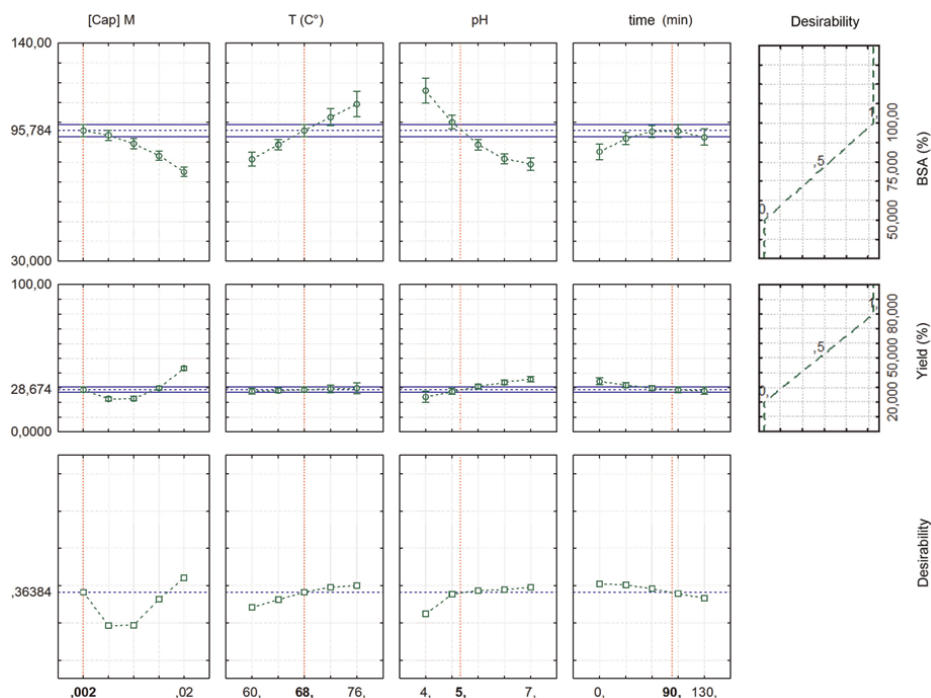


Figure 15.

Prediction profiles for estimated % BSA, yield, and desirability with $[Cap] = 0.002\text{ M}$, $T^\circ = 68^\circ\text{C}$, $pH = 5$, and $t = 90\text{ minutes}$.

7. Conclusions

Thermocoagulation was studied at the laboratory scale by modifying various process variables. A mathematical model was developed to determine the optimal operating conditions. The second-order full factorial model proved to be adequate for describing BSA purity. The most influential variables were pH and temperature. Maximum purity, close to 100%, was obtained at $[Cap] = 0.002\text{ M}$; $T^\circ = 76^\circ\text{C}$; $pH = 4.75$; and $t = 130\text{ minutes}$. Regardless of the temperature used, high purity could be achieved as long as the solution was maintained at a low pH. Increasing the temperature also improved purity, although to a lesser extent.

For BSA yield, a second-order model was also applied, excluding the quadratic terms for T° , pH, and time, and removing the interaction terms $[Cap] \times \text{time}$ and $pH \times \text{time}$. This reduced model was appropriate for describing yield behavior. The variables with the greatest effect were $[Cap]$ and pH. As with purity, time had a negligible impact on BSA yield. According to the model, the optimal conditions for achieving a maximum yield of 73.77% were: $[Cap] = 0.02\text{ M}$; $T^\circ = 60^\circ\text{C}$; $pH = 7$; and $t = 130\text{ minutes}$. Yield increased consistently with increasing pH and caprylate concentration. The highest yields were obtained when both variables were at their maximum values, while lower temperatures also had a favorable effect.

With respect to the compromise solution between BSA purity and yield, the optimization software suggested that the optimal conditions were $[Cap] = 0.02\text{ M}$; $T^\circ = 76^\circ\text{C}$; $pH = 7$; and $t = 0\text{ minute}$, under which a purity of 81.2% and a yield of 61.6% were achieved. When the goal was to prioritize purity, the variables $[Cap] = 0.002\text{ M}$

and $t = 90$ minutes were fixed. In this scenario, optimal values for $T^\circ = 76^\circ\text{C}$ and $\text{pH} = 7$ led to a purity of 97.4% and a yield of 26.9%, demonstrating that very high purity could be attained at the expense of yield.

One of the major advantages of this modeling approach is the ability to manipulate input variables and predict outcomes depending on the desired characteristics of the final product. The use of Response Surface Methodology (RSM) not only enabled the optimization of multiple process variables simultaneously but also provided a comprehensive understanding of the interactions between them. This modeling strategy is particularly useful in the development of scalable and efficient purification protocols, where a balance between product quality and process efficiency is essential.

Furthermore, this study contributes valuable insight into the thermal stability and separation behavior of BSA, offering a practical and reproducible method for its purification from bovine plasma. The ability to generate high-purity BSA through thermocoagulation, with tunable yield, makes this method a cost-effective and industrially viable alternative to more complex chromatographic processes, especially in settings where high-throughput or limited resources are considerations.

In summary, BSA, as the predominant plasma protein in cattle, plays a fundamental role in maintaining osmotic balance and mediating the transport of a wide range of endogenous and exogenous compounds. Its structural and functional characterization not only enhances the biochemical understanding of bovine physiology but also underpins practical applications in veterinary medicine. These include its use in animal reproduction, diagnostic assays, hypoalbuminemia, therapeutic formulations, vaccines, and as a model protein for the study of metabolic processes and pathological conditions in cattle. Therefore, research on BSA contributes both to fundamental science and to the advancement of veterinary practice and animal health.

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Conflict of interest

The authors declare no conflict of interest.

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*Rita Payan Carreira,
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