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ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE



MVSc THESIS

**CASE REPORTS ON SELECTED CLINICAL CASES OF ANIMALS IN AND
AROUND BISHOFTU, ETHIOPIA**

BY:

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JUNE 2023
BISHOFTU, ETHIOPIA

ADDIS ABABA UNIVERSITY

COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

DEPARTMENT OF VETERINARY CLINICAL STUDIES

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AROUND BISHOFTU, ETHIOPIA**

BY

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A thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Clinical Medicine

**DEPARTMENT OF CLINICAL STUDIES, MVSc IN VETERINARY CLINICAL
MEDICINE**

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LISTS OF ABBRVIATIONS

AAU	Addis Ababa University
BW	Body Weight
CM	Clinical Mastitis
CMT	California Mastitis Test
CPV	Canine Parvovirus
CVMA	College of Veterinary Medicine and Agriculture
DNS	Dextrose Normal Saline
EL	Epizootic Lymphangitis
FMD	Foot and mouth disease
GIP	Gastrointestinal parasite
HBS	Hemorrhagic Bowel Syndrome
IVF	Intravenous Fluid
ND	Newcastle Disease
OPV	Ovine Papilloma Virus
PCR	Polymerase Chain Reaction
PH	Potential of hydrogen
SPANA	Society for the Protection of Animal Abroad
VTH	Veterinary Teaching Hospital
XLD	Xylose Lysine Deoxycholate

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ABSTRACT

The majority of Ethiopians rely on livestock for their livelihood, but its viability and sustainability are limited by a variety of constraints, the most significant of which are livestock diseases. This case study was done with the objective of handling and organizing case reports on selected clinical cases involving various animals in and around Bishoftu town, Ethiopia. The study was conducted from November 2022 to June 2023 in clinics: Professor Feseha Gebreab Memorial Veterinary Teaching Hospital (VTH) and Society for the Protection of Animals (SPANNA) Equine Clinic found in the College of veterinary medicine and agriculture (CVMA) and on a private farm in Bishoftu town and its vicinity. The examination method involves owner complaints, clinical examinations, and postmortem examinations. Animals were treated with broad-spectrum antimicrobials and supportive drugs based on a tentative diagnosis. Simultaneously, samples from the individual animals were collected and processed to reach a definitive diagnosis. The cases were followed until the animal recovered, either in the clinic or at home. A total of 51 animals were examined, of which 43.1% were ruminants and 56.9% were equine, swine, chicken, and canine. Based on clinical findings and laboratory results, the diseases in the presented cases were caused by bacteria, viruses, fungi, protozoa, parasites, metabolic disorders, and tumors. Of the treated animals during the study 80.4% of the treated animals were recovered, 15.7% (six chickens, one horse, and one puppy) died, and two heifers remained refractory. It was observed that the primary causes of clinical cases were bacteria, followed by viruses, metabolic abnormalities, and parasites. In general, most animal diseases might be cured with prompt and appropriate care and treatment. In this study, delay of admission of diseased animals, lack of confirmatory diagnosis and appropriate treatment were the cause of delay recovery and animal death. It is recommended to perform a confirmatory diagnosis in order to initiate efficient treatment and control measures that protect the health and welfare of animals and restrict the transmission of disease.

Keywords: *Case description, Case management, Treatment outcome.*

1. INTRODUCTION

Ethiopia's economy is based on agriculture, which also happens to be the sector that generates the most foreign exchange. More than 85% of Ethiopian rural households, who engage in subsistence crop and animal production, rely on this sector as their main source of income (Adam *et al.*, 2018). Ethiopia has the largest livestock population in Africa and has an estimated 70.3 million cattle, 42.9 million sheep, 52.5 million goats, 11.3 million equines, 8 million camels, and 60.50 million poultry (CSA, 2020; Mekuriaw and Harris-Coble, 2021). 65% of Ethiopia's population depends on the livestock industry for their livelihood, contributing 12 and 40% of the country's total and agricultural Gross domestic product (GDP), respectively. Additionally, the industry generates 12–15% of all export revenue, ranking second in importance. (Jibat *et al.*, 2015).

The animal's production and productivity are greatly affected by livestock disease, limited grazing resources, climate changes, and lack of access to veterinary services (Nuvey *et al.*, 2023). Ineffective control of livestock diseases, poor veterinary services, and disease management strategies adopted by farmers are some of the factors that result in direct economic losses due to excessive animal mortality (Ashfaq *et al.*, 2015). They also affect the animals' reproductive and productive performance and may also contribute to antimicrobial resistance (Lamy *et al.*, 2012). According to Asresie and Zemedu (2015), the yearly livestock mortality rates for cattle, sheep, and goats are 8–10%, 14–16%, and 11–13%, respectively. Among diseases affecting the livestock sector of the country, Viral diseases such as Foot-and-mouth disease, Capri pox virus diseases, PPR, Contagious Ecthyma, AHS, and rabies are more common, while bacterial diseases such as Anthrax, blackleg, CBPP, CCPP, Pasteurellosis, IBK, Brucellosis, clinical mastitis, dermatophilosis, actinobacillosis, actinomycosis, foot rot and abscess are also reported (Asmare *et al.*, 2016). Additionally, gastro-intestinal tract (GIT) parasites and tick born haemoparasitic diseases such as Babesiosis, Theileriosis, Anaplasmosis and coudriosis have been reported in different regions of the country (Nejash, 2016).

Veterinary clinical studies seek to advance scientific knowledge and find the most effective methods for preventing, diagnosing, and treating diseases and other disorders that could affect

animals. A clinical investigation further contributes to the understanding of animal diseases and the discovery of new techniques of diagnosing and treating those diseases. Outcomes or findings from clinical studies can help future animal care by providing information regarding the benefits and risks of therapeutic, preventive, or diagnostic products or interventions currently applied. Furthermore, because some animal diseases are zoonotic, studying these diseases through veterinary clinical studies might help provide information for better public health services. Generally, individual animal diagnosis and treatment are critical in order to minimize suffering, enhance welfare, promote production, reduce the danger of zoonosis, satisfy owners and control disease spread (Cannavan, 2014).

Previous research conducted in the study area at the College of Veterinary Medicine and Agriculture Veterinary Teaching Hospital (CVMA-VTH) and Ada'a district veterinary clinic revealed that nearly all patients (96.6%) received drug therapy after being tentatively diagnosed without receiving a correct laboratory-supported diagnosis (Beyene *et al.*, 2015). This led in the emergence of antimicrobial-resistant disease-causing organisms and the possibility of drug residue, both of which had an impact on public health (Takele *et al.*, 2015). As a result, confirmed diagnosis and proper drug prescription are required to protect the health of both animals and humans. In addition, the majority of cases are curable and treatments are affordable, thus it is crucial to take action. Therefore, this case study was carried out with the following objectives:

General objective

The general objective of this work was to handle and organize case reports on selected clinical cases of different animals in and around Bishoftu, Ethiopia.

Specific objectives

- ✚ To verify the primary cause of animal diseases
- ✚ To treat the animals by the available drug
- ✚ To give professional advices to the owner of animals
- ✚ To document clinical animal diseases for future references and studies

2. MATERIALS AND METHODS

2.1. Study Area and Study Animals

This case study was conducted from November 2022 to June 2023 at Professor Feseha Gebreab Memorial-VTH and SPANA animal clinics located at Addis Ababa University College of Veterinary Medicine, Bishoftu campus. The study also included clinically diseased animals from surrounding peasant associations near Bishoftu town. Bishoftu is located in the East Shewa zone of the Oromia region, 47.9 km south-east of Addis Ababa. The absolute location of Bishoftu is $8^{\circ} 45' N$ latitude and $38^{\circ} 59' E$ longitude. Topographically, the town is located in tepid to cool sub-moist mid-highland at an altitude of about 1920 meters above sea level with moderate weather conditions and experiences the mean annual rainfall, maximum, and monthly temperatures range between 801.3mm, $25.50^{\circ} C$, $23.7^{\circ} C$ in July, and $27.70^{\circ} C$ in May. Additionally, Bishoftu and its surroundings have variable and yet representative agro-ecologies of the country. Bishoftu town is known for its animal production and medication center. Cattle, small ruminants, poultry, equines, and pet animals are the major livestock species reared in the area. Most of the animals in the town are kept intensively, while those in the rural areas are kept semi-intensively. Frushka, household remnants, and straw are the regular feed of the animals kept intensively, while semi-intensively kept animals graze on communal grazing land.

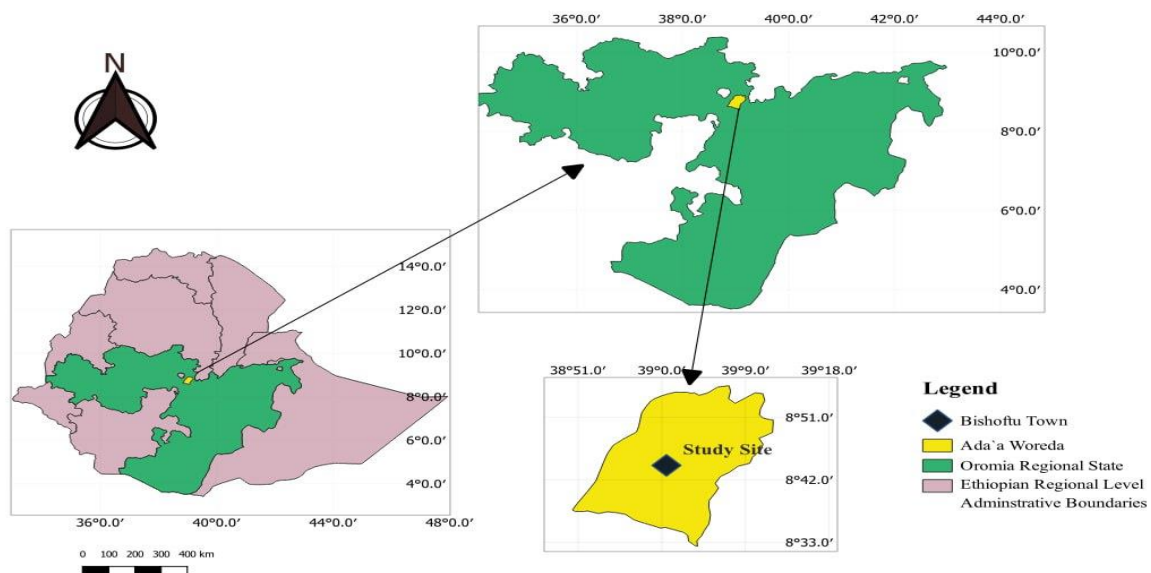


Figure 1: Map of study Area

2.2. Study Design and Approaches

Descriptive types of case study design were performed to understand the details of the study subject by describing the signaling of the affected animal and a detailed description of the clinical signs, diagnosis, treatment, and follow-up of individual animals. Clinically diseased animals which were coming to the clinics and observed on farm visit during the study period affected with frequently occurred and economically important diseases were purposively selected for the case study. Clinical examinations of the individual animal or groups of animals were begun with a history of the case and followed by a complete clinical examination, which included a broad search for abnormalities starting from head to tail (Abdeta *et al.*, 2015). Organs or systems involved, location and type of lesion present, and severity of diseases were identified thoroughly (Jackson and Cockcroft, 2002; Jana and Ghosh, 2013). The most important details are that tentative diagnoses were asserted based on the information obtained during the clinical examination and the owner's complaint. In some cases, illustrative samples from the affected individual were collected, and appropriate laboratory procedures were followed. After complete clinical diagnosis, postmortem examinations were continued and treatments were taken, but sometimes treatments were given empirically before definitive diagnosis due to time and logistical factors. Finally, advising the owner and following up took place until healing or death took place (Constable & Kenneth W. Hinchcliff). Stanley H. Done, Walter Grünberg, 2017; Jackson & Cockcroft, 2007). The information for all cases was obtained using the standard case recording format and compiled using the case report compilation format. Each case was supported with a summary, introduction, case description, laboratory investigations and findings, case management and treatment outcome, and discussion. Each case was supported with a picture of the animals during presentation and after recovery.

3. COMPILED CASES

A total of 21 cases comprised of 51 total animals were compiled in these case reports (table 2)

Table 1: Total number of animals examined and treatment outcome

Animals	Total	Recovered	Died	Refractory
Ruminants	22	20	0	2
Equine	2	1	1	0
Canine	3	2	1	0
Swine	8	8	0	0
Chicken	16	10	6	0
Total (in percentage)	51	41(80.4%)	8(15.7%)	2(3.9%)

Table 2: Diseases encountered during the study in percentage

Diseases	Number of cases	Number of affected animals	Percentages (%)
Bacteria	9	22	43.1%
Virus	5	18	35.3%
Fungus	1	1	2%
Protozoa	1	2	3.9%
Parasites	2	2	3.9%
Metabolic	2	5	9.8%
Tumor	1	1	2%
Total	21	51	100%

3.1. Case Reports on Bovine

3.1.1. Hemorrhagic bowel syndrome in exotic dairy cow

Abstract

Hemorrhagic bowel syndrome (HBS), also known as Jejunal Haemorrhage Syndrome (JHS), is an important acute enterotoxemic disorder of adult dairy cattle. The present case report describes the clinical signs, laboratory findings, and treatment outcome of the cow affected by HBS. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A 5-year-old exotic dairy cow on one of the smallholder dairy farms in Dukem with a history of profuse dark watery faecal matter and having completely stopped eating feed but drinking water one week ago was presented for diagnosis. Rectal temperature, heart rate, and respiration rate were measured and found to be within the normal range. Clinical signs during diagnosis include: dark watery diarrhoea, a sunken eye, depression, dehydration, and a pale conjunctival mucus membrane. Faecal and blood samples were collected, and faecal floatation technique and faecal culture on XLD, respectively, for detection of *Eimeria/Coccidian* oocytes and *Salmonella* were performed. Blood and faecal samples were also cultured on blood agar medium for the detection of *Clostridium*. It was negative for coccidiosis and salmonella, but growths of small and white colonies with hemolysis and gramme-positive small to medium-sized rod-shaped bacteria were observed, which was indicative of *Clostridium*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as hemorrhagic bowel syndrome. The cow was treated successfully with fluid therapy: Dextrose Normal Saline (DNS) and Ringer Lactate (10 ml/kg) for two days, I/V. Antibiotic: procaine penicillin G (20,000 IU) for five consecutive days, I/M. Anti-inflammatory: Meloxicam (0.5 mg/kg) for three days, I/M, and Multivitamin (8 ml) in a single shot, I/M. The improvement was noted one-week post-treatment, and the cow was fully recovered. Avoiding stress during early lactation, providing quality food, and having an adequate ratio of concentrate to roughage are recommended to reduce chance of exposure to infection.

Key word: Cow, *Clostridium*, Gram stain, HBS, treatment

Introduction

Hemorrhagic bowel syndrome (HBS), also known as jejunal hemorrhagic syndrome (JHS) is relatively common disease in dairy cattle which has high fatality rate ranging from 85 to 100% (Radostits *et al.*, 2007; Kirbas and Ozdemir, 2011). It is characterized by acute, sporadic and necro hemorrhagic enteritis that primarily affects the small intestine (Owaki *et al.*, 2015). The disease is first described in the US in 1991 and commonly seen in highly productive dairy cattle (Abutarbush *et al.*, 2004; Valgaeren, 2017). It is a multi-factorial disease. *C. perfringens* is a gram-positive, anaerobic, oxygen-tolerant, rod-shaped bacterium that is one of the causes of hemorrhagic enteritis (Lucey and Hutchins, 2004; Lebrun *et al.*, 2010). There are five types of *C. perfringens* (A, B, C, D, E), which are distinguished by the primary toxin types they generate (alpha, beta, iota, epsilon, and theta) (Bueschel *et al.*, 2003; Hamouda *et al.*, 2013).

C. perfringens type A is the most common and normal inhabitant of the intestine of most animals and humans. However, dietary changes or parasitism may produce a favorable growth environment, resulting in overgrowth and production of potent toxins, primarily α -toxin and β -toxin (Kirkpatrick *et al.*, 2001; Hamouda *et al.*, 2013; Peek *et al.*, 2018). *Aspergillus fumigatus* has also been suggested as the potential cause of HBS, as these organisms have been isolated from the lesions of clinical cases (Reeves *et al.*, 2004; Adaska *et al.*, 2014; Owaki *et al.*, 2015). The possible risk factors of HBS include: stage of lactation, season, herd size, forage quality, ration composition and feeding management (Godden *et al.*, 2003; Ewoldt and Anderson, 2005).

The pathogenesis of the HBS disease is associated with overfeeding of proteins and the overgrowth of the bacterium, *C. perfringens*, leading to toxin production and cell damage (Mamak and Borku, 2019). Inflammatory responses disrupt the intestinal barrier, leading to the secretion of plasma proteins and growth factors into the intestinal lumen and bacterial overgrowth, resulting in toxemia and death (Valgaeren, 2017; Elhanafy *et al.*, 2013). Affected animals generally have sudden onset of the disease with symptoms of anorexia, depression, decreased milk production, bloody to dark-red feces, abdominal distension, dehydration, weakness progressing to recumbency and sunken eye are the common findings in cows with HBS (Van Metre and Callan, 2005; Tajik *et al.*, 2010). Furthermore, pale mucous membranes,

elevated respiratory and heart rate, and atonic rumen are also reported other clinical findings in cows with HBS (Ceci *et al.*, 2006; Radostits *et al.*, 2007; Braun *et al.*, 2010).

Diagnosis of HBS is based on history, clinical signs, transabdominal ultrasonography and gross postmortem findings, but confirmation requires laboratory testing. ELISAs are one of the most important laboratory techniques for *C. perfringens* toxins detection (Kirkpatrick *et al.*, 2001; Dennison *et al.*, 2002; Uzal and Songer, 2008; Peek *et al.*, 2018). Common causes of intraluminal enteric hemorrhage in adult cattle include intussusception, volvulus, salmonellosis, bovine viral diarrhea and coccidiosis (Peek *et al.*, 2018). It is rare for HBS disease to be successfully treated, however sick animals have received immediate care with intravenous fluids, calcium salts, anti-inflammatory medications, antibiotics (penicillin), and surgery (Ceci *et al.*, 2006; Tajik *et al.*, 2010; Elhanafy *et al.*, 2013). Preventive strategies have not yet been established, and the precise etiology of HBS is unknown. For illness prevention, it is advised to utilize feed additives, practice proper nutritional management, and administer vaccinations (Mamak and Borku, 2019). “The present case report describes the clinical sign, laboratory findings and the treatment outcome of the cow that affected by Hemorrhagic bowel syndrome”.

Case description

An exotic dairy cow of around five years old was examined at Dukem in one of the smallholder dairy farms on December 17, 2022, with a history of profuse dark watery fecal matter and completely stopping eating feed but drinking water one week ago. On clinical examination, the body parameters of the cow were: temperature 38.4 °C, respiratory rate 24 breaths per minute, and pulse 64 beats per minute. The cow had dark diarrhea, a sunken eye, depression, dehydration, and a pale conjunctival mucus membrane (Figure 2). Based on the history and clinical findings, lists of differential diagnoses were made, including coccidiosis, hemorrhagic bowel syndrome, and salmonellosis, tentatively.



Figure 2: A dairy cow with profuse dark watery diarrhea

Laboratory investigation and findings

The fecal and blood samples were collected directly from the rectum and jugular veins, respectively. The samples were submitted and processed at the Microbiology and Parasitology Laboratory of Addis Ababa University College of Veterinary Medicine, Bishoftu. The fecal floatation technique was performed for the detection of Eimeria/Coccidian oocysts, and the result was negative. The fecal sample was cultured on xylose lysine deoxycholate (XLD) and incubated aerobically at 37°C for 24 hours for the detection of Salmonella and result was negative by the colonies' characteristics. On the same day, collected blood and fecal samples were cultured on blood agar medium and incubated anaerobically (candle jar method) at 37°C for 48 hours. Growths of small and white colonies with hemolysis were seen on the media (Appendix). Gram-positive small to medium-sized rods were seen under oil immersion (100X) on the prepared smears. These bacteria were identified as *Clostridium* (Figure 3). Finally, it was determined that hemorrhagic bowel syndrome by *Clostridium* was the final diagnosis based on the history, nature of the disease, clinical findings, and laboratory findings.

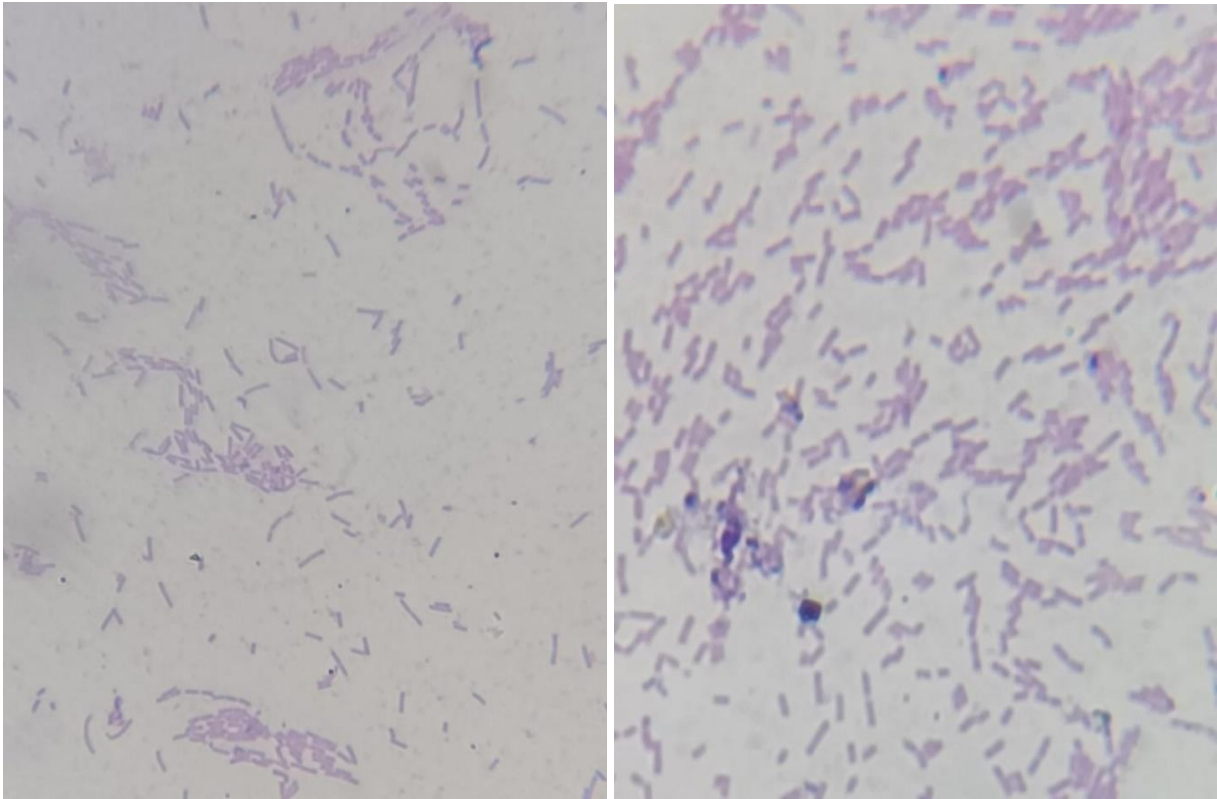


Figure 3: A Gram-positive bacilli or short rod-shaped bacteria

Case management and treatment outcome

Supportive therapy was used as a line of treatment, and the cow was treated with fluid therapy: Dextrose Normal Saline (DNS) and Ringer Lactate (Addis Pharmaceutical Factory PLC) at 10 ml/kg for 2 days, I/V. Antibiotic: procaine penicillin G (EPHARM, Addis Ababa, Ethiopia) at a dose of 20,000 IU for five consecutive days, I/M. Anti-inflammatory: Meloxicam (Ashish Life Science Pvt. Ltd.) at a dose of 0.5 mg/kg for 3 days, I/M and Multivitamin (Aether Center Beijing Biology Co., Ltd.) at 8 ml in a single shot, I/M. Forty-eight hours post-treatment, the cow was presented with a rectal body temperature of 38.4 °C. The cow's appetite also returned to normal. Generally, considerable change was appreciated post-therapy, the color of the feces returned to normal and after two week of post treatment the cow was fully recovered (Figure 4).



Figure 4: Indicating fully recovered cow with normal Colour of the faeces after three week of post treatment.

Discussion

The definitive cause of disease in cattle has not been identified (Lejeune, 2004), Initial reports (Godden *et al.*, 2001; Kirkpatrick *et al.*, 2001) speculated that *C. perfringens* type A was associated with HBS and more recently *A. fumigatus* has been implicated as well (Wang, 2004). Again, Kirkpatrick *et al.* (2001) have suggested that HBS is a multi-factorial disease.

The current case was asserted as haemorrhagic bowel syndrome based on the history, nature of the disease, clinical findings, and laboratory results (bacteriology examination). Stress associated with high-intensity milk production, increased energy in the ration, and decreased fiber in the diet have been mentioned previously by Callan (2002) and Berghaus *et al.* (2005) as risk factors for HBS. In the current case report, the history indicated that the cow developed the

case in the first week after parturition, so the stress associated with parturition caused immunocompromise that might have contributed to the occurrence of HBS because this bacterium (*C. perfringens*) is normal flora of the intestine of livestock (Smith *et al.*, 2019). This environment promotes bacterial multiplication, which results in the release of large amounts of toxins (Filho *et al.*, 2009). Dennison *et al.* (2002), and Abutarbush and Radostits (2005) reported that the alpha and beta toxin is the primary toxin responsible for inflammation of the intestine as well as partial loss of the mucosa with respect to hemolysis and necrosis.

In the current case study, clinical signs such as anorexia, depression, decreased milk production, dark watery diarrhea, dehydration, sunken eyes, and a dry muzzle were noted, which were similar to those described in the literature (Ceci *et al.*, 2006; Braun *et al.*, 2010; Owaki *et al.*, 2015) for hemorrhagic bowel syndrome. *Clostridium* was identified after anaerobically incubating the fecal and blood samples at 37°C for 48 hours in 5% blood agar media. Similar to other authors' findings (Dennison *et al.*, 2002; Callan, 2002; Tajik *et al.*, 2010), the colony features of *Clostridium* detected on blood agar showed tiny, white, dry colonies with hemolysis. The morphology of the isolated *Clostridium* showed gram-positive, short to medium-rod-shaped bacilli in Gram's staining. Several scholars, including Ceci *et al.* (2006), Hamouda *et al.* (2013), and Adaska *et al.* (2014), concurred with these findings. It is important to point out that the absence of a diagnostic kit in the compound prevented this study from doing further diagnostic tests to identify *A. fumigatus*, which is one of the other causative agents for HBS.

The cow in this case report was treated successfully with fluid therapy: dextrose-normal saline (DNS) and Ringer lactate (10 ml/kg) I/V to correct hydration status. Antibiotic: procaine penicillin G (20,000 IU) I/M. Anti-inflammatory: Meloxicam (0.5 mg/kg) I/M for pain relief and controlling inflammation in the gastrointestinal mucosa, and Multivitamin (8 mL) I/M to open the appetite. The improvement was noted one-week post-treatment, which is in agreement with a case reported by McGuirk (2014), Mamak *et al.* (2019), and Smith *et al.* (2019), they report medical management of intravenous fluid therapy, parenteral antibiotics, and nonsteroidal anti-inflammatory drugs were the main treatments for HBS in cattle.

In conclusion, *Clostridium* was the cause of HBS in this dairy cow case report. The cow's general clinical condition improved and started milking after full treatment. This indicates that, HBS in dairy cow could be successfully treated with aggressive multimodal therapy as shown in this case. Prevention of stress during early lactation, providing quality food and an adequate ratio of concentrate and roughage are recommended as feasible interventions.

Acknowledgement

I am grateful to Mr. Dereje Gudeta and Mis. Tesfanesh for their contribution and the owner of the animal for his valuable information during the follow up.

3.1.2. *Clinical mastitis in exotic cow*

Abstract

Mastitis is defined as an inflammatory reaction of udder tissue and is the most common disease in dairy cattle worldwide. This case report summarizes the case of clinical mastitis in this dairy cow. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A 6-year-old exotic dairy cow was examined at Bishoftu by one of the privately owned smallholders with a history of slight color changes in milk, reduced appetite, and depression that started four days ago. The rectal body temperature was raised (40.6 °C), while other parameters were within the normal range. Clinical signs during diagnosis include: left and right front quarters appeared slightly swollen, feeling pain and warmth during palpation, and the milk color changed. Milk samples were collected from all quarters: CMT test, catalase test, Milk culture on Mannitol salt agar, and Nutrient agar were performed. It was positive for CMT and catalase test, growths of colonies with a round, smooth, shiny, opaque, golden yellow color and gram-positive cocci arranged in grape-like clustered bacteria were observed which was indicative of *Staphylococcus aureus*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as clinical mastitis. The cow was treated with penstrip (1 ml/20 kg/day) for five successive days and dexamethasone (0.2 mg/kg/day) for three days, both I.M. The cow responded to the treatment, and two weeks later fully recovered. Although early intervention with an appropriate drug could cure the clinical mastitis case, pre- and post-milking teat dipping and keeping the animals' environment clean and dry could mitigate the risk of the problem occurring in the first place.

Keywords: *Cow, Clinical mastitis, Golden yellow Colonies, Grape like clusters, penstrip*

Introduction

Bovine mastitis is one of the most endemic infectious diseases of dairy cattle though worldwide (Petrovski *et al.*, 2006). It affects the milk production and quality, and the second most important next to reproductive disorders (Aweke and Mekbib, 2017; Abebe *et al.*, 2020). It is an inflammation of the parenchyma of the mammary gland and caused by *S. aureus*, *S. agalactiae*, *Mycoplasma spp*, *C. bovis*, *S. uberis*, *S. dysgalactiae*, *Pseudomonas spp*, and *E. coli* (Klimienė *et al.*, 2011; Ganguly, 2014; Kibebew K, 2017). These organisms are usually found in the environment of the cow; hence they can easily be contracted by the udder (Bradley, 2002).

Mastitis is universally classified as clinical and subclinical mastitis (Mungube, 2004; Siivonen *et al.*, 2011). There are several degrees of severity at which the clinical form of mastitis might manifest. Milder cases result in milk exhibiting clinical abnormalities such as clots, flakes, or color changes (Lakshmi, 2016). The clinical signs in most severe cases are fever, lack of appetite, melancholy, lethargy, decreased milk production, swelling, redness, and mammary gland atrophy (Fogsgaard *et al.*, 2015; Ganguly, 2014). Subclinical cases are not show any clinical signs and major cause of infection for other farm animals due to its difficulties in detection. When the cow is in heat or when environmental conditions change, many subclinical cases may develop into clinical cases, making the disease 15 to 40 times more prevalent than CM (Saroj *et al.*, 2015). It results in low milk quality and a 10–20% drop in total milk yield (Radostits *et al.*, 2007).

The most common diagnostic methods are CMT and bacteriological examination (Ganguly and Praveen, 2016; Moroni *et al.*, 2018). Intramammary infusion or parenteral injection of antibiotics such as streptomycin, ampicillin, cloxacillin, penicillin, and tetracycline are frequently used as the main treatments for mastitis (Bhosale *et al.*, 2014). The effective treatment of bovine mastitis depends on the antimicrobial susceptibility of the pathogens, the type of mastitis, the cattle breed, and the treatment regimen (Barkema *et al.*, 2006). Additionally, PCG (penicillin G) and PLM (pirlimycin), a lincomycin antibiotic that has just been approved as an antibacterial and is thought to be quite effective and is appropriately suggested as the first-line mastitis therapy (Kawai *et al.*, 2023; Tomanic *et al.*, 2023).

The most common mastitis control and prevention measures are keeping the hygiene of the milker and the machine, segregation of the infected animal, culling the chronically infected animal and use effective treatment (Jamali *et al.*, 2018). Mastitis results in significant milk losses (wasted milk), decreased milk quality, greater treatment and veterinary costs, a higher risk of mortality and culling of the afflicted dairy cows, and other costs to dairy farmers and the milk processing industry (Elmaghraby *et al.*, 2017; Moroni *et al.*, 2018). “This case report summarizes the case of clinical mastitis in dairy cow”.

Case descriptions

A 6 years old exotic cow was examined at Bishoftu in one of the privately owned smallholders on November 9, 2022, with a history of slightly colored milk, reduced appetite, a lesion on the teat, and depression that started five days ago. The cow was managed intensively and had previously been infected with FMD. Upon physical examination, the rectal body temperature was raised (40.6 C), while other parameters were normal. On palpation, the left and right front quarters showed signs of minor swelling, reddening, pain, and warming, as well as a slight milk color shift (figure 5). Based on the history and clinical findings, mastitis was tentatively diagnosed.



Figure 5: Indicating swollen, small lesion, firm, reddened, painful and warm teat.

Laboratory investigations and findings

The milk samples were collected into a sterile test tube from all four quadrants and checked immediately with CMT reagent; the samples from the left and right front quarters were positive (formed gel reaction) (figure 6 A), and CMT-positive samples were cultured on mannitol salt agar and nutrient agar and incubated aerobically at 37°C for 24 hr. Growths of colonies with round, smooth, shiny, opaque, golden yellow color were observed on both medium (Appendix) and stained by Gram's stain revealed Gram-positive cocci arranged in grape-like clusters under a 100x binocular microscope (figure 6 B). Again, the media was subjected to the catalase test (using 3% hydrogen peroxide) and showed positive results (i.e., production of bubbles) (Figure 7). Finally, it was concluded based on the history, clinical findings, and laboratory findings that the bacterium was *Staphylococcus aureus*, and the final diagnosis was clinical mastitis.

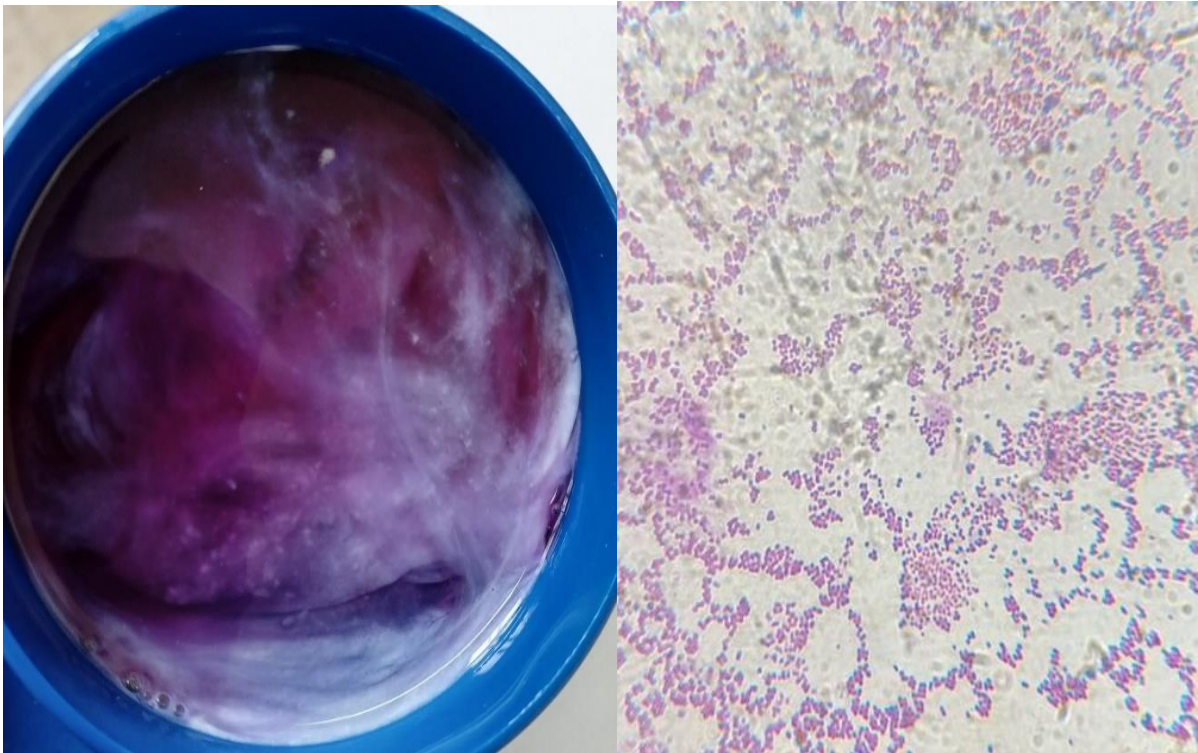


Figure 6: Indicating gel formed milk by CMT test (A) and Gram-positive cocci arranged in grape like clusters (*Staphylococcus aureus*) (B).



Figure 7: Indicating positive catalase test (i. e. production of bubbles)

Case management and treatment outcome

The cow was treated with penstrip (Chongqing Fantong Animal Pharmaceutical Co. Ltd., China) for five successive days at 1 ml/20 kg/day and dexamethasone sodium phosphate (Sokar Healthcare Pvt. Ltd., Gujarat, India) at 0.2 mg/kg/day for one day, both I.M. Besides the drug treatment, the owner was advised to strip the teat, wash the udder with warm water, and discard the dirty material cautiously. Post-treatment, fever, clotty milk from the affected teat, and swelling of the udder were not observed. Two weeks later, the cow was fully recovered (Figure 8).



Figure 8: Fully recovered teat and udder after one week of post treatment

Discussion

The current case was asserted as mastitis based on the history, clinical findings, and laboratory results (CMT, bacteriology examination, and catalase test). According to previous reports by Almaw *et al.* (2008), Iraguha *et al.* (2015), Mureithi and Njuguna (2016), and Rajabi *et al.* (2017), poor cow hygiene can contribute to the presence of mastitis pathogens on teat ends and increase the rate of new infections. In the present case study, the age, breed, lesions caused by a previous FMD infection, dirtiness of the udder and hind legs, and poor hygiene of the cows' environment could be contributed for disease occurrence. Fever, inappetence, depression, decreased milk yield, milk color change, swelling, redness, and feeling pain when palpating the mammary glands were among the clinical signs noted in this case report that were similar to those described in the literature (Saroj *et al.*, 2015; Fogsgaard *et al.*, 2015; Shashank *et al.*, 2020).

In the current case study, udder milk samples from each quarter were placed in each cup on the CMT paddle, and then equal amounts of the 3% CMT reagent were added to each cup and thoroughly mixed. There was a positive reaction (formation of gel), as stated in the CMT reports by Saidi *et al.* (2013), Mwabonimana *et al.* (2015), and Birhanu *et al.* (2017). The CMT-positive milk samples were cultured on nutrient agar and mannitol salt agar. The observed colony characteristics were smooth, shiny, opaque, and golden yellow, which was similar to the colony characteristics of *Staphylococcus aureus* found by Mekibib *et al.* (2010), Belayneh *et al.* (2013), and Ryman *et al.* (2021). The Gram staining characteristics were gram-positive, cocci-like, and arranged in grape-like clusters. These findings were in agreement with several authors, such as Haftu *et al.* (2012), Ganguly and Praveen (2016), and Brennecke *et al.* (2021). The isolates also revealed positive reactions in catalase tests, which was in agreement with the reports of Cortinhas *et al.* (2016), Birhanu *et al.* (2017), and Brennecke *et al.* (2021).

In the current case study, penstrip was administered parenterally (1 ml/20 kg/day) for five days along with dexamethasone (0.2 mg/kg/day) to manage the mastitis case of the affected cow. The improvement was noticed one week after treatment, and the cow was fully recovered, which is consistent with cases reported by Barkema *et al.* (2006), Bhosale *et al.* (2014), and Shashank *et*

al. (2020), who report intramammary infusion or parenteral administration of streptomycin, ampicillin, cloxacillin, penicillin, and tetracycline for mastitis cases in dairy cows.

In conclusion, *Staphylococcus aureus* was the cause of mastitis in this dairy cow case report. The cow's general clinical condition improved and started milking after full treatment. This indicates that, mastitis in dairy cow could be successfully treated with penstrip along with dexamethasone as shown in this case. Raising owner knowledge, creating a clean and dry environment for the animals, post-milking teat dipping, and isolating infected cows are recommended as workable interventions for the successful control and prevention mastitis case

Acknowledgement

I am grateful to Mis. Tsedale Teshome and Mis. Tesfanesh for their contribution and again the owner of the animal for his valuable information during the follow up.

3.1.3. Blackleg in a bull

Abstract

Blackleg is an infectious disease of cattle between 6 months and 2 years of age and is rare in other ruminants, with occasional cases occurring in animals outside this age range. It is caused by *Clostridium chauvoei* and is characterized by necrotizing myositis. This case report describes the clinical signs, laboratory findings, and treatment outcomes of the bull affected by blackleg. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A two years old local breed bull with a history of reduced feed intake and difficulty walking started a week ago was examined at FGM-VTH. The body temperature was raised (40 °C), while other parameters were within the normal range. Clinical observations were depression, dry muzzle, difficulty moving its limbs, pain reaction, and emphysematous swelling with crepitation sounds while palpating around the middle gluteal and the longissimus dorsi muscles. The collected exudate and blood sample were cultured on 5% sheep blood agar. Growths of small, white, dry, and narrow hemolytic colonies and gram-positive, numerous large rods, thick, straight round-ended, occurring singly or in short chains were observed which was indicative of *Clostridium*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as blackleg. The bull was treated with procaine penicillin G (20,000 IU/kg/day) for five consecutive days and dexamethasone (0.2 mg/kg/day) for three days, both intramuscularly, and was successfully cured at the end of the therapy. Although early intervention with an appropriate drug could cure the animal, vaccination of all susceptible animals against black leg before the anticipated danger period is recommended.

Keywords: Bull; Black leg; Gram positive, Procaine penicillin

Introduction

Blackleg is a very lethal, febrile bacterial illness that mostly affects cattle between the ages of 6 months and 2 years. It can also sometimes afflict sheep, goats, pigs, camels, deer, and horses, as well as sheep, goats, and animals outside of these age groups (Uzal *et al.*, 2003; Snider and Stern, 2011). The name 'blackleg' derives from the fact that the site of infection is often a leg muscle with the affected muscle dark in color (Ziech *et al.*, 2018). It was first reported in 1870 and is caused by the gram-positive, anaerobic, extremely pathogenic *C. chauvoei* bacterium, that produces endospores and creates endospores with a lemon-shaped shape (Quinn *et al.*, 2011).

The majority of blackleg cases occur in warm months, following soil disturbance, or during years with exceptionally high annual rainfall that can uncover and activate dormant spores. Additionally, the disease is enzootic in regions that have had floods in earlier times (Useh *et al.*, 2006; Huang *et al.*, 2013). The spores of *C. chauvoei* may contaminate the soil, survive for many years in the environment, and infect grazing animals. When silage, fodder, hay, or other feed is polluted with spore-containing soil, the illness can arise in housed animals less frequently (Sathish *et al.*, 2008; Falquet *et al.*, 2013). The most frequent exposure is likely through the ingestion of *C. chauvoei* spores, and due to the disease's pathophysiology, sick ruminants cannot spread the illness to other animals directly (Radostits, 2007).

The pathogenesis of the disease begins after ingestion of *C. chauvoei* from contaminated soil as dormant spores. After being consumed, *C. chauvoei* spores travel through the digestive system before reaching the blood and muscles. When they enter the muscle, they are phagocytized by local macrophages and can wait for the right circumstances for germination (Robson, 2007b; Pires *et al.*, 2017). As soon as *C. chauvoei* reverts to its vegetative form, it generates a number of toxins, including oxygen-stable and oxygen-labile hemolysins, DNase, hyaluronidase, and neuramidase, which can cause necrotizing myositis locally and a fatal toxemia systemically. Blackleg onset and the release of toxins can also occur (Tagesu *et al.*, 2019). The toxins from vegetative bacteria destroy surrounding tissue before entering the bloodstream and killing affected individuals within 12-48 hours. Additionally, they produce gas, which forms bubbles

in the muscles and causes the skin to crackle and exude odorous gas when the animal is necropsied. The toxins will turn the muscles dark red to black and spongy and cause muscle swelling, depression, and lameness (Robson, 2007b; Tagesu *et al.*, 2019). The majority of blackleg cases are acute or sub-acute, though cases can also occur chronically (Aiello and Moses, 2016). Cardiac lesions have similar clinical signs, such as increased lung sounds and dyspnea, but congestive heart failure signs such as jugular vein distension and brisket edema are rare (Groseth *et al.*, 2011; Snider and Stern, 2011).

The diagnosis of black leg is established by history, clinical signs and gross lesions coupled with cultural biological method, PCR and immunodetection methods, including FAT and IHC (Sasaki *et al.*, 2000; Sasaki *et al.*, 2002; Abreu *et al.*, 2017). The differential diagnosis for blackleg is anthrax and malignant edema (Assis *et al.*, 2005; Quinn *et al.*, 2011). Antimicrobials (drug of choice procaine penicillin) around affected tissues, aggressive surgical debridement to allow aeration along with supportive treatment can be of value (Disasa *et al.*, 2017; Constable *et al.*, 2017). Booster dose vaccination represents the main tool of controlling the disease (Rychener *et al.*, 2017). Most of the vaccines can be found in a polyvalent formulation with *C. novyi*, *C. septicum*, and *C. sordellii* (Tolera *et al.*, 2019). In many parts of the world, it results in significant financial losses for cattle farmers. “This case report describes clinical signs, Laboratory findings and treatment outcomes of the bull that affected by blackleg”.

Case description

A two years old local breed bull from Bishoftu, Kebele 09, was brought to Professor Feseha Gebreab Memorial-VTH on December 7, 2022 with the history of reduced feed intake and difficulty of walking, starting a week ago. Up on physical examination the body temperature was raised (40. oC), while other parameters were within the normal range. The bull was very depressed; the muzzle was dry; the bull had difficulty moving its limbs; and there was pain reaction and emphysematic swelling with crepitation sound while palpating around the middle gluteal and the longissimus dorsi muscles (Figure 9). Therefore, based on the history and clinical findings, black leg was tentatively established, although other acute cases were doubted (malignant edema).



Figure 9: Indicating emphysematic swelling (A) and Dry muzzle (B)

Laboratory investigations and findings

The exudate sample from swellings and blood samples from the jugular vein were collected aseptically and submitted to the veterinary microbiology laboratory of Addis Ababa University College of Veterinary Medicine, Bishoftu. The bacteriological culture was made on 5% sheep blood agar and incubated anaerobically (candle jar method) at 37 °C for 48 hr. Following incubation for 48 hours, growths of small, white, dry colonies and narrow hemolysis were observed on medium (Appendix). The smear prepared with Gram stain revealed gram-positive large rods and thick, straight, and round-ended bacteria occurring singly or in short chains under a 100X binocular microscope and that determined to be *Clostridium* (Figure 10). Finally, it was concluded based on the history, clinical findings, and laboratory findings that the final diagnosis was black leg.

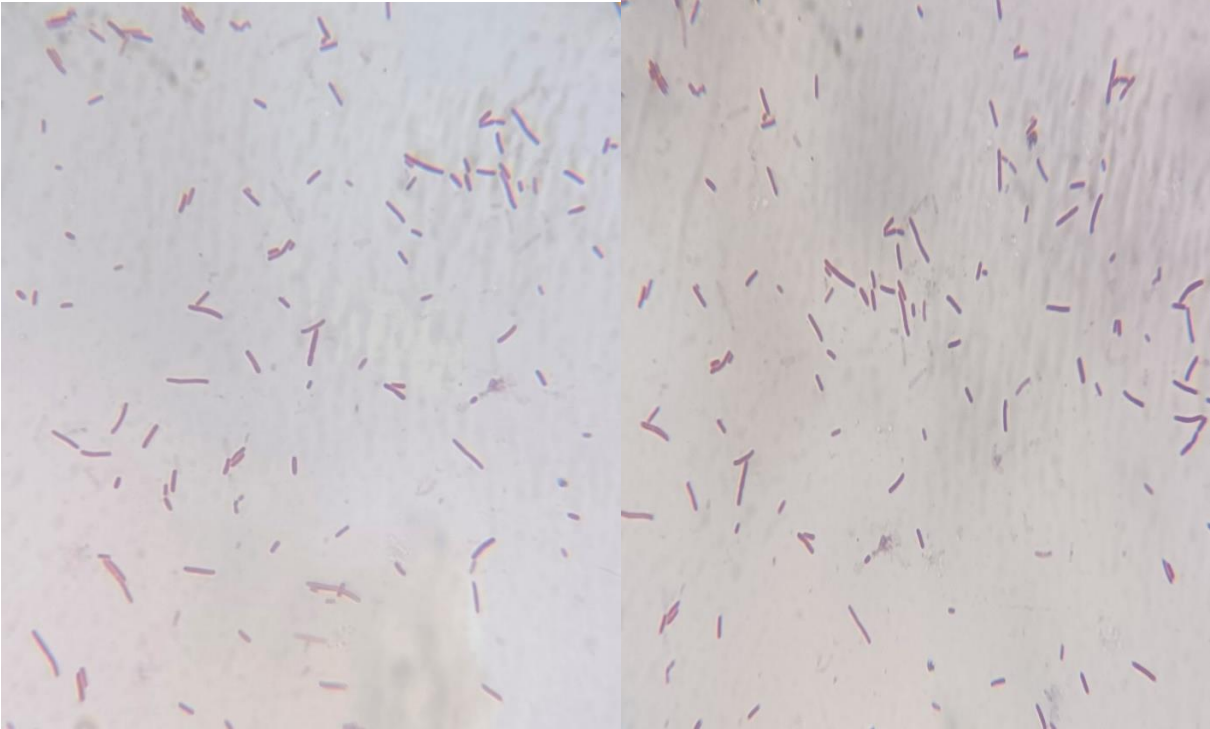


Figure 10: Indicating gram-positive and large rod-shaped bacteria

Case management and treatment outcome

The bull was managed with procaine penicillin G (EPHARM, Addis Ababa, Ethiopia) at a dose of 20,000 IU for five consecutive days and dexamethasone sodium phosphate (Sokar Healthcare Pvt.Ltd. Gujarat India) at a dose of 0.2mg/kg/day for three consecutive days both intramuscularly. Besides, the treatment the owner was advised to manage the bull at home until recovered from the disease. On the 2nd day visit the rectal body was reduced to 39.2° C and the appetite were also improved (as the owner reported). However, on the 3rd day of therapy, rectal body temperature was returned to normal. At the end of therapy, the bull was fully recovered (figure 11).



Figure 11: Indicating moist muzzle (A) and feeling of feeding of fully recovered bull after a month during follow-up period (B).

Discussion

Blackleg is an acute, febrile, and highly fatal disease of cattle caused by *Clostridium chauvoei*. In different reports, blackleg cases occurred in animals aged between 6 months and 2 years (Groseth *et al.*, 2011; Abreu *et al.*, 2017). Similarly, in this case report, the disease was found in a 2-year-old bull. This could be due to the anaerobic environment that may occur due to being hit around the thigh muscle during plowing. Based on the history, clinical signs, and laboratory results, the current case was declared to be a black leg. The clinical symptoms seen in this case report, including the sudden onset of the disease, depression, anorexia, lameness, and a crepitation sound in the area of swelling, were similar to those described in the literature (Snider *et al.*, 2011; Wickramasinghe *et al.*, 2014; Abreu *et al.*, 2017; Ziech *et al.*, 2018).

Tissue from the damaged area maintained in a screw-top bottle would have been the proper sample to send to the lab, but the owner refused. The fluid that was accumulated in the infected area was very minimal (it was only enough to do the smear). Since the bull was febrile (which could be caused by bacteremia), blood was taken in a vacutainer tube, and an anaerobic culture on 5% sheep blood agar for 48 hours in an anaerobic jar was done for confirmation of this case. Similar samples and gram stain results were reported in the literature (Lakho *et al.*, 2016; Ambhore *et al.*, 2018; and Nekrasov *et al.*, 2023).

The present case was successfully treated with procaine penicillin G at 20,000 IU for five successive days, coupled with dexamethasone (0.2 mg/kg/day) to reduce pain and fever. The improvement was noted forty-eight hours post-treatment, which is in agreement with a case reported by Nazir Zahid *et al.* (2012) and Datta and Karmakar (2017). Early intervention with an appropriate drug could cure the animal; a better option to control the disease would be getting the animals vaccinated against blackleg.

Acknowledgement

I am grateful to Dr. Mehari, Dr. Taye, and Mis. Tesfanesh for their contribution and again the owner of the animal for his valuable information during the follow up.

3.1.4. Babesiosis in a cow and puppy

Abstract

Babesiosis is a worldwide tick-borne disease of animals that is caused by hemoprotozoan apicomplexan parasites of the genus *Babesia*. This case report describes the clinical signs, laboratory findings, and treatment outcomes of a babesiosis case in a cow and a puppy. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A six-year-old local breed cow and a four-month-old male puppy were presented to Professor Feseha Gebreab Memorial-VTH with the history of blood-mixed urine, vomiting (the puppy), and decreasing feed intake, which started three days ago (the cow) and two weeks ago (the puppy). The cow had a fever, red urine, pale mucous membranes, tenesmus, and depression. Clinical signs during diagnosis of puppy include: ticks over its body, dehydrated, poor physical condition, lethargic, difficult to stand, and had icteric conjunctival and oral mucous membrane. Blood samples were taken and it was positive (presence of intraerythrocytic piroplasms) was observed in both cases, which was determined as *Babesia*. Babesiosis was the final diagnosis based on the history, clinical diagnosis and laboratory findings. The cow was treated with a single shot of diminazene aceturate (7.9mg/kg), 20% oxytetracycline (0.1ml/Kg) and dexamethasone sodium phosphate (0.2mg/kg/day), I/M and one week later the cow was fully recovered. The puppy was treated with fluid resuscitation by intravenous infusion of Ringers lactated (10 ml/Kg) solution along with diminazene aceturate and dexamethasone sodium phosphate but the puppy collapsed and died after 12 hr of treatment. Early detection and opportune treatment are recommended for successful management of babesiosis cases.

Keywords: *Babesiosis, Cow, Geimsa stain, Puppy, Red urine, Treatment*

Introduction

The protozoan parasites of the genus *Babesia*, some of which also have zoonotic importance, are the cause of the tick-borne hemoprotozoan disease known as babesiosis, which affects many species of animals and has a global distribution (Laha *et al.*, 2015). Bovine babesiosis has a huge economic effect due to loss of meat and milk production of infected animals and death. The parasite in vertebrate hosts' remains in erythrocytes and the disease is characterized by haemolytic anemia and fever, with occasional hemoglobinuria and death (Mosqueda *et al.*, 2012). It has two phases in its life cycle: the first occurs inside the host RBCs, where the sporozoites transform into piroplasms, and the second takes place inside the tick vector (Uilenberg G, 2006). It has a 10- to 21-day prepatent phase, followed by a temporary parasitemia and a secondary parasitemia that develops later (de Gopegui *et al.*, 2007).

All species of *babesia* are naturally transmitted from animal to animal through the bites of hard ticks (*Ixodidae*). Among domestic and wild mammals, cattle, horses, sheep, goats, swine, cats, and dogs are susceptible to *babesia* infection (Yadav *et al.*, 2019). It is more prevalent in tropical and subtropical countries due to the high permanence of tick vectors (the tick population is very high), as the environment and climatic conditions favor the survival, reproduction, and distribution of the tick vectors. The disease is the second most common widespread blood-borne disease of animals after trypanosomes and is prominently also emerging as a zoonosis in humans (Leiby, 2011; Gohil *et al.*, 2013).

The disease has complex relationships exist between the disease's vector, host, and environmental factors. Despite the fact that babesiosis is generally thought of as a tropical illness, outbreaks may happen anywhere with moderate climates (Ijaz *et al.*, 2013). The transmission is depending on the presence of tick vectors and animal infestations. According to Razmi and Nouroozi (2010), the parasite within the tick vector can be transmitted through the ova of mother ticks (transovarian transmission). The disease's clinical signs are severe and include different degrees of anorexia, lethargy, anemia, mild jaundice, and hemoglobinuria. *Babesia* species and host resistance affect mortality (Rahbari *et al.*, 2008).

Babesiosis can be diagnosed based on history, clinical symptoms, and the ability to identify parasites in tissue or blood smears using the Giemsa staining method (Bal *et al.*, 2016). It is treated by removing the parasite from the body, correcting anemia, along with supportive care. Babesiocidal drugs, however diminazene aceturate and imidocarb dipropionate are the most often employed (Halder and Gupta, 2021).

The control and prevention of the disease depends on tick control, immunization of susceptible hosts and use of chemoprophylaxis (Perez de Leon *et al.*, 2012). The economic losses caused by the disease are due to direct effects such as decreased milk production and body weight, costs for controlling measures, and losses due to tick-borne diseases (Pfeffer *et al.*, 2018). “This case report describes the clinical sign, laboratory findings and treatment outcomes of babesiosis case in cow and puppy”.

Case description

Case 1

A local breed cow around six years old from Bishoftu, Dhenkaka Kebele, was brought to Professor Feseha Gebreab Memorial-VTH on February 3, 2023, with a history of voiding blood mixed urine, tenesmus, and reduced feed intake that started three days ago. The cow had ticks over her body previously, which she managed extensively. On clinical examination, the body parameters of the cow were: temperature was 40.5 °C, respiratory rate was 24 breaths per minute, and heart beat was 120 beats per minute. Clinical signs were red discoloration of urine, tenesmus, depression, and pale conjunctival mucus membrane. Again, on rectal examination, there was no problem in the bladder (Figure 12). Based on the history and clinical findings, lists of differential diagnoses were made, including blacken fern poisoning, urinary tract infection, and babesiosis, tentatively.



Figure 12: Indicating red discoloration of the urine (A) and examination of rectal body temperature(B).

Case 2

The puppy around 4 months of age from Bishoftu, Kebele 15, was examined at Professor Feseha Gebreab Memorial-VTH on February 6, 2023, with a history of completely stopping feeding, red colored urine, vomiting, and bloody diarrhea that started two weeks ago. The puppy was treated for parvo previously but did not respond. The physical examination revealed that the rectal body temperature was 34 °C, the respiratory rate was 20 breaths per minute, and the heart rate was 54 beats per minute. The puppy was lethargic, unable to stand, had icteric conjunctival and oral mucous membranes, was dehydrated, had poor body conditions, and had a tick infestation over different parts of the body (Figure 13). Based on the previous treatment history and clinical findings, lists of differential diagnoses were made, including canine parvovirus disease (CPV), liver problems, and babesiosis, tentatively.



Figure 13: Indicating tick infestation over the ear(A), Emaciated (B), yellow discoloration of the eye(C) and mucous membrane of the mouth(D).

Laboratory investigations and findings

Blood samples were aseptically collected by vacutainer needle from the jugular vein of the cow and by capillary tube from the ear vein of the puppy. A thin blood smears were prepared from both animal and stained by Giemsa's stain. The Giemsa's stain from the cow sample, revealed elongated, rounded dividing forms and pear shape with an acute angle intraerythrocytic

piroplasms (figure 14 A), and the Giemsa's stain from the puppy sample, revealed highly destructed RBC, round, oval or irregular shaped intraerythrocytic piroplasms, mostly located in pairs and more than pairs in some erythrocytes were observe under 100x binocular microscope (figure 14 B). Finally, it was concluded based on the history, clinical examination and laboratory findings (morphological characteristics of the parasite), the definitive diagnosis of the both cases were Babesiosis caused by *Babesia*.

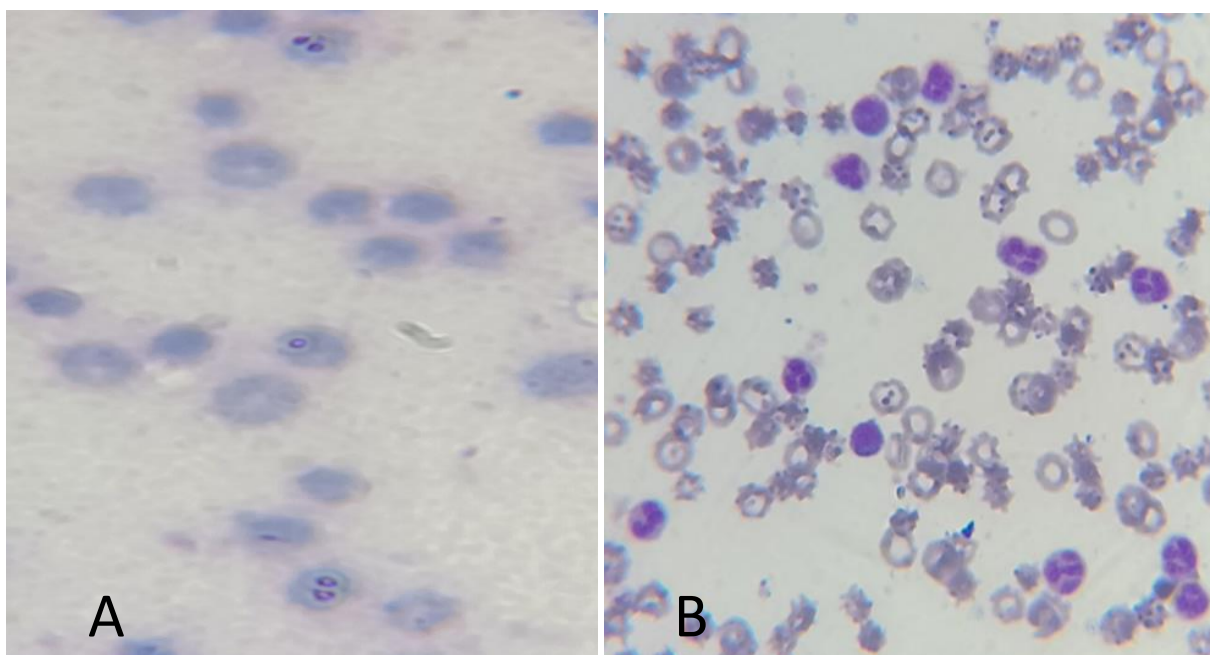


Figure 14: Giemsa-stained blood smear of infected cow (A) and puppy (B)

Case management and treatment outcome

The cow was treated with Diminazen Di acetate (Hebei New Century Pharmaceutical Co., Ltd, China) 1ml per 20kg BW (2.36g/300kg.BW) dissolved in 15 ml of sterile water, 20% oxytetracycline (Shijiazhuang Fengqiang Animal Pharmaceutical Co., Ltd) at (0.1ml/Kg) a single shot I/M and Dexamethasone sodium phosphate (Sokar Healthcare Pvt.Ltd. Gujarat India) 0.2mg/kg/day, all a single shot I/M and the cow was responded for the treatment. The puppy was treated promptly and vigorously with the fluid resuscitation by intravenous infusion of Lactated Ringer's solution (Addis Pharmaceutical factory PLC) at (10 ml/Kg) along with diminazene acetate and Dexamethasone sodium phosphate but the puppy collapsed and died

after twelve hours of treatment (as the owner report). Forty-eight-hour post treatment, the cow was presented with rectal body temperature of 38.6°C; the color and the consistency of the urine were changed in to normal; stops tenesmus and the appetite returned to normal. The cow was Fully recovered after one week of post treatment (figure 15).



Figure 15: Fully recovered cow after one month during follow-up period.

Discussion

A number of authors from throughout the world, including those from Ethiopia (Laha *et al.*, 2012; Tufani *et al.*, 2015), have previously reported cases of babesiosis infection. The spread of *B. bovis* is associated with temperate climate vector ticks like *B. microplus* ticks and is therefore quite distinct from the distribution of *B. bigemina*, claim Talkhan *et al.* (2010) and Ziapour *et al.* (2011). As a result, *B. bigemina's* spread is associated with tropical and subtropical ticks, primarily *B. decoloratus* (Wadhwa *et al.*, 2008).

The cow and puppy in this case study were diagnosed based on the history, clinical observation, and microscopic examination of the smears. The cow in the case study showed a high fever, red

urine, pale mucous membranes, and general weakness as clinical symptoms. These clinical results are comparable to those previously reported by Rani *et al.* (2010) and Hemaswathy *et al.* (2020). The findings of Salem and Farag (2014), Kumar *et al.* (2015), and Teodorowski *et al.* (2022) all supported the clinical symptoms displayed by the puppy in the current case study. According to Boozer and Macintire (2005), anemia is frequently caused by both intravascular and extravascular hemolysis. One of the canine babesiosis consequences that is most frequently described is jaundice (Keller *et al.*, 2004; Yadav *et al.*, 2011).

Giemsa-stained thin blood smears from cows showed elongated, rounded dividing forms and the distinctive pear shape with an acute angle in the erythrocytes, which identified the parasite as *B. bigemina*. Prior studies by Venu *et al.* (2015), Vijayakumar *et al.* (2017), El-Bahy *et al.* (2018), and Agrawal *et al.* (2023) revealed similar microscopic examination results. The microscopic examination of the stained peripheral blood smears from the puppy used in the present case report was similar to different literature previously used for the diagnosis of Babesia infection in dogs (Sivajothi *et al.*, 2014), and similar microscopic examination results were previously reported by Yogeshpriya *et al.* (2014), Kumar *et al.* (2015), and Sudhakara *et al.* (2016). The two types of Babesia, large and small, can be distinguished using a blood smear based on the size and morphology of the intraerythrocytic piroplasm, which are the primary parameters in diagnosing *Babesia spp.* (Solano-Gallego and Baneth, 2011). The laboratory diagnosis by microscopic examination of the smear in the present case report detects high parasitemia by *Babesia* through it.

The affected cow in this case study was treated with diminazene aceturate (7.9 mg/kg) along with 20% oxytetracycline (0.1 ml/kg) and dexamethasone sodium phosphate (0.2 mg/kg/day). The cow became afebrile after 48 hours of therapy, changed the color of the urine to normal, and showed complete clinical recovery after one week of post-treatment. Zintl *et al.* (2003), Laha *et al.* (2012), and Hemaswathy *et al.* (2020) suggested the most commonly used chemotherapy for *babesia* infections in cattle is diminazene aceturate. Long-acting oxytetracycline has been shown to have a prophylactic effect against *babesia* infection (Tufani *et al.*, 2015). The present case study suggests that early detection as well as specific chemotherapy and prophylactic therapy are essential for successful management of babesiosis

in affected animals. Uneventful recovery from babesiosis following proper treatment without undue delay has been reported by many workers (Bhikane *et al.*, 2001; Tufani *et al.*, 2009; Rani *et al.*, 2010).

The puppy in this case study received the same treatment regimen as Mosqueda *et al.* (2012), and Yogeshpriya *et al.* (2014), which included fluid resuscitation using an intravenous infusion of Lactated Ringer's solution (10 ml/kg), diminazene aceturate, and dexamethasone sodium phosphate. Unlike the above outliers, which report good response and recovery of dogs affected by babesiosis, the puppy in this case study did not respond to the treatment and died after 12 hours of post-treatment. The puppy in this case study was very young and highly infected with the *babesia* parasite that causes blood loss by destruction of the red blood cell, and again, highly infested by the tick that causes blood loss during the feeding process of the tick. The subsequent death may be related to a lack of treatment with blood transfusion due to the absence of kits that are used for blood transfusion and the identification of blood types in animals in our hospital.

In conclusion, the laboratory results of this case report confirm the presence of Babesia in the blood of both cases. Clinical findings correlated with a blood smear examination could be beneficial for the early diagnosis and Specific chemotherapy and prophylactic therapy are essential for the successful management of babesiosis cases as shown in this case report. Blood transfusion is recommended for severely affected animals by this disease.

Acknowledgement

I am grateful to Dr. Tilaye, Dr. Mehari, Ms. Adisu Wakuma and the owners of the animals for their valuable information during laboratory diagnosis and follow up.

3.1.5. Clinical hypocalcaemia/milk fever in a late pregnant ewe and a lactating Cow

Abstract

Milk fever (parturient paresis) is an important metabolic disorder of animals around the time of calving because of the high volume of milk produced during this time and the subsequent demand for calcium. This case report describes the clinical management of hypocalcemia (milk fever) in a late-pregnant ewe and a lactating cow. History and clinical diagnosis are routine diagnostic procedures for this disease. The cow was examined at home with a history of sternal recumbency and inability to stand that started after parturition, and the late pregnant ewe was presented with a cart to Professor Feseha Gebreab Memorial-VTH with a history of inability to stand that started 10 hours ago. The ewe was managed intensively and gave birth twice per year. Physical examinations showed vital parameters were within the normal range. Sternal recumbency with the head-turning into her left flank sign was observed when examining the cow. The ewe was depressed, recumbent, and unable to stand, and the neck musculature caused the neck to curve in an S shape when the head was extended. Both cases were symptomatically diagnosed as hypocalcemia and treated successfully with calcium borogluconate (400 mg/ml) intravenously. After the completion of therapy, both animals successfully responded. Therefore, an opportune IV calcium borogluconate institution has reduced the likelihood of complications and death due to hypocalcemia. Proper dietary management is recommended for the control and prevention of metabolic-related diseases.

Keywords: Cow; Calcium borogluconate; Ewe; Hypocalcaemia

Introduction

One of the metabolic illnesses that affects adult cows most frequently within 48 hours following parturition is milk fever, also known as post-parturient hypocalcemia or parturient paresis, which occurs from severe hypocalcemia. However, it can also happen weeks before or after parturition (Anteneh *et al.*, 2012; Engdawork, 2019). Age, physical condition, and the mineral content of the food surrounding calving (including metabolic alkalosis, hypomagnesemia, and others) are some of the risk factors for a cow acquiring this disease (Goff, 2008; Daresjo, 2020; Sammad *et al.*, 2022). When milk production begins, the mammary gland uses up more calcium than it can replenish from the pool of calcium in the plasma and extracellular fluid. Cows respond rapidly to hypocalcemia by secreting more parathyroid hormone. Conversely, if the response to parathyroid hormone is reduced, which lowers PTH secretion, poor gut calcium absorption inhibits calcium ionization and reduces abomasal motility and rumen function (Goff *et al.*, 2014; Venjakob *et al.*, 2018). According to Chamberlin *et al.* (2013), clinical hypocalcemia has also been linked to an increased chance of developing a number of other common postpartum conditions, including mastitis, metritis, retained placenta, and displaced abomasum.

Milk fever is the condition occurring in all dairy cows within clinical and sub clinical conditions (Venjakob *et al.*, 2018). The most severe form of hypocalcemia, clinical hypocalcemia, is characterized by a plasma calcium content of less than 5 mg/dl and causes a cow to be unable to stand. Because of this, a cow with milk fever becomes recumbent, has a decreased appetite, and may possibly go into a comatose state or pass away (Daresjo, 2020). The incidence of these diseases in sheep flocks typically low, less than 3%, and sporadic, while mortality rates during outbreaks may be as high as 20% (Robertson *et al.*, 2022). About 50% of all multiparous cows and 25% of heifers have subclinical hypocalcemia, which is defined as plasma Ca concentration 8 mg/dL. Subclinical hypocalcemia also causes less severe disturbances in blood Ca and does not have any obvious signs of milk fever (Reinhardt *et al.*, 2011; Rodriguez *et al.*, 2017). Grazing sheep frequently have subclinical deficiencies in calcium and magnesium (Edwards *et al.*, 2018; Robertson *et al.*, 2022).

Diagnosis of hypocalcaemia is based on history, clinical signs, Plasma Ca concentration and rapid response to treatment. Plasma Ca concentration was determined by atomic absorption spectrophotometer (Kimura *et al.*, 2006). Treatment of milk fever (hypocalcemia) should be done early administration of calcium borogluconate via I/V. The huge weight of the cow's body during prolonged recumbency can, in as little as 4 hours, result in a "crush syndrome" impact on the lower appendages. This results in the downer cow syndrome by causing ischemia of the muscles and nerves and necrosis of these tissues (Murray *et al.*, 2006; Goff, 2008).

Milk fever can be prevented through proper management. Multiple strategies have been utilized to control and prevent hypocalcemia through nutritional management including feeding anionic salts, low calcium ion diets, Oral drenching of easily absorbed calcium and vitamin D supplementation (Amaral-Phillips, 2014). Other possible less specific control measures for the prevention of milk fever include management practices such as: Body condition control, controlling dietary carbohydrate intake peripartum, Shortening of the dry period, Prepartum milking and Reduced milking in early lactation (Thilsing-Hansen *et al.*, 2002). “This case report describes the clinical management of hypocalcaemia (milk fever) in late pregnant ewe and a lactating cow”.

Case descriptions

Case 1

An exotic bred cow weighing around 450kg from Bishoftu, was examined in one of privately owned smallholder dairy farm on February 17, 2023 with the chief complaint of inability to stand after parturition. The cow was managed intensively. A physical examination has revealed that the rectal body temperature of the cow was 37.8 °C, while the heart rate and respiratory rates were 64 beats per minute and 32 breaths per minute, respectively. Sternal recumbency with the head turning into the left flank was observed (Figure 16). Based on the history of parturition and clinical findings, the case was tentatively asserted as hypocalcaemia.



Figure 16: A sternal recumbent cow with hypocalcaemia (A), and Mammary-vein calcium-borogluconate institution (B)

Case 2

A late-pregnant local breed ewe from Bishoftu was presented to Professor Feseha Gebreab Memorial-VTH on February 6, 2023, with a history of inability to stand and a distended abdomen that started 10 hours ago. The ewe was managed intensively and gave birth twice per year. On physical examination, the rectal body temperature was 37.5°C , the respiratory rates were 32 breaths per minute, and the heart rate was 80 beats per minute. The ewe was depressed, recumbent, and unable to stand, and the neck musculature caused the neck to curve in an S shape when the head was extended (Figure 17). Based on the stage of pregnancy and clinical findings, the case was tentatively asserted as pregnancy toxemia even though hypocalcaemia was also doubted.



Figure 17: Indicating Pregnant ewe with sternly recumbent, asymmetrical presentation of the head and abdominal distention

Case management and treatment outcome

A therapy of 40% calcium borogluconate 400 mg/ml (Peru Pharmaceutical Corp. S.A.Av.) at a dose of 1 ml/kg body weight was managed slowly in the jugular and mammary veins. The cow was treated with the slow administration of 500 mL of calcium borogluconate into the mammary vein over a period of 40 minutes and recovered after treatment. For the ewe, 30 ml of the same preparation was given intravenously into the jugular vein, and she responded very well. Eructation was observed in ewes following IV institution. Then, five minutes later, the ewe was able to stand, defecate, urinate, and start walking. Muscle tremor, particularly of the shoulder regions, which later extends to the whole body, was appreciated in the cow. Finally, the cow stood by herself after one hour of post-treatment. Therefore, based on the treatment outcome and exhibited signs, it was confirmed that the animals suffered from hypocalcaemia (Figure 18).



Figure 18: After calcium borogluconate administration of the cow and ewe.

Discussion

Hypocalcaemia was diagnosed in the present case report based on the stages of production, clinical findings and rapid response to calcium borogluconate therapy. Hypocalcaemia is an acute metabolic disease particularly in adult sheep and lactating cow, which is caused by an inadequate supply of metabolizable calcium during the peak demand (Goff *et al.*, 2014; Venjakob *et al.*, 2018).

Clinical signs of hypocalcemia are categorized into three stages. Stage I hypocalcaemia will manifest in the animal as light nervousness, hypersensitivity, anorexia, weakness, shifting of weight, and shuffling of the back feet (Ali *et al.*, 2020; Ibrahim and Kirmani, 2021). This could progress to Stage II, which is characterized by flaccid paralysis-related sternal recumbency. Clinical signs of stage II last for one to twelve hours. Affected sheep or cows may stretch their heads or turn their heads toward their flanks. Due to an imbalance in the tone of the neck muscles, the neck bends in a S shape when the head is stretched. there may be visible fine muscular tremors, particularly in the triceps muscles (Gray *et al.*, 2007; Kavitha *et al.*, 2014), as were the cases in the present case report.

Early detection and sufficient treatment with calcium borogluconate, just like in the current case report, reduce the chance of the case developing and dying (Chiwome *et al.*, 2017). As shown in current cases after successful therapy, animals with milk fever exhibit a typical pattern of response to calcium borogluconate administration, including belching, muscle tremor, a decrease of heart rate, an increase of heart sounds, muzzle sweating, defecation, and urination (Mavrogianni and Brozos, 2008; Sasaki *et al.*, 2013; Akalu, 2017; Pannu *et al.*, 2022).

In conclusion, hypocalcemia is a common metabolic disease in late pregnant ewes and early nursing cows due to the increased need for calcium during this time; therefore, early and appropriate calcium borogluconate therapy will lower the risk of complications and death from hypocalcemia, as shown in this case report. Limit Ca⁺ intake 3-4 weeks before calving is recommended for decreases the incidence of the disease.

Acknowledgments

I am grateful to Addis Ababa University College of Veterinary medicine, Veterinary Teaching Hospital, in particular, Dr. Cheru Talila, Dr. Mehari, and Mr. Dereje Gudeta.

3.1.6. Actinobacillosis in Ox and Ram

Abstract

Actinobacillus lignieresii has been recognized for many years as the cause of actinobacillosis in cattle and sheep. This case report describes the clinical signs, laboratory findings, and treatment outcomes of an actinobacillosis case in Ox and Ram. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A seven-year-old local breed ox and a one-year local breed ram from Bishoftu were brought to the Professor Feseha Gebreab Memorial-VTH with a history of decreasing feed intake, neck swellings, and salivation that began two days ago (for the ox) and five days ago (for the ram), respectively. Physical and clinical examination of the both animals include: fever, a firm, painful swelling at the base of their mandibles, some salivation, a swollen sub-mandibular lymph node, a dry muzzle, and no oral lesions. Exudate samples were collected; culture on MacConkey agar and Nutrient agar was performed. Gram-negative, small rods, or coccobacilli, occurring as single and paired bacteria were observed, which was indicative of *Actinobacillus*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as Actinobacillosis. Both cases were treated with penstrip at 1 ml/20 kg for five successive days, I/M. In addition to the penstrip, the ox was given dexamethasone sodium phosphate (0.2 mg/kg/day) I/M, and the ram's pus swelling was surgically removed and managed with daily applications of tincture iodine till healing. Two weeks post-therapy, both animals fully recovered. Prompt treatment for those that have the disease and decreasing the amount of dry feeds are recommended to prevent the spread of the disease.

Key word: *Actinobacillosis, Diagnosis, Gram stain, MacConkey agar, Ox, Ram, Treatment*

Introduction

Actinobacillosis is an uncommon disease of sporadic occurrence with worldwide distribution and Sheep, pigs, and horses are more rarely affected by the disease than cattle (Andreazza *et al.*, 2013). *Actinobacillus lignieresii*, a natural inhabitant of ruminants' upper respiratory and digestive systems, is the cause of the disease in ruminants (Radostits *et al.*, 2007; Aziz *et al.*, 2019). It is a gram-negative, coco-bacillus, aerobic bacteria, first recognized as a pathogen in South America in the early 20th century, characterized by pyogranulomas formation in various soft tissues (Smith *et al.*, 2009). Actinobacillus infection occurrence was associated with the feeding of dry fibrous forage that may injure the mucous membranes of the oral cavity, favoring the colonization of deeper tissues by *A. lignieresii* (Dhand *et al.*, 2003; Caffarena *et al.*, 2018).

Common clinical manifestations of the disease in animals include the development of pyogranulomas lesions in the mouth, tongue, or forestomach, which extend to local lymph nodes in the head and neck (Rycroft and Garside, 2000; Uzal *et al.*, 2016). The involvement of other organs is considered to be atypical (Holzhauer and Roumen, 2002). Atypical cases are described by several authors and characterized by skin lesions (Milne *et al.*, 2001; Margineda *et al.*, 2013), respiratory system lesions (Agnelo *et al.*, 2009), hind limbs lesions (Relun *et al.*, 2019) and generalized lymph nodes and organs lesions (Kasuya *et al.*, 2017). Furthermore, when widespread lesions affect the lips, palate, throat, nasal pits, and face, the result is a clinical picture known as the hippo-like face (Scheid *et al.*, 2020).

Actinobacillosis is diagnosed based on the isolation and identification of the causing organism, as well as lesions resembling the disease, such as the appearance of several firm, yellowish nodules encircled by fibrous tissue (Margineda *et al.*, 2013; Tessele *et al.*, 2014). However, molecular identification and genetic sequencing is important in the elaboration of the diagnosis especially in atypical cases of the disease (Kish *et al.*, 2014; Scheid *et al.*, 2020). Other diseases such as nocardiosis, which causes pyogranulomatous lesions in the skin and actinomycosis characterized by pyogranulomatous osteomyelitis, especially in the mandible (Relun *et al.*, 2019), should also be differentiated from actinobacillosis.

Treatment of affected animals with penicillin-streptomycin (penstrip) combination and amoxicillin–clavulanic are effective (Kish *et al.*, 2014). Once more, *A. lignieresii* was resistant to erythromycin and amikacin and showed intermediate sensitivity to penicillin G, streptomycin, and flumequine (Relun *et al.*, 2019). There is no scientific evidence of the vaccination in preventing this disease. The disease has widespread distribution especially in tropical and subtropical climates including Ethiopia. Keeping in view the economic losses and widespread distribution of actinobacillosis, “This case report describes the clinical sign, laboratory findings and treatment outcomes of Actinobacillosis case in Ox and Ram”

Case descriptions

Case 1

A seven-year-old local breed ox from Bishoftu, kality Kebele, was brought to Professor Feseha Gebreab Memorial-VTH of Addis Ababa University on February 7, 2023, with a history of decreasing feed intake, swellings on the left side of the neck region, and salivation that started two days ago. The ox was reared extensively, and such swelling was previously observed in neighboring household ox. Upon physical examination, the rectal body temperature was 40 °C, respiration was 36 breaths per minute, and the heart rate was 72 beats per minute. The ox had hard and painless swelling at the base of the mandibular region of the left side, slight salivation, a swollen sub-mandibular lymph node, a dry muzzle, and no lesion in the oral cavity on examination (Figure 19). Immature pus with blood was aspirated with a 22-gauge needle. Based on the previous history and clinical findings lists of differential diagnoses were made, actinobacillosis and actinomycosis, tentatively.



Figure 19: Indicating Swelling at the base of mandibular region.

Case 2

A one-year-old local breed ram from Bishoftu, Kebele 5, was brought to Professor Feseha Gebreab Memorial-VTH of Addis Ababa University on February 24, 2023, with a history of decreasing feed intake and swelling on the right side at the base of the mandible that started five days ago. The ram was reared extensively. Upon physical examination, the rectal body temperature was 40.6 °C, respiration was 28 breaths per minute, and the heart rate was 108 beats per minute. Examination revealed that the ram had swelling at the right side's base of the mandible, swollen parotid lymph nodes, and no oral lesions. (Figure 20). Again, white, creamy pus was aspirated with a 22-gauge needle. Based on the history and clinical findings lists of differential diagnoses were made, actinobacillosis and local trauma, tentatively.



Figure 20: Indicating Swelling at the base of mandibular region.

Laboratory Diagnosis and Result

The exudate samples were collected aseptically from both cases and processed at the veterinary microbiology laboratory of Addis Ababa University College of Veterinary Medicine, Bishoftu. The bacteriological culture was made on MacConkey agar medium and incubated aerobically at 37 °C for 24 hours. Again, colonies were randomly picked and subcultured again on nutrient agar at 37 oC for 24 hours (Appendix). The smear was prepared with Gram stain, and Gram-negative coco-bacilli bacteria were observed under the 100x oil objective lens of a binocular microscope, and that was *Actinobacillus* (Figure 21). Finally, based on the history, clinical findings, and laboratory findings, the final diagnosis was actinobacillosis.

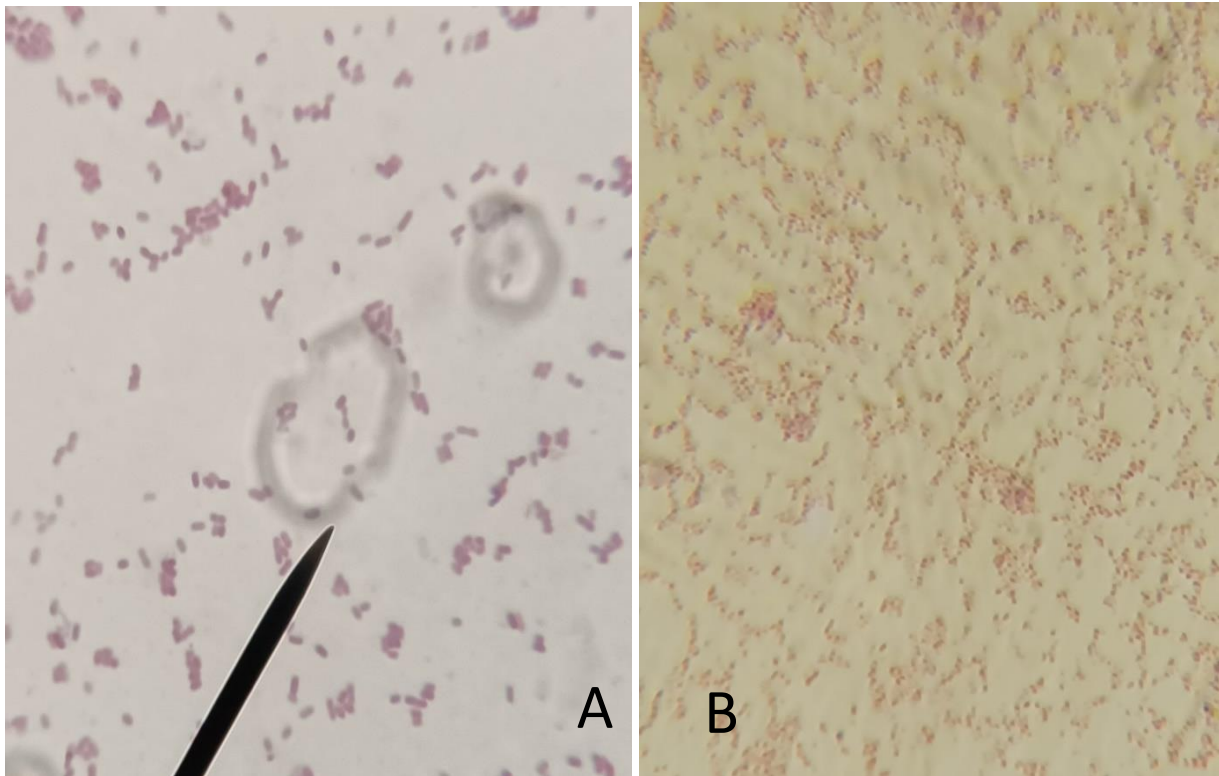


Figure 21: Gram negative coco-bacilli bacteria from the ox (A) and ram sample(B).

Case management and treatment outcome

Both cases were treated with penstrip (Chongqing Fantong Animal Pharmaceutical Co. Ltd., China) at 1 ml/20 kg for five successive days, I/M. In addition to the penstrip, the ox was given dexamethasone sodium phosphate (Sokar Healthcare Pvt. Ltd., Gujarat, India) at 0.2 mg/kg/day for one day, I.M., and the ram's pus swelling was surgically removed and managed with daily applications of tincture iodine till healing. Besides the treatment, the owners of both animals were advised to manage their animals intensively by feeding soft food until they recovered to prevent further trauma in the oral cavity. In the next few treatment days, the owners reported that their animals started to eat well and that their body temperatures returned to normal. On the 5th day of treatment, the mandibular swelling of the ox disappeared, and the surgical wound of the ram was well healed. Two weeks post-therapy, both animals were fully recovered (Figure 22).



Figure 22: Fully recovered ox (A) and ram after one month of post treatment (B).

Discussion

Actinobacillosis has often been described as a granulomatous disorder that develops slowly and sporadically (Smith, 2009). Actinobacillosis in cattle and sheep is known to be brought on by the type species, *A. lignieresii* (Rycroft and Garside, 2000). The current cases were asserted as actinobacillosis based on the history of previous infection, clinical signs and laboratory findings. In this case report, the history suggested that the disease may have been brought on by feeding hard, fibrous hay and straw to animals that had already been infected. The dry hay and straw caused oral lesions, and the route of entry of the organisms was likely an abrasion or wound in the inner part of the oral cavity and/or skin of the neck, supporting earlier reports by Albornoz and Sali (2012), and Salvaggio *et al.* (2023), who assert that *A. lignieresii* enters deep tissues via traumatic erosions, ulcers, and penetrating lesions caused by hard fibrous hays, straw, and plant awns.

The clinical signs seen in both cases, including pyogranulomas swelling in the subcutaneous tissue of the left ox and right ram at the base of the mandibular area of the neck region close to the jugular furrow, slight salivation, and swollen sub-mandibular lymph nodes, all are consistent with the clinical signs of the disease described in the literature (Milne *et al.*, 2001; Albornoz and Sali, 2012; Cahalan *et al.*, 2012; Salvaggio *et al.*, 2023). In contrast to the previous literature, the ox in this case study was feverish and no abscesses formed, it could be due to the acute nature of the disease. Infection can disseminate to the lymphatics and may sometimes spread to deep organs. Even though actinobacillosis is more commonly associated with cattle and less so with sheep, it can infect any exposed soft tissues, especially those in the head and neck area (Farghali *et al.*, 2020; Scheid *et al.*, 2020), as the report stated in these cases.

The morphological confirmation of *Actinobacillus* by colony and gram stain in the current case report is consistent with laboratory findings in the literature (Milne *et al.*, 2001; Taghipour *et al.*, 2010; Al-Katib and Dennis, 2009; Kumar *et al.*, 2016). As for the methods, the growth ability on MacConkey's agar seemed to be a most delicate feature, although only the selection of the convenient medium was the matter. Gram-negative, small rods or coccobacilli, occurring

single and paired, are characteristics of *Actinobacillus*, the cause of actinobacillosis in both cases in the current case report.

Due to early treatment with penstrip (20 mg/kg/day) in the current case study, both cases successfully responded to treatment. Similar treatments have been used in the literature (Milne *et al.*, 2001; Angelo *et al.*, 2009; Luna-Castro *et al.*, 2012; Cahalan S, *et al.*, 2012; Tessele *et al.*, 2014; Aziz *et al.*, 2019) with variable result. In addition to the penstrip, the ox was given dexamethasone sodium phosphate (0.2 mg/kg/day), and the ram's pus swelling was surgically removed and managed with daily applications of tincture iodine till healing. Both animals fully recovered after treatment.

In conclusion, the animal's general clinical condition was improved. This indicates that Actinobacillosis in animals could be successfully treated with penstrip along with dexamethasone and wound management, as shown in this case. Preventing the contamination of feed by diseased animals, prompt treatment for those that have the disease, and keeping infected animals isolated from the herd until they have fully recovered are recommended to prevent the spread of the disease.

Acknowledgement

I am grateful to Dr. Bethy, Dr. Taye for their contribution and the owner of the animal for his valuable information during the follow up.

3.1.7. Ulcerative lymphangitis in bull

Abstract

Ulcerative lymphangitis is a bacterial infection of the lymphatic vessels of the skin in horses, cattle, sheep, and goats. *C. pseudotuberculosis* is the classical cause of the disease. The present case report describes the clinical signs, laboratory findings, and treatment outcome of the bull that was affected by ulcerative lymphangitis. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A two years old local breed bull from Bishoftu, Kebele 15, was brought to Professor Feseha Gebreab Memorial-VTH with a history of swelling of the left hind limb, circular lesions, difficulty of movement, and reduced feed intake a week ago. The bull was reared extensively, and such lesions had previously been observed in the neighbor's household ox. The rectal body temperature was raised (40.4 °C), while other parameters were within the normal range. Clinical signs during diagnosis include: swollen precrural lymph nodes, and oedematous and nodular swellings along the course of lymphatic ducts, which were ulcerated and oozing out creamy pus that was odorless, thick, and blood-tinged. The collected exudate sample was cultured on 5% sheep blood agar. Growths of small, white, dry, and hemolytic colonies and gram-positive, short-rod, and also pleomorphic rod bacteria in typical 'Chinese letter'-like arrangements were observed which was indicative of *Corynebacterium*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as ulcerative lymphangitis. The bull was treated with Penstrip (1 ml/20 kg/day) for five successive days, I/M with the application of tincture iodine on the lesions daily till healing, and the bull was successfully cured. Early intervention with appropriate drugs and wound management could cure the ulcerative lymphangitis case. Avoiding environmental contamination from ruptured abscesses is recommended to prevent the spread of the disease.

Keyword: Bull, Chinese letter, Gram positive, Penstrip.

Introduction

Ulcerative lymphangitis is a common and economically important skin disease with worldwide distribution (Ameni and Terefe, 2004; Mamman *et al.*, 2011; Abdullahi *et al.*, 2019). It is caused by bacterial infection of the cutaneous lymphatic vessels and has been reported in sheep, goats, cattle, buffalo, camelids, humans and especially in equines (Spier, 2006; Tejedor *et al.*, 2008). Even though infection was first recognized in 1888 as a cause of bovine lymphangitis, it is rarely seen in cattle (Aroch *et al.*, 2003). In cattle the infection has been reported in cutaneous, mastitis, and visceral forms (Yeruham *et al.*, 2003; Sood *et al.*, 2003). The causative organisms of the disease are *C. pseudotuberculosis*, *C. pyogenes*, *Streptococcus spp.*, *Fusobacterium necrophorum*, *Pseudomonas aeruginosa*, *C. equi*, *Mannhiemia hemolytica* (Spier, 2006; Radostits *et al.*, 2007; Zavošti *et al.*, 2009).

The route of infection in animals remains undetermined, but animal-to-animal transmission through direct contact, insect mechanical vectors, or contaminated environment could be suspected (Pratt, 2005; White, 2006; Markey *et al.*, 2013). The pathogenic process of *C. pseudotuberculosis* begins with colonization and replication within lymph nodes, resulting in the formation of pyogranuloma (Marques, 2021). This cycle is followed by death of the host cell and release and spread of bacterial via the lymphatic or circulatory system, allowing the pathogen to infect visceral organs and lymph nodes, where it eventually causes the development of lesions (Stefanska *et al.*, 2010; Markey *et al.*, 2013).

The most common clinical form affecting cattle is cutaneous excoriated granuloma, which manifests as lymphadenitis and lymphangitis with abscess formation and ulceration on the hind limbs (Smith, 2002; Yeruham *et al.*, 2012). It can also cause lameness and deformity of the limbs, which can affect performance (Scott and Miller, 2003). Small ruminants may also exhibit abscessation and enlargement of superficial or internal lymph nodes, which may coexist in the same animal (Baird and Fontaine, 2007). When infection becomes chronic, the affected lymph nodes exhibit characteristic encapsulated abscesses with a concentric ring appearance (Quinn *et al.*, 2011). The diagnosis of ulcerative lymphangitis is based on the clinical signs and laboratory findings such as isolation and identification of the organism having a typical Chinese letter

configuration in pus samples (McCluskey and Cunningham, 2001; Vikas *et al.*, 2017; Spier, 2019). Local treatment of ulcers is common and effective, but surgical drainage and parenteral injection of procaine penicillin G (20,000 to 80,000 IU/kg BW, bid, IM) are also recommended (Rose and Hodgson, 2000; Brown and Bertone, 2002; Radostits *et al.*, 2007).

Control and preventive measures include disinfectants, improving hygienic conditions, quarantine and isolation of infected animals, fly control, and wound management (Smith, 2002; Scott and Miller, 2003). The disease causes economic losses such as condemnation of carcasses, additional meat inspection, devaluation of hides, and decreased milk and wool production (Kumar *et al.*, 2012; Haas *et al.*, 2017; Jens *et al.*, 2022). Additionally, humans can be infected with *C. pseudotuberculosis* causing lymphadenitis (Baird and Fontaine, 2007; Quinn *et al.*, 2011). “The present case report describes the clinical sign, laboratory findings and the treatment outcome of the bull that affected by Ulcerative lymphangitis”.

Case description

A two years old local breed bull from Bishoftu, Kebele 15, was brought to Professor Feseha Gebreab Memorial-VTH on November 7, 2022, with a history of swelling of the left hind limb, circular lesions, difficulty of movement, and reduced feed intake that started a week ago. The bull was reared extensively, and such lesions had previously been observed in the neighbor’s household ox. Upon physical examination, the heart rate was 78 beats per minute, the respiratory rate was 30 breaths per minute, and the rectal temperature was 40.4 °C. The bull had swollen precrucial lymph nodes and oedematous and nodular swellings along the course of lymphatic ducts, which were enlarged, ulcerated, and oozing out creamy pus that was odorless, thick, and blood-tinged (figure 23). Based on the history and clinical findings, ulcerative lymphangitis was tentatively diagnosed.



Figure 23: Indicating a swollen precrucial lymph node, subcutaneous oedematous swelling (A), and nodular lesions with abscesses along the course of the lymphatic duct (B).

Laboratory investigations and findings

The exudate sample was collected in a sterile bottle from the lesion under sterile conditions. The bacteriological culture was made on 5% sheep blood agar and incubated aerobically at 37 °C for 24 hours. After 24 hours, the growth of small, white, dry, and hemolytic colonies was observed (Appendix) and again Colonies were randomly picked and subcultured on nutrient agar at 37 °C for 24 hours to obtain a pure culture, which was stained by Gram's stain, which revealed Gram+ positive, short rod, and also pleomorphic rod bacteria in a typical 'Chinese letter'-like arrangement under a microscope with an X100 objective lens. Finally, based upon the history, clinical findings, and laboratory findings (colony and gram stain morphological characteristics), the pathogen was identified as *Corynebacterium* (Figure 24). It was finally diagnosed as a case of ulcerative lymphangitis.

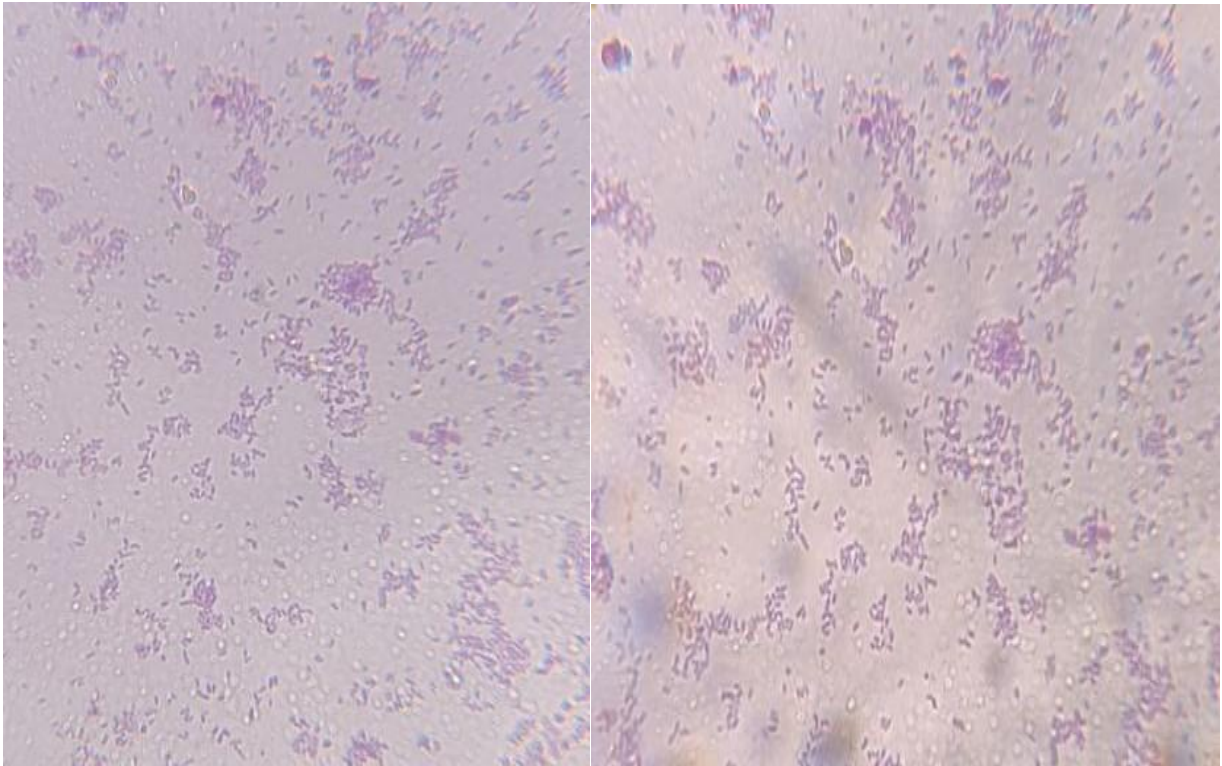


Figure 24: Gram-positive small rods with typical “Chinese letter"-like arrangements (*Corynebacterium*).

Case management and treatment outcome

The bull was treated with Penstrip (a combination of procaine penicillin and dihydrostreptomycin) (Chongqing Fantong Animal Pharmaceutical Co. Ltd., China) at a dose of 1 ml/20 kg/day for five successive days, I/M, with application of tincture iodine on the lesions daily till healing. Besides the drug treatment, the owner was also advised to segregate and nurse the young bull at home until he fully recovered. On the third day of the visit, the rectal body temperature was reduced from 40.4 °C to 38.2 °C, and movement also improved. At the end of therapy, the edematous swelling had decreased and the lesions were slightly dried (Figure 25A). One month later, the bull was fully recovered, and the lesions also disappeared (Figure 25B).



Figure 25: Decreased edematous swelling and dried lesions at the end of therapy (A) and fully recovered bull after one month of post-treatment (B).

Discussion

Ulcerative lymphangitis is caused by *C. pseudotuberculosis* and is characterized by edematous swelling, pyogranulomatous reactions, abscess formation, and ulcerative lymphangitis (Selim, 2001). The current case was asserted to be ulcerative lymphangitis based on the history, clinical findings, and laboratory results. According to Pratt *et al.* (2005), Spier (2006), and Mohamed and Reda (2015), abrasions or wounds in the skin or mucous membranes (in horses) are thought to be the portal of entry for soilborne *C. pseudotuberculosis*. These abrasions and injuries may have facilitated the entry of the disease, leading to the invasion of lymphatic vessels and the development of abscesses along their course. As was evident from the clinical sign observed in the present case report, the organism's virulence is increased by the different extracellular exotoxins that are produced by the organism. These exotoxins damage blood vessels and inhibit phagocytes, which helps the infection spread and exacerbates the disease.

The clinical symptoms seen in this case report, including a swollen precrural lymph node and oedematous and nodular swellings along the course of lymphatic ducts that were enlarged, ulcerated, and oozing out creamy pus that was odorless, thick, and blood-tinged, were similar to those described in the literature by Abou (2001), and Vikas *et al.* (2017), in bovine. Our preliminary diagnosis based on the clinical picture of the typical lesions was confirmed by the pathogen's identification as *Corynebacterium*, which confirmed ulcerative lymphangitis, and the colony characteristics (growths of small, white, dry, and hemolytic colonies) and gram stain morphological characteristics (gram positive, short rod, and also pleomorphic rod bacteria in typical "Chinese letter" like arrangement). These results supported the reports that *C. pseudotuberculosis* is the typical cause of ulcerative lymphangitis made by Abou (2001), Sood *et al.* (2012), and Steerforth and Marutsov (2017) in cattle, and by Zavošti *et al.* (2009) and Olajide and Alaba (2023) in horses.

The earlier reports by Singathia *et al.* (2011), and Vikas *et al.* (2017) suggested the applicability of daily dressing of an open abscess with a tincture of iodine with the administration of penicillin (15,000 IU/kg) and streptomycin (3 mg/kg) in the therapy of ulcerative lymphangitis, resulting in complete recovery in horses and cattle. The current therapeutic finding is consistent with the previous reports, and a re-examination of the bull on day 21 after therapy showed that the bull responded well to the line of treatment and recovered gradually. There was no evidence of cutaneous lesions (nodules and/or abscesses) on different parts of the body.

In conclusion, the general clinical condition of the bull was improved, and had gained weight. This indicates that ulcerative lymphangitis in cattle could be successfully treated with penstrip along with wound management as shown in this case. Avoiding environmental contamination from ruptured abscesses is recommended to prevent the spread of the disease because the organism's ability to survive in soil and on fomites ensures its continued presence.

Acknowledgement

I am grateful to Dr. Mehari, Mis. Frehiwot, and Mis. Tesfanesh for their contributions and to the owner of the animal for their valuable information during the follow-up.

3.2. Case Reports on Ovine

3.2.1. *Contagious ecthyma (orf) in sheep:*

Abstract

Contagious ecthyma is an acute, highly contagious, zoonotic, debilitating, and economically important viral non-systematic eruptive skin disease of small ruminants worldwide. It is caused by the gene *parapoxvirus*. This case report describes the clinical management of contagious ecthyma (orf) in a sheep. The local breed male sheep of around one year was presented at Professor Feseha Gebreab Memorial-VTH with a history of lesions on the mouth and reduced feed intake that started five days ago. The rectal body temperature was raised (41°C), while other parameters were within the normal range. Clinical signs during diagnosis include: multifocal crusty, ulcerative, and proliferative painful lesions of various sizes on the external surfaces of the mouth, lips, muzzle and nostrils, and lesions were vascular and bled easily. The case was clinically diagnosed as Contagious ecthyma. The case was treated symptomatically with 10% oxytetracycline (10 mg/kg/day) for five consecutive days, dexamethasone (0.2 mg/kg/day) for three days both intramuscularly, and application of tincture iodine on the scab lesions daily till healing, and the sheep was fully recovered. The application of hygienic measures is recommended for control and prevention of contagious ecthyma.

Keywords: *Contagious ecthyma, Parapoxvirus, Sheep.*

Introduction

Contagious ecthyma (Orf) is an acute, contagious, debilitating and important zoonotic skin disease that affects domestic and wild ruminants, especially sheep and goat worldwide (Azad *et al.*, 2016; Dalal *et al.*, 2021). It is also known as contagious pustular stomatitis, contagious ecthyma, ecthyma contagiosum, infectious pustular dermatitis (Wet and Murie, 2011; Lovatt *et al.*, 2012), and orf. Orf virus (ORFV), the prototype of the genus *Parapoxvirus* (PPV), is the etiological agent of contagious ecthyma, and four strains (OVIA82, OV-SA00, D1701 and NZ2) of Orf have been sequenced completely (Mercer *et al.*, 2006; McGuire *et al.*, 2012). The genome is a 138-kbp linear double-stranded DNA containing 132 putative genes (Mondal *et al.*, 2006; Mercer *et al.*, 2006).

Orf virus infection in kids and lambs' results in more severe disease than in adult animals, with up to 100% morbidity and 10% mortality (Martins *et al.*, 2014; Gao *et al.*, 2016). Risk factors include age, congestion due to increased stocking density, increased orphaned lambs, stress, immunosuppressive diseases, prolonged parturition and forage weed (thorny plants) (Onyango *et al.*, 2014; Abdullah *et al.*, 2015; Gao *et al.*, 2016). The virus can survive for up to 17 years in places with a dry climate and can persist for extended durations on the wool of animals and contaminated materials. Viruses spread through touch, entering through damaged skin, and reproducing in epidermal cells (Abrahamo *et al.*, 2009). The incubation period of this disease may vary from 4 to 8 days and the viral replication leads to oedematous and granulomatous inflammation of dermal cells with an increase in temperature (Spyroua and Valiakosb, 2015).

The clinical features of the infection vary, with lesions usually localized around the mouth, within the buccal cavity and the nostrils in lambs (Kumar *et al.*, 2015). In adult animals, lesions can be found in the teat orifice, vulva, skin-vaginal junction and preputial orifice (Gouletsou and Fthenakis, 2010; Billinis *et al.*, 2012). Lesions follow a well-defined development pattern, with local erythema followed by formation of papules, vesicles, postules and scabs. As lesions resolve, scabs become dry and shed, with no scar remaining at the lesion site (Sadiq *et al.*, 2017). Usually, lesions heal spontaneously within 3-4 weeks, but animals may be unwilling to nurse, eat, or walk (De La Concha-Bermejillo *et al.*, 2003).

Orf can be diagnosed based on clinical signs, histopathology, and electron microscopy (Nandi *et al.*, 2011). Serological tests such as AGPT, CFT, ELISAs, and SNT can also be used (Hosamani *et al.*, 2009; Magana *et al.*, 2014). Differential diagnoses include bluetongue, foot and mouth disease, pox, papillomatosis, staphylococcal dermatitis, and dermatophilosis (Dalal *et al.*, 2021). The lip lesions of Orf infected animals were treated with topical application of 10% Potassium permanganate solution, sodium permanganate and salicylic acid ointment, and antibiotics like penicillin and chloramphenicol in cases complicated with stomatitis or enteritis (Onyango *et al.*, 2014; Kumar *et al.*, 2015). One of the most efficient strategies to prevent Orf virus infection is vaccination (Smith and Sherman, 2009). “This case reports describe the clinical management of contagious ecthyma (orf) in a sheep”

Case description

The local breed male sheep of around one year was presented at Professor Feseha Gebreab Memorial-VTH of Addis Ababa University on January 23, 2023, with a history of lesion on the mouth and reduced feed intake that started five days ago. The sheep were managed semi-intensively with other sheep. Physical examination revealed that the sheep was febrile, with a rectal body temperature of 41 °C and 34 breaths per minute and 78 beats per minute respiratory and heart rates, respectively. On clinical examination, there were multifocal crusty, ulcerative, and proliferative painful lesions of various sizes on the external surfaces of the mouth, lips, muzzle, and nostrils. Lesions were vascular and bled easily (Figure 26). Based on the history, clinical findings (lesions), and symptoms, contagious ecthyma (Orf) was tentatively diagnosed.



Figure 26: Multifocal crusty, ulcerative, and proliferative lesions of various sizes on the mouth, lips, muzzle, and nostrils.

Case management and treatment

The sheep received intramuscular treatments of 10% oxytetracycline (Shanghai Thongren Pharmaceutical Co., Ltd., China) at a dose of 10 mg/kg for five consecutive days and dexamethasone (Sokar Healthcare Pvt. Ltd., Gujarat, India) at a dose of 0.2 mg/kg/day for three consecutive days. Until the wound was healed, the sheep also received daily applications of an iodine tincture. The sheep began eating well and had a rectal temperature that was within the usual range (38.7 °C) 72 hours after therapy. The sheep's lesions on the mouth, lips, muzzle, and nostrils had fully recovered by the time the treatment was finished out (the fifth day). Once more, the sheep was entirely healed after a month (Figure 27). The disease was contagious ecthyma (Orf) based on the history, clinical signs (characteristics of the lesions), and the location or site where the lesions occurred.



Figure 27: Fully recovered young sheep from orf after one month during follow-up.

Discussion

Contagious ecthyma is a zoonotic viral disease of sheep and goats in many countries, including our country, Ethiopia. The affected sheep in this case report showed multiple painful lesions of varying sizes that were crusty, ulcerative, and proliferative on the exterior surfaces of their mouths, lips, muzzles, and noses. In previous reports by De La Concha-Bermejillo *et al.* (2003), Saravanan *et al.* (2007), Zhang *et al.* (2010), Gelaye *et al.* (2016), Peralta *et al.* (2018), Tedla *et al.* (2018), and Kumar *et al.* (2022), comparable clinical symptoms were seen.

The diagnostic approach to cases of the Orf virus has been thoroughly explained in the paper by Abdullah *et al.* (2015). However, in this case report, the history and clinical signs along with the distinctive lesions played a major role in the diagnosis. An Orf virus typically exhibits dramatic distinguishing characteristics that are simple to detect and identify, leading to a presumptive and rapid clinical diagnosis (Kumar *et al.*, 2015). It is important to point out that the absence of a diagnostic kit in the compound prevented this study from doing any diagnostic tests to identify the specific pathogen.

Since there is no particular therapy for this viral infection, the focus of all therapies is always on secondary infections. In this case report, sheep were treated with parenteral administration of 10% oxytetracycline (10 mg/kg/day) and dexamethasone (0.2 mg/kg/day), as well as daily application of tincture iodine to the scab lesions until healing. This treatment was aimed at reducing the severity of the lesions and speeding recovery, as removal of the scab may delay healing. This approach agrees with Schwarz (2007), Nandi *et al.* (2011), Pugh and Baird (2012), Onyango *et al.* (2014), and Spyroua and Valiakosb (2015), who reported that supportive treatment that includes the application of ointments topically and parenteral antibiotic administration against secondary infection during Orf cases speeds recovery. In the present case study, the lesions resolved within one week, and the sheep showed a good prognosis after treatment.

In conclusion, a combination of broad-spectrum parenteral 10% oxytetracycline with dexamethasone and topically applied tincture iodine is beneficial in treating secondary infections, as shown in this case. The application of hygienic measures is recommended for the control and prevention of contagious ecthyma.

Limitations

No laboratory confirmation was made for this disease due to lack of facilities and hence treatment was done based on clinical picture only (symptomatic therapy).

Acknowledgement

My grateful appreciation goes to Dr. Cheru, Mis. Lamrot and the owner of the animal for their valuable information during the follow up.

3.2.2. *Cutaneous papillomatosis (wart) in Ewe*

Abstract

Cutaneous papillomatosis (wart) is a tumor caused by *papillomaviruses*, that are known to infect humans and domestic animals and cause proliferation of the stratified squamous epithelium of the skin or mucosa. This case report describes the clinical signs and treatment outcomes of a cutaneous papillomatosis (wart) case in Ewe. A 4-year-old local breed ewe from Bishoftu, Kebele 09, was presented at Professor Feseha Gebreab Memorial-VTH with a history of multiple growths on the chest region and difficulty in movement that started six months ago. Rectal temperature, heart rate, and respiration rate were measured and found to be within the normal range. Clinical signs during diagnosis include: multiple hard, dry, and rounded finger-like projections on the left side of the chest region. The case was clinically diagnosed as cutaneous papillomatosis (wart). The ewe was treated symptomatically by ligating the base of every wart with silk, applying a tincture of iodine to the ligated area with autohemotherapy, and administering Ivermectin (0.2 mg/kg). The ewe was followed for 4 months, and the medications showed significant improvements. Post-therapy, the ewe responded well to the line of treatment and recovered gradually. There was no evidence of warts on different parts of the body. Therefore, the combination of immunotherapy (autohemotherapy and ivermectin) with ligating the base of every wart with silk and applying a tincture of iodine to the ligated area is a critical therapeutic tool for the treatment of warts. Hygiene measures, minimizing direct contact, and avoiding contaminated equipment are recommended to prevent the spread of the disease.

Key word: *Cutaneous papillomatosis, Ewe, Finger-like projections, Immunotherapy.*

Introduction

Cutaneous papillomatosis (wart) is a tumor and an economically significant disease in the world (Ann *et al.*, 2000). *Papillomaviruses* are a diverse group of small, non-enveloped, circular, double-stranded DNA viruses that are known to infect humans or domestic animals in which they cause proliferation of the stratified squamous epithelium of the skin or mucosa (Dean *et al.*, 2001; Antonsson, and Hansson, 2002; Al-Sabaawy and Al-Sadi, 2021). Ovine papillomaviruses (OaPVs) have been suggested to be associated with skin tumors and comprise four members (OaPV1, OaPV2, OaPV3 and OaPV4) (Rector *et al.*, 2005; Alberti *et al.*, 2010; Tore *et al.*, 2017). OaPV1, OaPV2 and OaPV4 form OaPV species three within the genus *Delta-papillomavirus*, while OaPV3 belongs to the genus *Dyokappa-papillomavirus* (De Villiers *et al.*, 2004; Rector *et al.*, 2004; Al-Salihi *et al.*, 2020).

The virus is mostly spread by direct contact, but it can also spread horizontally through indirect means through iatrogenic mistakes, infected food, and contaminated equipment (MacLachlan *et al.*, 2017; Ugochukwu *et al.*, 2019). Though less frequently, PV DNA has been found in samples of healthy skin (Rollison *et al.*, 2021). The agent may cause cutaneous lesions with a variety of morphologies, including filiform, peduncular, atypical, and squamous papillomas (Daudt *et al.*, 2018). Squamous cell carcinoma in sheep is more common in the ears, head, neck, back, udder, teat base, and belly, as well as in anatomical locations absent of wool and pigmentation (Fava *et al.*, 2001; Rollison *et al.*, 2021; Cacciotto *et al.*, 2022). Genetic factors may play a significant part in the cause of skin cancer in animals (Goldschmidt M, 2002). It is unclear what age domestic animals have their peak occurrences (Al-Sabaawy and Al-Sultan, 2022).

PV infection is usually characterized by a latency period, during which no virus particles are produced and the host exhibits no manifest clinical symptoms. Upon stimulus, the latent infection may undergo reactivation and subsequent virions assembly and release, leading to the appearance of symptoms and lesions developing fine finger-like projections and are extremely painful (Maglennon and Doorbar, 2012; Maglennon *et al.*, 2014). The diagnosis of *Papillomavirus* infection can mostly be done by clinical and histopathological examination, with electron microscopy and immune histochemical analysis being successful methods

(Catroxo *et al.*, 2013; Dorttas and Dagalp, 2020). PCR technique is also frequently used in molecular detection of the agent and taxonomy studies (MacLachlan *et al.*, 2017). Autohemotherapy and surgical attempts have been used to treat papillomatosis, and many methods such as antibiotics, chemotherapeutic, immune modulator and antiparasitary drugs have been tried in papillomatosis treatment (Ugochukwu *et al.*, 2019). Immunotherapies target specific immune system cells, while some have an overall impact on the immune system (Mohammed *et al.*, 2022). ‘‘This case report describes the clinical sign and treatment outcomes of Cutaneous papillomatosis (wart) case in in Ewe’’.

Case description

A 4-year-old local breed ewe from Bishoftu, Kebele 09, was presented at the Professor Feseha Gebreab Memorial-VTH of Addis Ababa University on November 21, 2022, with the history of multiple finger-like growths on the chest region and difficulty in movement that started six months ago. On physical examination, the rectal body temperature, respiratory, and heart rates were within the normal limits. The ewe had hard, dry, and rounded finger-like projections, or cauliflower appearances, on the left side of the chest region (Figure 28). Based on the history and typical clinical findings, it has been tentatively concluded to be ovine cutaneous papillomatosis or wart.



Figure 28: Indicating hard, dry and rounded finger-like projections(wart).
Case management and treatment outcome

The ewe was given autohemotherapy (about 6 ml of blood collected aseptically from the jugular vein and mixed with the blood from the affected area, administered intramuscularly), two injections of ivermectin (Sheyang Sunvictor Pharmaceutical Co., Ltd., China) at a dose of 0.2 mg/kg, administered subcutaneously, over a two-week period, and ligating the base of each wart with silk and dousing the ligated area in an iodine tincture. The ewe was followed for 4 months; there was no evidence of warts on different parts of the body, and she recovered gradually (Figure 29).

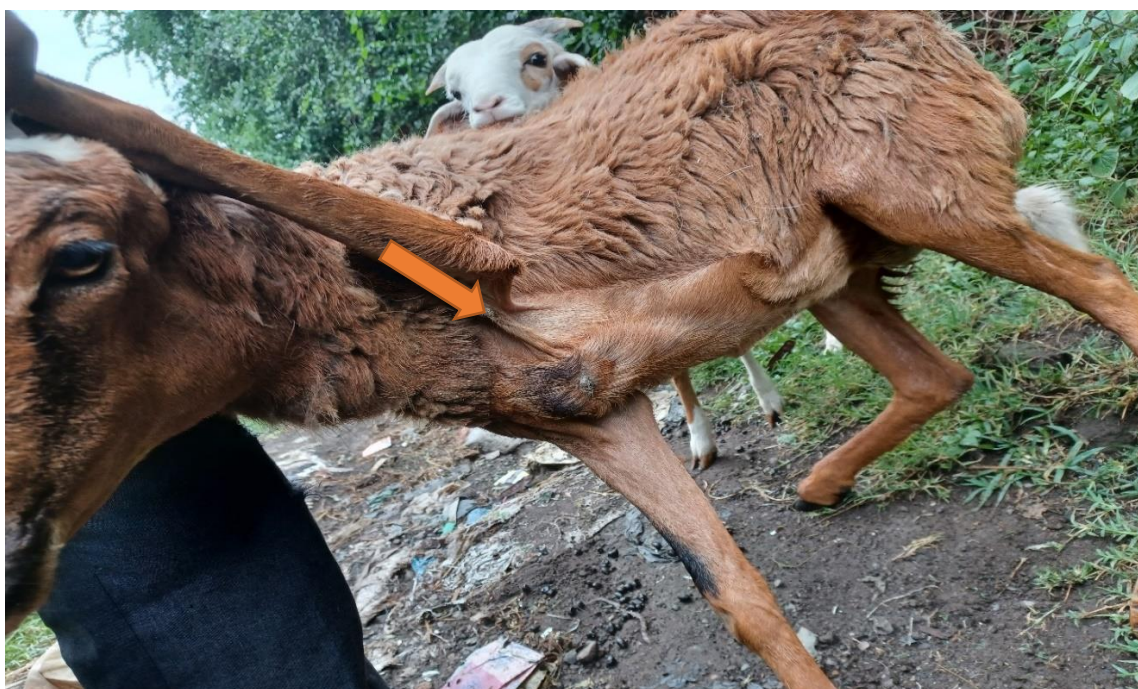


Figure 29: Indicating recovered ewe after four months of follow-up.

Discussion

Cutaneous papilloma induced by *papovaviruses* and occurs mainly on the head, neck and thoracic limb and is flat, circumscribed with a crusty surface and ring-wormlike in appearance (Smith *et al.*, 2002). Based on the history and clinical findings, the current case of ewe was diagnosed as cutaneous papillomatosis (wart). Additionally, the length of time that the fingerlike projections grew was also considered. The ewe in the present case report showed similar clinical

manifestations indicated in various literature, including hard, dry, and rounded finger-like projections or cauliflower appearance (Moharram *et al.*, 2019; AM Farghali *et al.*, 2020; Al-Sabaawy and Al-Sultan, 2022).

The earlier studies by Aslan and Oruc (2010), Jameel *et al.* (2011), Dhule (2013), Feyisa (2018), Banerjee and Ghosh (2021), and Bozkurt *et al.* (2023) suggested the use of tincture of iodine with administration of Ivermectin (0.2 mg/kg) and autohemotherapy in the treatment of papillomatosis (wart) and resulted in full recovery in bovine, horse, and goat. According to the current therapeutical finding, which is consistent with earlier findings, the ewe in the current case report was treated by ligating the base of each wart with silk, covering the ligated region with an iodine tincture, giving autohemotherapy, and administering Ivermectin (0.2 mg/kg). The ewe was followed for 4 months, and the medications showed significant improvements. Post-therapy, the ewe responded well to the line of treatment and recovered gradually. There was no evidence of warts on different parts of the body.

In conclusion, ovine papillomavirus may be identified from a clinical examination by the presence of warts of different sizes that are keratinized, have a cauliflower-like look, and range in color from black to gray, as shown in this case. The combination of immunotherapy (autohemotherapy and ivermectin) with ligating the base of every wart with silk and applying a tincture of iodine to the ligated area is a critical therapeutic tool for the treatment of warts, as shown in this case. Hygiene measures, minimizing direct contact, and avoiding contaminated equipment are recommended to prevent the spread of the disease.

Acknowledgement

I am grateful to Mr. Dereje Gudeta for his contribution and the owner of the animal for his valuable information during the follow up.

3.1.3. Grain overload in rams and a calf

Abstract

Ruminal lactic acidosis is a condition that arises in ruminants due to the ingestion of excessive quantities of easily digestible carbohydrate-rich feed, eating a diet poor in fiber, and failing to produce buffer, leading to physiological derangements that can be fatal in severe cases. This case report describes the clinical signs, laboratory findings, and treatment outcomes of grain overload in a calf and two rams. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A local breed calf and two rams from Bishoftu were admitted to Professor Feseha Gebreab Memorial-VTH with a history of becoming off-feed, diarrheal, and reluctant to move after accidentally consuming the grain a few hours ago. Bloating, lethargy, nasal discharge, crackling sound, ataxia, anorexia, total rumen atony, diarrhea, fast breathing, and pulse were among the physical and clinical examination findings. Ruminal fluid samples were collected, pH of the rumen fluids and protozoal activity was examined, it shown acidic and had no protozoal activity. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as acidosis. The animals were treated with indigestion powder, 100 mg dissolved in 1 L of water for a calf, and 25 mg dissolved in 1 L of water for rams, PO. Multivitamin (4–8 ml) for calves and (0.4–0.8 ml) for rams, as well as 20% oxytetracycline at (0.1 ml/kg) were administered, I/M, for prophylaxis. Additionally, 40% dextrose for rams was given, I/V, for electrolyte imbalance and dehydration. Improvement was noticed on the very next day and complete recovery was observed after three days. Early and aggressive treatment with an appropriate alkalinizing agent could cure the animals. Early admission of animals to veterinary clinics is recommended to reduce animal deaths.

Key Words: *Calf, Grain overload, Rams, Rumen Ph; Rumen Microbes*

Introduction

Grain overload, also known as "Lactic Acidosis" or "Acute Carbohydrate Engorgement", is a disorder of microbial fermentation in the rumen of ruminants such as cattle, sheep and goats (Nagarajam *et al.*, 2007). Clinical definitions of ruminal acidosis are based on rumen pH cut points (Plaizier *et al.*, 2008). In clinically diseased ruminants, the morbidity rate ranges from 10 to 50%, and the mortality rate from lactic acidosis can be as high as 90% when left untreated, compared to 30 to 40% when it is properly treated (Dagnaw Fenta *et al.*, 2023).

Acidosis is caused by suddenly consuming excessive quantities of carbohydrate-rich feed, eating a diet poor in fiber, failing to produce buffering, producing weak VFA, and producing lactic acid in the rumen (Rodostitis *et al.*, 2000). The severity and clinical result are determined by the type and amount of carbohydrate-rich feed (Gentile *et al.*, 2004). Due to group competitiveness, which encourages the animals to consume more feed, group feeding exacerbates the disease (Rodostitis *et al.*, 2007). The rumen microbes multiply rapidly with the feeding of concentrates (Chen *et al.*, 2011), resulting in an increase in starch availability and a high rate of short-chain fatty acid (SCFA) release (Schwaiger *et al.*, 2013). This can lead to accumulation and decrease of ruminal pH, promoting the growth of *Streptococcus spp* and *Lactobacillus spp* (Bramley *et al.*, 2005; Asefa *et al.*, 2020). The production of lactic acid by these bacteria further reduces rumen pH, resulting in fermentation stasis, absorption of D- and L- lactate and metabolic acidosis (Silvestre *et al.*, 2023).

Acidosis is a condition that can present in both the clinical and sub-clinical forms. Reduced milk fat content, feed conversion efficiency, feed intake, intensity of roughage degradation, and lameness are the signs of sub-clinical acidosis (Lean *et al.*, 2000; Bramley *et al.*, 2005). Clinical acidosis presents as indigestion, rumen stasis, anorexia, depression, ataxia, abdominal distension, diarrhea, and weakness and ranges in severity from moderate to severe. It may be deadly in a few extreme cases in less than a day. Carbohydrate engorgement causes aberrant rumen distension, lactic acidosis, and rumen atony, which can result in the retention of fermented gases and maybe animal death (Bashir *et al.*, 2015). It causes a high death rate in

small ruminants and is one of the most significant clinical emergencies (Radostits *et al.*, 2000; Revathi *et al.*, 2015).

Diagnosis of clinical rumen acidosis is based on history of exposure to offending feedstuffs, clinical signs, and ancillary diagnostic tests (Alam *et al.*, 2014; Snyder and Credille, 2017). For mild instances of acidosis, reducing the amount of grain fed and giving hay to promote salivation are two treatments (Oetzel, 2017). Additional therapy for metabolic acidosis includes oral antacids and oral electrolyte solutions. For severe cases, it is advised to significantly reduce the amount of grain offered, administer IV fluids, and make water or balanced electrolyte solutions free of lactic acid available (Arora *et al.*, 2011; Meyer and Bryant, 2017; Constantin *et al.*, 2018).

Management strategies that induce rumen acidosis in certain animals are often addressed in order to prevent it (Hassan *et al.*, 2023). If an animal is not habituated to high-concentrate diets, it is better to restrict its access to easily digested feedstuffs. Avoid making sudden feed changes, and if you must, do it gradually because it may take several weeks for the rumen microbes to adjust to new diets (Snyder and Credille, 2017). “This case report describes the clinical sign, laboratory findings and treatment outcomes of grain overload in calves and rams”.

Case descriptions

Case 1

On March 22, 2023, a local-breed male calf from Bishoftu was taken to Professor Feseha Gebreab Memorial-VTH with the history of the calf being off-feed, diarrheal, and reluctant to move after accidentally ingesting the dissolved grain a few hours earlier. The calves were managed extensively. Clinical observations were bloating, lethargy, nasal discharge, grunting sound, ataxia, anorexia, complete rumen atony, diarrhea, rapid respiration, and pulse. Foam when inserting the stomach tube into the rumen (Figure 30). Based on the history of feed and clinical findings, acidosis was tentatively diagnosed.



Figure 30: Indicating mucoid nasal discharge (A) and blotting (B).

Case 2

On March 20, 2023, two local breed rams from Bishoftu, kebele 12, were hospitalized at the Professor Feseha Gebreab Memorial-VTH with a history of depression, head pressing, and profused watery diarrhea following consumption of the digested powder of bread, which began 10 hours earlier. The rams were managed semi-intensively, and one ram passed away after ingesting for a period of time. Clinical observations were lethargy, nasal discharge, head pressing, recumbency, absence of ruminal motility, diarrhea, hyperpnea, tachycardia, and foam when inserting the stomach tube into the rumen (Figure 31). Based on the history of feed and clinical findings, acidosis was tentatively diagnosed.



Figure 31: Indicating severe diarrhea.

Laboratory Diagnosis and Result

Samples of ruminal fluid were taken using a stomacher bag and sent to the multipurpose lab of the college of veterinary medicine at Addis Ababa University in Bishoftu. The samples of ruminal fluid that had been taken were immediately tested for ruminal pH using litmus paper and for protozoal activity using a 10X microscope. The rumen fluid pH of the calf was 5.5 (figure 32 A), while that of the ram was 4 and 5-5.5 (figure 32 B). The microscopic examination of the rumen fluid revealed no protozoal activity. Finally, the definitive diagnosis of acidosis was reached based on the history, clinical examination, and laboratory results.



Figure 32: Indicating rumen fluid pH of calve (A) and rumen fluid pH of rams (B).

Case management and treatment outcome

The cases were treated by drenching indigestion powder (Chengdu Qiankun Veterinary Pharmaceutical Co., Ltd.) at 100 mg per liter of drinking water for the calf and 25 mg per liter of drinking water for the rams, as well as multivitamin (Aether Center Beijing Biology Co., Ltd.) at 4–8 ml and 0.4–0.8 ml in a single shot, I/M, and 20% oxytetracycline (Shijiazhuang Fengqiang Animal Pharmaceutical Co., Ltd.) at 0.1 ml/kg in a single shot, I/M. In addition to the abovementioned medications, 40% dextrose was also given to the rams through an IV.

Besides the drug treatment, the owners were advised to manage their animals by providing roughage feed until they recovered to prevent further acid formation in the rumen. Improvement was noticed the very next day. On the second day of treatment, they started feeding, the bloat disappeared, the diarrhea stopped, and complete recovery was observed after three days in all cases. Early admission of their animals to veterinary clinics was advised for the owners to prevent the deaths in this case (Figure 33).



Figure 33: Fully recovered calf and rams.

Discussion

On the basis of history, clinical signs, and laboratory examination the current cases were diagnosed as rumen acidosis. According to the current case report, a history of grain consumption was strongly indicative of severe ruminal lactic acidosis. After consuming a high-carbohydrate diet in large amounts, the lactate-producing rumen bacteria (*Streptococcus species*) multiply and ferment the readily available carbohydrate, leading to a significant accumulation of lactic acid (Panciera *et al.*, 2007; Tufani *et al.*, 2013; Schwaiger *et al.*, 2013).

The clinical symptoms described in the current case report within 10 hours of grain consumption include blotting, lethargy, nasal discharge, grunting sound, ataxia, anorexia, complete rumen atony, diarrhea, rapid respiration, and pulse. These symptoms have also been described in the literature (Plaizier *et al.*, 2008; Steele *et al.*, 2009; Jafari *et al.*, 2011; Bashir *et al.*, 2015). According to the severity of the acidosis, this rise in heart rate may be caused by the toxic effects of lactic acid, decreased plasma volume, and circulatory failure (Radostits *et al.*, 2000; Kofler *et al.*, 2023). In extreme situations, it may reach 155 beats per minute. This increase in respiration may be caused by increased blood carbon dioxide (CO₂) tension and lower blood pH, stimulating the respiratory center (Reddy *et al.*, 2014; Revathi *et al.*, 2015).

In the present case report, the ruminal fluids were collected using a stomach tube connected to a plastic bottle. The sheep's rumen fluid had a pH between 3 and 5, whereas the calf's had a pH of 5.1. This low pH value clearly indicates an acidic environment in the rumen. A pH value less than 5.5 is non-physiological and detrimental for normal rumen microbes (Abd El-Roaf *et al.*, 2007; Golder *et al.*, 2014; Huot *et al.*, 2023). Due to the low pH, gram-positive bacteria (*Streptococcus spp.*) can multiply rapidly and *Lactobacilli* can utilize the carbohydrate to produce huge quantities of lactic acid (Tufani *et al.*, 2013; Revathi *et al.*, 2015). The microscopic examination of rumen fluid in this case report showed no protozoal activity. Previous studies by Martin *et al.* (2006) reported a sharp decline in protozoan count in ruminants with ruminal acidosis. The death of microflora occurs due to acidic conditions, as microflora best thrive in neutral media of 6.4–7.0 (Steen, 2001; Minuti *et al.*, 2014; Bashir *et al.*, 2015).

In the present cases report, the cases were treated by drenching Indigestion powder 100mg dissolved in 1L water for calve and 25mg dissolved in 1L water for rams was pumped into rumen by stomach tube to neutralize the acidity, Multivitamin (4 ml) for calf and (1 ml) for each ram and 20% oxytetracycline (0.1ml/Kg) for prophylaxis. In addition to the above drugs the rams were also administered 40% dextrose through IV to counter dehydration and electrolyte imbalance. Magnesium hydroxide and Sodium bicarbonate are advised as potent alkalinizing agent to be used in ruminants as an antacid (Smith and Correa, 2004; Abalaka *et al.*, 2020). Improvement was noticed on the very next day and complete recovery was observed after three days. Early and aggressive treatment with appropriate alkalinizing agent could be cured the animals.

Acknowledgement

I am grateful to Dr. Tedros, Mis. Dereje Gudeta and the owners of the animals for their valuable information during the follow up.

3.2.4. *Listeriosis in a sheep*

Abstract

Listeriosis is a zoonotic infectious disease caused by *L. monocytogenes* with a low incidence but a high case fatality rate. This case report describes the clinical management of listeriosis in sheep. A female sheep of around 7 months was admitted to Professor Feseha Gebreab Memorial-VTH with a history of circling movement and reduced feed intake that started three days ago. The sheep were managed semi-intensively and fed household leftovers, straw, and vegetables from the market. The rectal body temperature was febrile (40.8 °C), while other parameters were within the normal range. Clinical observations were difficulty standing, difficulty balancing the head region, left-sided circling movement, head pressing, and congested conjunctival mucus membrane. Listeriosis was tentatively diagnosed based on the history and clinical findings. The case was treated symptomatically with 10% oxytetracycline (10 mg/kg/day) for five consecutive days and dexamethasone (0.2 mg/kg/day) for three days, both I/M, and the sheep showed early recovery within six days. Hence, early and aggressive treatment with appropriate antibiotics could cure the animals. Providing good-quality feed and assessing the feeding environment are recommended to minimize contamination.

Keywords: *sheep, circling movement, listeriosis, treatment*

Introduction

Listeriosis is one of the most important bacterial infections diseases worldwide, caused by *L. monocytogenes* and represents a risk to public health since it affects ruminants and humans (Oevermann *et al.*, 2010; Sarfraz *et al.*, 2017; Farag *et al.*, 2021). *L. monocytogenes* is a gram-positive, facultatively anaerobic, rod-shaped intracellular bacteria that does not produce spores or capsules (Ryser and Marth, 2007). It can happen when sheep kept on pasture consume low-quality silage contaminated with the bodily fluids of domestic and wild animals, or if they are raised in an unclean environment (Driehuis, 2013). The bacteria are extensively dispersed in the environment, and it is believed that decaying plant materials are where they naturally live as saprophytes (Schoder *et al.*, 2012; Lang Halter *et al.*, 2013) and can multiply when temperature and humidity conditions are ideal. There are now 20 species of *Listeria* in the family *Listeriaceae*, which are well suited to soil, water, and plants (Linke *et al.*, 2014; Orsi and Wiedmann, 2016; Rodriguez *et al.*, 2021).

Listeria infection's pathogenesis and symptoms. Following ingestion, *L. monocytogenes* can enter the intestinal mucosa and cause infections, which can include septicaemia, meningitis, encephalitis or uterine infections (Ho *et al.*, 2007; Constable *et al.*, 2016). Rapid progression, with death occurring 24–48 hours after the first signs. Clinical symptoms that sheep exhibited included loss of balance, profuse drooling, facial paralysis on one side, tremors, head deviation, lethargy, nystagmus, lateral recumbency, paddling, and shortness of breath (Braun *et al.*, 2002; Ribeiro *et al.*, 2022). Additionally, uveitis, keratitis, and ocular infections are also possible results (Nightingale *et al.*, 2004). uterine infections frequently result in subclinical mastitis, abortion, stillbirth, or septicaemia in newborns (Rodriguez *et al.*, 2021).

Diagnosis of listeriosis is based on history of silage consumption, recognizable symptoms, and laboratory test results (Siddiqui *et al.*, 2018). Additionally, *Listeria* species are identified in laboratories using conventional cultivation techniques including isolation, FA, inoculation, serological testing, and PCR (Conly and Johnston, 2008; Kundul and Ame, 2022). Combining antibiotics like Trimethoprim, Tetracycline, and supportive care is the best course of therapy (Braun *et al.*, 2002; Pal and Awel, 2013).

Listeriosis management and preventive strategies include hay and straw used as bedding should be thoroughly inspected; water irrigation of animals must be clean, fresh, transparent and, of course, free from infectious agents; Barns are disinfected using a solution of 5% creolin solution, 20% fresh slaked lime, or 2-3% sodium hydroxide on healthy farms once a month; livestock and locations where animals are aborted or killed should be cleaned and disinfected for listeriosis (Ilkhomovich, 2021). Additionally, isolate sick animals, dispose of spoiled silage (Hunter, 2020). The economic importance of disease due to production loss abortion, treatment cost, reduces welfare and trade of animal and they're by product (Sepahvand *et al.*, 2022). “This case report describes a clinical case of listeriosis in young sheep and its treatment outcome”.

Case description

A female sheep of around 7 months was admitted to Professor Feseha Gebreab Memorial-VTH of Addis Ababa University on December 4, 2022, with a history of circling movement and reduced feed intake that started three days ago. The sheep were managed semi-intensively with other sheep, and the sheep were fed household leftovers, straw, and vegetables from the market. Physical examination revealed that the sheep was febrile, with a rectal body temperature of 40.8 °C and 36 breaths per minute and 72 beats per minute respiratory and heart rates, respectively. On clinical examination, the sheep showed symptoms of difficulty standing, difficulty balancing the head region, left-sided circling movement, head pressing, and regurgitation of food material (Figure 34). Based on the history and clinical findings (circling), lists of differential diagnoses were made: cineriosis, botulism, listeriosis, and poisoning, tentatively.



Figure 34: Difficulty to balance the head in Listeriosis affected sheep.

Case management and treatment

The sheep was treated with 10% oxytetracycline (Shanghai Thongren Pharmaceutical Co., Ltd., China) at a dose of 10 mg/kg/day for five successive days and dexamethasone (Sokar Healthcare Pvt. Ltd., Gujarat, India) at a dose of 0.2 mg/kg/day for three days, both intramuscularly. After receiving therapy for 24 hours, the young sheep was able to stand by herself, begin walking, and begin eating healthily. Rectal body temperature was within the normal range (38.7 °C) at the end of the treatment (the fifth day), and the young sheep movement had improved. The sheep was completely recovered at the two-week checkup post therapy (Figure 35). Because the other differential diagnoses described above did not respond to therapy with 10% oxytetracycline, listeriosis was determined to be the cause of the condition.



Figure 35: Recovered young sheep at the end day of the medication (the 5th day).

Discussion

Listeriosis is a zoonotic infectious disease of both humans and animals caused by *L. monocytogenes* (Radoshevich and Cossart, 2018). It is associated with environmental contamination or contact with fluids and fecal shedding of animals that have been exposed to *L. monocytogenes* (Frag *et al.*, 2021; Rodriguez *et al.*, 2021). Young sheep can be affected when they are losing and changing their teeth, leading to lesions in the gums that the bacteria can penetrate (El-Beskawy *et al.*, 2010; Ribeiro *et al.*, 2022). In the present case report of listeriosis, based on information collected from the owner, the case may be associated to intake of infected vegetables and other feed, and the age of the sheep.

The Clinical presentations of listeriosis exhibited by the affected young sheep in this study were nervous manifestations. This is in line with earlier studies by Kumar *et al.* (2007) and Ribeiro *et al.* (2022), which found that sheep up to 6 months old had more pronounced nervous listeriosis symptoms than adult sheep did, and that their mortality rate was significantly higher than that of sheep older than a year.

The reports of Schleich (2019) and Kundul and Ame (2022) have extensively elucidated that the diagnostic approach for cases of listeriosis infection depends on the isolation of the *L. monocytogenes* from a normally sterile site, usually blood or cerebrospinal fluid, by gram stain result, which is gram-positive coccobacilli (characteristic of *L. monocytogenes*); however, in the current case report, diagnosis was made primarily based on the history of feeding management, exhibited clinical signs, and treatment response of the sheep.

Recovery from listeriosis depends on the stage and severity of the condition, with early and less severe cases responding to higher doses of antibiotic therapy (Srinivasan *et al.*, 2005; Brugère-Picoux, 2008; Hamidi *et al.*, 2020; Ribeiro *et al.*, 2022). Treatment with oxytetracycline or a combination of oxytetracycline and dexamethasone has been reported (Braun *et al.*, 2002; Conter *et al.*, 2009; Kundul and Ame, 2022) like in the case described in this report. The current case report was less severe and managed early hence the young sheep was successfully responded to 10% oxytetracycline (0.2 mg/kg/day) and dexamethasone (0.2 mg/kg/day) treatment.

In conclusion, early and aggressive treatment with antibiotics can cure the animals from Listeriosis case as shown in this case, that good quality and assessment of the feeding environment are important measures to minimize contamination, that exchange of feed that served as food for sheep is another control measure, and that manure treatment methods such as chemical disinfection and biogas production are recommended measures for prevention and control of listeriosis.

Limitations

No laboratory confirmation was made for this disease due to lack of facilities and hence treatment was done based on clinical picture only (symptomatic therapy).

Acknowledgement

My grateful appreciation goes to Dr. Taye and the owner of the animal.

3.2.5. *Salmonellosis in sheep*

Abstract

Salmonellosis can affect all species of domestic animals, including sheep, and is an economically important zoonotic pathogen worldwide. This case report describes the clinical signs, laboratory findings, and treatment outcomes of salmonellosis in sheep. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. Two local breed sheep from Bishoftu were presented to Professor Feseha Gebreab Memorial-VTH with a history of profuse watery fecal matter and reduced feed intake, started three days ago. The sheep were managed semi-intensively and fed household leftovers, straw, and vegetables from the market. Both sheep were febrile, and the other parameters were within the normal range. clinical signs include diarrhea which was watery to pasty, foul-smelling, and contained mucus, and depression. Fecal samples were collected, culture on xylose-lysine-desoxycholate (XLD) agar was performed. Growths of black colonies with a highly transparent zone of reddish color and Gram-negative, short rod-shaped bacteria arranged in single, paired, or short chains were observed which was indicative of *Salmonella*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as salmonellosis. Both sheep were treated successfully with Sulphamethoxazole-Trimethoprim (1 ml/16 kg/day), I/M and 40% dextrose (10 ml/kg), PO. The improvement was noted one week after treatment and fully recovered as the owner report by phone call. Hence, early and aggressive treatment with appropriate antibiotics could cure the animals. Avoiding contaminated household left-over feed is recommended to minimize the incidence of the disease.

Key Word: *Diarrhea, Salmonellosis, Sheep, Treatment*

Introduction

Salmonellosis can affect all species of domestic animals including sheep and economically important zoonotic pathogens worldwide (Balasubramanian *et al.*, 2019; Hawwas *et al.*, 2022). The disease is concerned as one of the most prevalent diseases in small ruminants in areas of intensive management system and poor husbandry system (del Carmen Ferreras *et al.*, 2007; Ayaa, 2016). It is mostly an enteric infection, which has clinical and subclinical forms (Acha and Szyfres, 2001). Age and serotype have an impact on the severity of the disease and the clinical symptoms of *Salmonella* infection in small ruminants (Uzzau *et al.*, 2001). There are more than 2600 identified *Salmonella* serovars are identified globally (Jajere, 2019; Farouk *et al.*, 2021). The disease is characterized by watery and mucoid diarrhea with the presence of fibrin and blood (Khairy and Marvet, 2019).

The American veterinarian Daniel Elmer Salmon, who discovered *Salmonella enterica* serotype Choleraesuis in pigs for the first time in 1885, gave the genus *Salmonella* its name (Girma, 2015). It is under the family of *Enterobacteriaceae*, which consists of rod-shaped, Gram-negative, oxidase negative, non-spore forming, predominantly motile bacteria (Jay *et al.*, 2003; Ibrahim *et al.*, 2022). The serotypes have a broad host range. Some serotypes are highly adapted to animal hosts, such as *Salmonella gallinarum* in poultry, *Salmonella abortusovis* in sheep (Ohl and Miller, 2001; Santos *et al.*, 2001). Feco-oral transmission is the most common route to get a disease. Feed, water, pasture, wastes; wild animals, etc. can serve for the transmission of the pathogen into farm animals which in turn serve as a source for human infection (Ibrahim *et al.*, 2022). Animals can also become infected via the conjunctiva or the respiratory tract, but these routes of transmission are rare (Brugère-Picoux and Lefloc'h Soye, 2014; Benzaouche *et al.*, 2021).

The organisms in the rumen are rapidly growing and multiplying, passing into the small intestine and adhering to the epithelial cells of the small intestine cause enteritis, diarrhea, and enterotoxins (Teferi, 2020). The bacteria penetrate mucous membranes, invade Peyer's patches, mesenteric lymph nodes, and enter the blood, reaching all organs. When the organism enters the bloodstream, a febrile reaction occurs and the acute phase of the disease develops 24 - 48 hours.

Death from the disease usually occurs due to shock, septicemia, endotoxemia, dehydration and acidosis (Radostitis *et al.*, 2007).

Salmonellosis presents with varied clinical signs that may depend on the infecting dose, health of the host, *Salmonella* serovar/strain, and other factors (Chiu *et al.*, 2004). Acute enteric salmonellosis is common in adult sheep which presents as fever, anorexia, depression, and diarrhea, while young animals were noted to develop septicemia (Sharma *et al.*, 2001). Infections of ewes with serotype *s. abortusovis* were found to suffer from stillbirth, merits, placental retention, or peritonitis (Sharma *et al.*, 2001; Murray *et al.*, 2010). Diagnosis of salmonellosis based on Clinical findings and confirmed through laboratory diagnosis (Schott *et al.*, 2001; Warnick *et al.*, 2003; Barrow and Feltham, 2003).

Tetracycline's, Nitro furazone, Fluorquinolone, sulfadimidine and trimethoprim are effective treatments for salmonellosis (Adams, 2001; Radostitis *et al.*, 2007; Ahmed, 2022). Attenuated DNA recombinant live *Salmonella* vaccines combined with comprehensive control and Prevention strategy in animals, feed, water and animal foodstuff implementation of hygienic measures will help reduce salmonellosis (Mastroeni and Menager, 2003; Kemal *et al.*, 2015). Salmonellosis causes significant economic loss in farm animals due to the cost of clinical disease, diagnostic laboratory cost, cleaning and disinfection, and control and prevention (Benzaouche *et al.*, 2021; Pal *et al.*, 2020). "This case report describes the clinical sign, laboratory findings and treatment outcomes of salmonellosis in sheep".

Case description

Two local breed sheep from Bishoftu were presented to Professor Feseha Gebreab Memorial-VTH on November 24, 2022, with a history of profuse watery fecal matter and reduced feed intake that started three days ago. The sheep were managed semi-intensively and fed household leftovers, straw, and vegetables from the market. On clinical examination, both sheep were febrile, and the other parameters were within the normal range. Both sheep had diarrhea and depression. The diarrhea was watery to pasty, often foul-smelling, and contained mucus (Figure

36). Based on the history and clinical findings, lists of differential diagnoses were made, including coccidiosis and salmonellosis, tentatively.



Figure 36: Diarrheic sheep affected by salmonellosis.

Laboratory investigation and findings

The fecal samples were collected directly from the rectum by using sterile swabs and sterile surgical gloves. The collected fecal swabs were putted in the test tubes, which contain transport media of peptone water, and incubated aerobically at 37 oC for 24 hr at the microbiology laboratory of Addis Ababa University College of Veterinary Medicine, Bishoftu. From fecal samples collected by sterile surgical gloves, a direct fecal examination technique was performed for the detection of *Emeria* and *Coccidian* oocytes, and the result was negative. Following incubation, the bacteriological culture was made on xylose-lysine-desoxycholate (XLD) agar and incubated aerobically at 37°C for 24 hours, revealing growths of black colonies with a highly transparent zone of reddish color (ANNEX). Again, colonies from XLD were randomly picked and subcultured on nutrient agar at 37 oC for 24 hours and stained with gram stain; revealed gram-negative short rod bacteria were seen under oil immersion (100 X) on the prepared smears (Figure 37). These bacteria were identified as *Salmonella*. Finally, it was

determined that salmonellosis was the final diagnosis based on the history, nature of the disease, clinical findings, and laboratory findings.

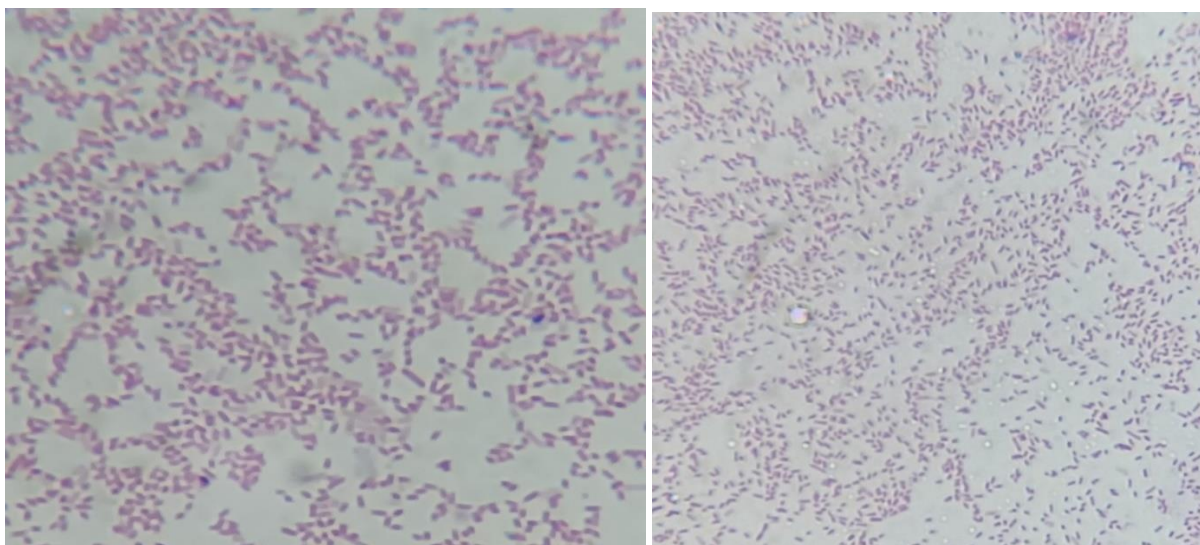


Figure 37: Indicating gram-negative short rod bacteria.

Case management and treatment outcome

Both sheep were treated with Sulphamethoxazole-Trimethoprim (Chengdu Qiankun Veterinary Pharmaceutical Co., Ltd., China) at a dose of 1 ml/16 kg/day for the subsequent five days, IM, and 40% dextrose at a dose of 10 ml/kg, PO. On the second day of therapy, the sheep started taking feed, but the evacuation of runny feces continued with a slight change. However, on the fifth day of therapy, the diarrhea stopped. Both sheep were followed by a phone call after they finished their treatment period, and one-month post-treatment, both sheep were fully recovered (as the owner reported).

Discussion

Diarrhea is an important problem in young domestic animals although its etiology is not well understood since several agents may be involved concurrently (Nasr *et al.*, 2014). The current case was asserted as Salmonellosis based on the history, clinical findings and laboratory result (bacteriology examination). The clinical signs observed in this case report; diarrhea and

depression. The diarrhea was watery to pasty, often foul smelling, and contain mucus were similar signs of the Salmonellosis mentioned on literatures (Van Metre *et al.*, 2000; Uzzau, 2013; Nasr *et al.*, 2014; Mokhbatly *et al.*, 2022; Hafez *et al.*, 2022; Hawwas *et al.*, 2022).

In the present case study, to confirm the presence of *Salmonella*, the fecal samples were cultured on xylose-lysine-desoxycholate (XLD) agar and incubated aerobically at 37°C for 24hr and *Salmonella* was identified. The colony characteristics of *Salmonella* observed on XLD showed black colonies and a highly transparent zone of reddish color was similar to the findings of other authors (Clark *et al.*, 2004; El-Twab *et al.*, 2016; Farouk *et al.*, 2021; Ibrahim and Abdul-Kareem, 2022; Gaffar *et al.*, 2022). In Gram's staining the morphology of the isolated *Salmonella* appeared Gram-negative, pink-colored, tiny rod-shaped appearance arranged in single or paired or short-chain. These findings were in agreeable with several authors such as Muktar *et al.* (2015), Raghavendra *et al.* (2017), Sarker (2018), and Sabur *et al.* (2021).

The present case was treated successfully with Sulphamethoxazole- Trimethoprim (1ml/16kg/day) I/M and 40% dextrose PO. The improvement was noted one week post treatment which is in agreement with different literatures (Kumaresan *et al.* (2012), Manzoor *et al.* (2018), and Carter *et al.* (2021), they report Sulfadimidine is the drug of choice for salmonellosis case in calves.

In conclusion, early and aggressive treatment with antibiotics can cure severe cases of Salmonellosis as shown in this case. Assessment of the feeding environment and household left over feed are recommended to minimize contamination.

Acknowledgement

I am grateful to Mis. Tsedale Teshome, Mis. Tesfanesh and the owner of the animal for their valuable information during the diagnosis and follow up.

3.2.6. Mixed parasitic infection by *monezia* and *strongyle spp* parasite in ewe

Abstract

Gastrointestinal parasite (GIP) infections are among the most important health problems in pasture-based livestock production systems. Among cestodes infections in small ruminants, *Moniezia* infections are the most common and widely distributed. This case report describes the clinical signs, laboratory findings, and treatment outcomes of mixed parasitic infection case in ewe. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A 4-year-old local breed ewe from Bishoftu, Kaliti Kebele, was brought to Professor Feseha Gebreab Memorial-VTH with a history of reduced feed intake and reduced body condition one month ago. The ewe was managed extensively with other flocks of sheep. Rectal temperature, heart rate, and respiration rate were measured and found to be within the normal range. Clinical signs during diagnosis include: dullness, weakness, poor body condition, and depression. Fecal sample was collected, and faecal floatation and sedimentation techniques were performed. It was positive for *Monezia* and *Strongyle* oocytes. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as mixed parasitic infection. The ewe was managed successfully with Albendazole 300mg (7.5 mg/kg) and Ivermectin (0.2 mg/kg) both in a double dose (within a two-week interval). The ewe appeared healthy and had gained weight as the owner reported by phone. Effective deworming sessions twice or three times a year are recommended as prophylactic measures for the prevention of the parasitic infection.

Key Word: Anthelmentic, Ewe, Feces, Flotation

Introduction

Gastrointestinal parasite infections are a global problem for both small and large-scale farmers but their impact is greater in Sub-Saharan Africa in general due to the availability of a diverse range of agro-ecological factors suitable for diversified hosts and parasite species (Singh *et al.*, 2017; Fayisa *et al.*, 2020). Ethiopia has high prevalence rates of gastrointestinal (GI) nematode infections, with an estimated prevalence of 75.8% (Asmare *et al.*, 2016). Endoparasites are parasites with a major economic impact that greatly harm small ruminants reared in extensive and intensive production systems. In addition, helminthes refers to a complicated disease brought on by nematode, cestode, and trematode parasites, even though the parasite can infect any grazing sheep or goat (Aliyu *et al.*, 2020).

The genus *Moniezia* belongs to the family *Anoplocephalidae* and the genus *Strongyloides* belong to the order *Rhabditida*, have a worldwide distribution (Taylor *et al.* 2015; Verocai *et al.*, 2020). Due to the prevalence of mites eating *Moniezia* eggs, *Moniezia expansa* infection in sheep is more prevalent in the winter, while juvenile forms (metacestodes) are present in the intermediate hosts, adult forms (hermaphrodites) are only present in the final host (Schuste, 2001; Abdelnabi *et al.*, 2011). Strongyle infections have significant financial implications in adult sheep also affect newborns or animals with compromised immune systems (Gathuma *et al.*, 2007). The transmission can be trans mammary, cutaneous or by the ingestion of the L3 (Verocai *et al.*, 2020).

The life cycle of cestodes is thought to be indirect, and it starts with the intermediate host (a free-living oribatid mite) ingesting feces carrying *Moniezia* eggs. Following consumption, the oncosphere (first generation larva) emerges from the egg, penetrates the gut, and grows into a cysticercoid larva (second generation larva). By accidentally ingesting the mite while grazing, the definitive host becomes afflicted. The cysticercoid larva adheres to the wall of the small intestine by the scolex after the arthropod has been digested within the ruminant. When it reaches maturity, it begins to reproduce automatically and begins to produce eggs (which contain the onchosphere), which are then expelled singly with the feces, collectively, or inside the proglottid (Verocai *et al.*, 2020). The Pathogenesis *Moniezia spp* in heavy infection the

intestine becomes solid mass by the parasite and this causes enterotoxaemia then death (Radositis *et al.*, 2008).

The majority of the time, *Monieziasis* and *Strongyloidiasis* in sheep do not present any clinical signs or symptoms, but large loads may cause reductions in feed intake, thriftiness, poor coat, nebulous digestive disorders such mild diarrhea or constipation dysentery, and occasionally anemia in young animals (Radositis *et al.*, 2008). Diagnosis GIP on sheep is based on fecal examinations beyond the clinical sign, and the presence of worm eggs or larvae is the most common routine aid for diagnosis (Gupta and Singla, 2012; Rajpoot *et al.*, 2017). Anthelmintics like albendazole, fenbendazole, febantel, mebendazole, benzimidazoles, ivermectin, niclosamide, Praziquantel are highly effective against *GIP* (Taylor *et al.*, 2015; Barda *et al.*, 2017; Verocai *et al.*, 2020; Hürlimann *et al.*, 2023).

GI parasite control and prevention interventions are alternating grazing of different host species, integrated rotational grazing of different age groups, and alternation of grazing and cropping can give an economic advantage when combined with anthelmintic treatment (Tibbo, 2006). Carbamate insecticides such as carbaryle and propoxur are used to control the mites, which act as intermediate hosts (Zeryehun, 2012). “This case report describes the clinical sign, laboratory findings and treatment outcomes of mixed parasitic infection case in ewe”.

Case description

A 4-year-old local breed ewe from Bishoftu, Kaliti Kebele, was brought to Professor Feseha Gebreab Memorial-VTH on November 5, 2022, with a history of reduced feed intake and reduced body condition that started two weeks ago. The ewe was managed extensively with other flocks of sheep. On clinical examination, the rectal body temperature, respiratory, and heart rates were within the normal limits. There was dullness, weakness, poor body condition, and depression (Figure 38). Based on the history and clinical findings, lists of differential diagnoses were made, including fasciolosis, and another GIT parasite, tentatively.



Figure 38: Indicating depressed ewe with poor body condition

Laboratory investigation and findings

A fecal sample was collected directly from the rectum and immediately processed at the veterinary parasitology laboratory of Addis Ababa University College of Veterinary Medicine, Bishoftu. Both the sedimentation and floatation methods were applied, but the sedimentation method result was negative; there was no egg. Fortunately, the floatation technique was found helpful, and no further investigations were performed. Accordingly, typical *Monezia* and *Strongyle* oocytes were appreciated under a microscope with an X10 objective lens (Figure 39). Therefore, based on the history, clinical findings, and laboratory results, a definitive diagnosis of mixed parasitic infection was made, and a treatment regimen was arranged.

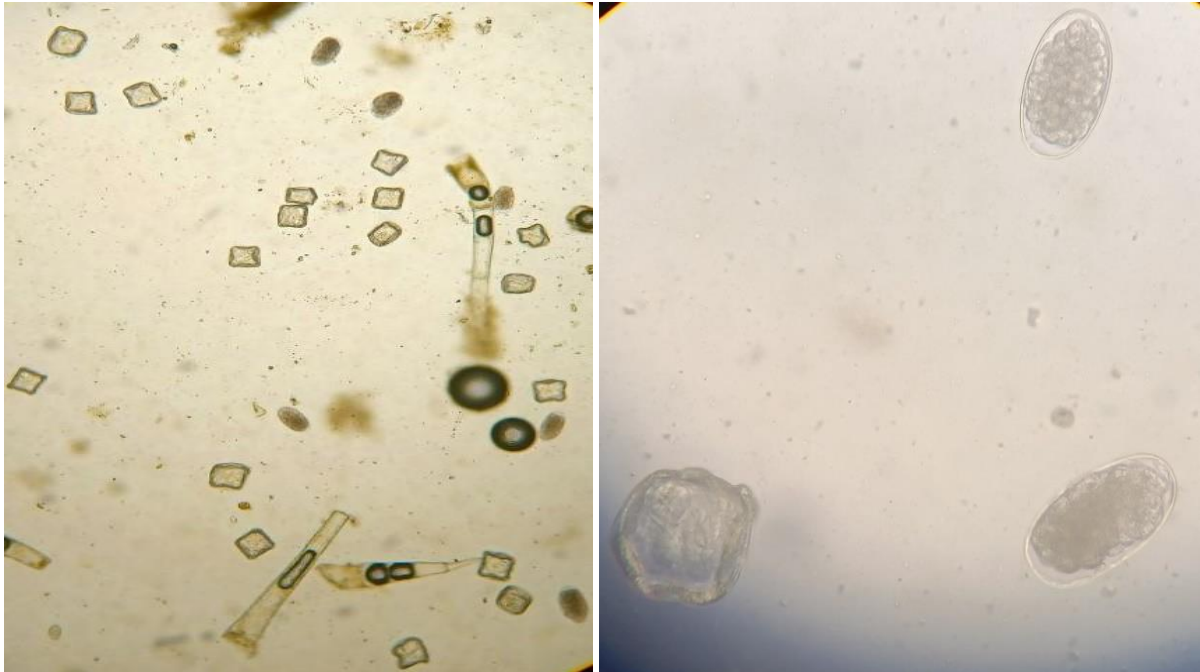


Figure 39: Indicating *moniezia* and *strongyle* spp egg under 10X and 40X magnification

Case management and treatment outcome

Sub clinically infected ewe was administered Albendazole 300mg (Chengdu Qiankun Veterinary Pharmaceutical Co., Ltd) at 7.5mg/ kg double dose (two-week interval) with double shot (within two weeks interval) of Ivermectin (Sheyang Sunvictotor Pharmaceutical Co., Ltd. /China) at a dose of 0.2mg/kg subcutaneously. The appetite of the ewe was returned to normal. Generally, considerable change was appreciated on the body condition of the ewe after one month of post treatment (phone call owner report).

Discussion

Gastrointestinal parasite (GIP) infections are among the most important health problems in pasture-based livestock production systems. Pasture-based production systems that raise small ruminants have long incurred economic losses due to parasitic diseases (Arzik *et al.*, 2022). Among cestodes infections of small ruminants, *Moniezia spp.* infection is the most common and widely distributed (Gizachew *et al.*, 2015; Sharma *et al.*, 2020; Atiyah and Azzal, 2022).

Nguyen *et al.* (2012), based on a PCR study in Vietnam, described *M. expansa* as a dominant species in sheep.

In the present case study, the observed clinical signs are dullness, weakness, poor body condition, and depression. Similar observations were made by Chamuah *et al.* (2013), Raghavendra *et al.* (2018), and Zainalabidin *et al.* (2021). The dullness, weakness, and emaciation of the ewe observed in the present case study could be attributed to hypoglycemia, hypoproteinemia, and loss in body muscles during severe GI parasitic infection (Ahmed *et al.*, 2015; Liu *et al.*, 2007; Kar *et al.*, 2007). Among the intestinal parasites observed in this study, infection with *Moniezia expansa* was predominant in this case report.

In the present case study, a fecal sample was taken and examined with the flotation method, and it was positive for *Moniezia expansa* and *Strongyles* eggs, which were highly infested by this parasite. This examination technique was previously supported by Arulmozhi *et al.* (2011), Abdel-Megeed *et al.* (2014), Raghavendra *et al.* (2018), and Sirag (2019). The high infestation of *Moniezia expansa* and *Strongyles* parasites in ewes observed in this study could be a result of the existing grazing system adopted by the farm, where no systematic grazing pattern is followed and there is no pasture rotation or resting.

In the subclinically infected ewe in the present case study, the mixed infection was successfully managed with Albendazole 300mg (7.5 mg/kg) and Ivermectin (0.2 mg/kg) both in a double dose (within a two-week interval). Similar treatment was used in the literature (Chamuah *et al.*, 2013; Sheikh, 2021; VanHoy *et al.*, 2022; Madhu *et al.*, 2022) with variable results. In conclusion, effective deworming sessions twice or three times a year is recommended as prophylactic measures.

Acknowledgement

I am grateful to Ms. Gebeyehu, Mis. Tigest and the owner of the animal for his valuable information during the follow up.

3.2. Case Reports on Caprine

3.3.1. Infectious Keratoconjunctivitis in Doe and Heifers

Abstract

Infectious keratoconjunctivitis (IKC) is a highly contagious ocular inflammatory condition that is often reported in domestic small and large ruminants. This case report describes the clinical, laboratory, and therapeutic aspects of infectious keratoconjunctivitis in Heifers and Doe. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. The Doe was presented to Professor Feseha Gebreab Memorial-VTH with an inflamed left eye accompanied by excessive lacrimation, mild corneal opacity, conjunctivitis and photophobia. Two heifers were examined at Bishoftu on a privately owned farm with an inflamed right eye accompanied by lacrimation, photophobia, cloudiness, corneal opacity, and blindness of the right eye in both heifers. Ocular swabs were collected, culture on 5% sheep blood agar were performed. Growth of small, grey-white, rough cells surrounded by a wide area of hemolysis and gram-negative thin rods to coccus-like structures that have a tendency to occur as diplobacilli or in short chains were seen which was indicative of *Moraxella*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as Infectious keratoconjunctivitis. In all cases, 1% tetracycline hydrochloride eye drops were applied to the affected eyes twice daily for five days, along with 10% oxytetracycline (1 mL/10 kg), I/M. The Doe was fully recovered two weeks after treatment but the heifers were not responded for the treatment and once again treated with ciprofloxacin and dexamethasone eye drops (two drops twice daily) for five days, only lacrimation ceased. Treat the animals early, remove the carrier animals, and improve sanitary conditions in the animal's environment are recommended in order to prevent a reoccurrence.

Keywords: *Diagnosis, Doe, Heifers, Infectious Keratoconjunctivitis, Treatment*

Introduction

Infectious keratoconjunctivitis (IKC), also called "pink eye," is an economically important disease that affects the eyes and membranes of many animals, including cattle, sheep, goats, dogs, and cats. It occurs in many parts of the world with low mortality and high morbidity (Gupta *et al.*, 2015; Jesse *et al.*, 2017). It is a highly contagious disease that often affects one or both eyes, is severe, and spreads rapidly (Hammadi, 2015). In cattle, the most important causative agents of infectious bovine keratoconjunctivitis are *Moraxella bovis* and *Mycoplasma spp* (Loy *et al.*, 2021; Karthik *et al.*, 2021). Among sheep and goats, the causative agents of this disease are *Mycoplasma spp.*, *Chlamydophila spp.*, *Staphylococcus aureus*, *Moraxella caprae*, *Moraxella ovis*, *Listeria monocytogenes*, and *Pseudomonas aeruginosa* (Evans *et al.*, 2008; Giangaspero *et al.*, 2010; Abdullah *et al.*, 2015; Karthikeyan *et al.*, 2022).

IKC is a disease that presents throughout the year, but peaks in autumn and summer due to increased UV radiation (Snowder *et al.*, 2005). Risk factors include flies, immune status, virulence of the pathogenic organisms involved, stress, sunlight and irritant factors such as pollen and tall grasses may predispose or exacerbate the disease (Fernandez-Aguilar *et al.*, 2017, Santos *et al.*, 2022). Within a herd of ruminants, the disease is disseminated either directly through contact with contaminated objects or indirectly through flies' conjunctival exudates. Light coloured animals have higher incidence of the disease and there is a resistance in cattle with more pigmentation at ocular margin (Snowder *et al.*, 2005).

Clinical symptoms of ICK have been linked to contagiousness, a sudden start, excessive lacrimation, conjunctival inflammation, corneal opacity or ulceration, blindness, behavioral deviations, and even death of the animal (Evans *et al.*, 2008; Hadani *et al.*, 2013; Gupta *et al.*, 2015). The cornea may burst in the most serious cases, which might result in irreversible blindness. When blood vessels enter the cornea and extend toward the ulcer, the healing process starts. While animals with minor pinkeye can recover in five weeks, those with severe infections might suffer months or even years of corneal damage (Rodríguez, 2006).

A diagnosis of ICK is based on the history, presenting clinical signs and laboratory confirmation by the isolation of the causative agent in culture with subsequent immunologic identification, or by using molecular techniques such as PCR (Giangaspero *et al.*, 2010; Kowalski *et al.*, 2017; Santos *et al.*, 2022). Successful treatment of ICK is usually achieved by administering both topical and systemic antibiotics and non-steroidal anti-inflammatory agents (Clark, 2013; Regnier *et al.*, 2013). It can be prevented and controlled using Fly control, Clipping the pastures, Disinfectants and Vaccination techniques (Haile and Ayano, 2021). It causes significant economic losses to livestock farmers due to treatment costs, lowered production and mortality. “This case report describes clinical, laboratory and therapeutic aspects of infectious keratoconjunctivitis in heifers and doe”.

Case description

Case 1

Two exotic heifers around two years old were examined at Bishoftu in one of the privately owned farms on March 1, 2023, with a history of white discoloration of the eye, eye discharge, and reduced feed intake that started one month ago. The heifers were managed intensively, and such cases happened on the farm every year. Physical examination revealed that the heifers' parameters were in the normal range: a rectal body temperature of 38.8 °C, 32 breaths per minute, and 80 beats per minute respiratory and heart rates, respectively. Ocular examination showed lacrimation, photophobia, cloudiness, corneal opacity, and blindness of the right eye in both heifers (Figure 40). Infectious keratoconjunctivitis was tentatively diagnosed based on the history and clinical findings.



Figure 40: Indicating lacrimation and Corneal opacity.

Case 2

An adult local breed doe from Bishoftu, Kebele 05, was admitted to Professor Feseha Gebreab Memorial-VTH on March 1, 2023, with a history of fear of sun light, eye discharge, red eye, and reduced feed intake that started three days ago. The doe was managed extensively with other goats, and such an infection previously occurred in another goat. Physical examination revealed that the goat was slightly febrile, with a rectal body temperature of 39.9 °C and 32 breaths per minute and 92 beats per minute respiratory and heart rates, respectively. Ocular examination

showed lacrimation, mild corneal opacity, conjunctivitis, photophobia, and conjunctival and corneal hyperemia of the left eye (Figure 41). After ruling out traumatic injuries, a tentative diagnosis of infectious keratoconjunctivitis was made based on the history and clinical findings.



Figure 41: Indicating lacrimation, mild corneal opacity and conjunctivitis.

Laboratory investigation and findings

Ocular swabs from both cases were collected aseptically by using sterile swap, then immediately putted in the test tube, which contains transport media of peptone water, and submitted to the veterinary microbiology laboratory of Addis Ababa University College of Veterinary Medicine, Bishoftu, and immediately incubated aerobically at 37 °C for 24 hours. Following incubation, the bacteriological culture was made on 5% sheep blood agar and incubated at 37 °C for 24 hours. The growth of small, grey-white, rough cells surrounded by a wide area of hemolysis was observed (Appendix) and stained by Gram's stain, which revealed gram-negative thin rods to coccus-like structures and a tendency to occur as diplobacilli or in short chains under a 100x binocular microscope (Figure 42). This finding suggests *Moraxella*. Finally, based on the

history, clinical examination, and laboratory findings, the definitive diagnosis was infectious keratoconjunctivitis.

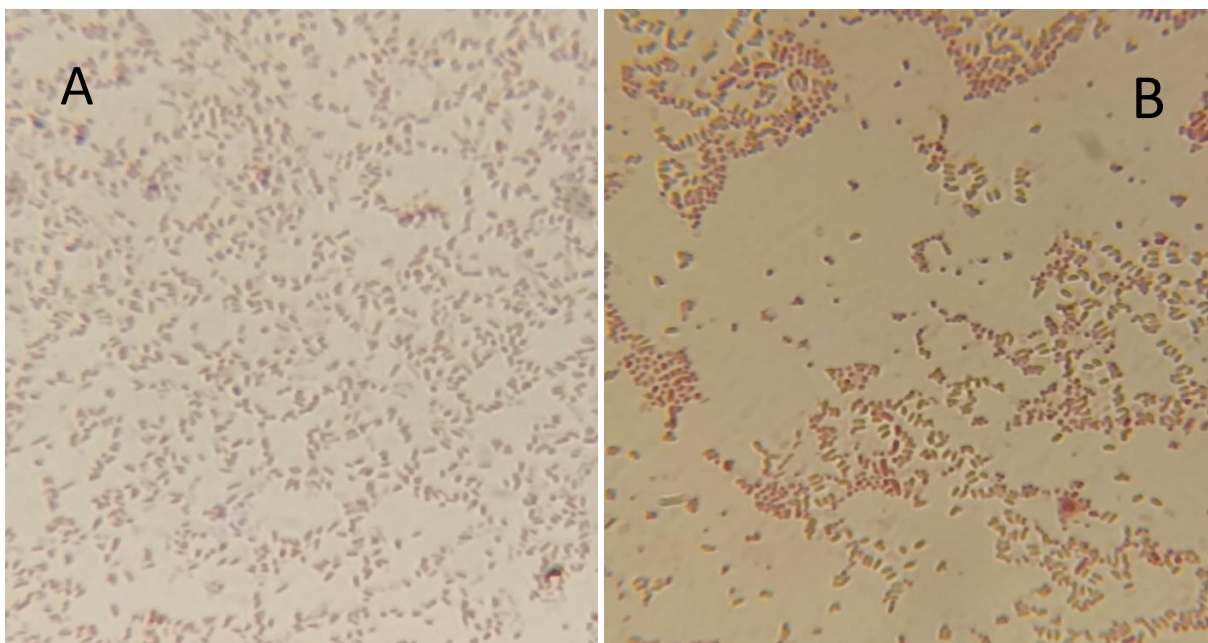


Figure 42: Indicating Gram- negative, short rod- coco bacilli bacteria from heifers' (A) and Doe sample (B).

Case management and treatment

All cases were treated with 10% oxytetracycline (Shanghai Thongren Pharmaceutical Co., Ltd., China) at a dose of 10 mg/kg for five successive days, and 1% tetracycline hydrochloride (Galentic Pharma India Pvt. Ltd.) at a dose of two drops twice a day was topically applied on the conjunctiva for five days. The owner was advised to improve the sanitary conditions in and around the goat pen and manage the goat intensively until it fully recovered. The doe responded well to the antibiotic therapy, and tremendous recovery was made within 2 weeks of presentation. On routine follow-up, there was no lacrimation or hyperemia, and the conjunctiva were normal (Figure 43). The heifers did not respond to the treatment and once again treated with ciprofloxacin and dexamethasone eye drops (FDC Limited) at two drops twice a day for five days, but only lacrimation was stopped. The owner was advised to treat the animals early, remove the carrier heifers, and improve sanitary conditions on the heifer's farm in order to prevent reoccurrence.



Figure 43: Fully recovered doe after two weeks of post treatment and unrecovered heifer after the second therapy, only lacrimation stopped.

Discussion

Infectious keratoconjunctivitis is a serious problem in bovines and small ruminants, caused by *Moraxella* and *Mycoplasma spp.* appear to be the most important. The season and flying insects are prime predisposing factors that contribute to the transmission of the disease. Face flies have been reported to be excellent mechanical transmitters of infectious diseases such as IKC from an infected animal to a susceptible animal (Abdullah *et al.*, 2013; Aguilar *et al.*, 2019). In the current case report, as indicated in the history, it is very likely that a source of infection exists near where these cases were kept and was transmitted to the animals through mechanical means, probably by flying insects.

The clinical stages of IKC are categorized into five different stages in ruminants. The goat with stage one and two IKC will present with photophobia, conjunctivitis and lacrimation (Ojo *et al.*, 2009; Abdullah *et al.*, 2014; Karthik *et al.*, 2017; Zaitoun *et al.*, 2021; Wang *et al.*, 2022), as was the doe case in the present case report. This may go on to Stage 5, which is characterized

by corneal oedema, corneal opacity and ulceration, excessive lacrimation, photophobia, and blindness (Alberti *et al.*, 200; 6 Jesse *et al.*, 2017; Kneipp, 2021), as was the heifer's case in the present case report.

In the present case report, the bacteriological cultures were made on 5% sheep blood agar. The growth of small, white, rough cells surrounded by a wide area of hemolysis was observed and stained by Gram's stain, which revealed gram-negative thin rods to coccus-like structures and a tendency to occur as diplobacilli or in short chains. Prior studies (Akerstedt and Hofshagen, 2004; Hadani *et al.*, 2013; Meekins *et al.*, 2017; Angelos *et al.*, 2021) on *Moraxella spp.* described similar colony and gram stain morphological characteristics.

In the present case report, use of tetracycline hydrochloride 1% (two drops twice a day) and 10% oxytetracycline (10 mg/kg/day) successfully managed IKC in a doe as previously recommended (Mughal *et al.*, 2015; Jesse *et al.*, 2017; Pandey, 2018). As the disease progresses without treatment, ocular discomfort and blindness may happen (Rodrguez, 2006; Kneipp, 2021), as was the heifer's case in the present case report. The present case study suggests that early detection as well as specific chemotherapy and prophylactic therapy are essential for successful management of infectious keratoconjunctivitis case in animals.

Inconclusion, Infectious keratoconjunctivitis is a serious problem in bovines and small ruminants. Early diagnosis and antimicrobial treatment increase the recovery rate, as shown in this case report. Treat the animals early, remove the carrier animals, and improve sanitary conditions in the animal's environment are recommended in order to prevent a reoccurrence and spread of the disease.

Acknowledgement

My grateful appreciation goes to Dr. Mehari, Mis Tesfanesh and the owner of the animal for their valuable information during the treatment, diagnosis and follow up period.

3.4. Case Reports on Swaine

3.4.1. Foot and Mouth Disease in pigs and ox: Symptomatic therapy

Abstract

Foot and Mouth Disease is an acute, extremely contagious, and highly communicable viral disease of all cloven-hooved animals and is caused by the genus *Aphthovirus*. This case report describes the clinical signs and treatment outcomes of FMD cases in an ox and pigs. A seven-year-old local breed ox from Bishoftu, Kebele 12, was presented to Professor Feseha Gebreab Memorial-VTH with a history of refusing to eat feed and drooling of saliva that started three days ago. The pigs were examined at Bishoftu on one of the privately owned farms with a history of difficulty in movement and lesions on the mouth and feet that started two days ago. Physical and Clinical examination of the ox revealed fever, salivation, and more than three ruptured circular lesions (erosions) on the upper gum and the tongue. Physical and Clinical examination of the pigs revealed fever, lameness, stamping of the feet, preferring to lie down, vesicular eruptions on their feet and snoot, as well as an unruptured vesicle on their snoot. Both cases were clinically diagnosed as FMD and treated symptomatically with 10% oxytetracycline (10 mg/kg/day) for five consecutive days with application of tincture iodine on the erosions of the ox gum and 2% of the citric acid solution was used for disinfecting the pigs farm where the case was observed and restricting the movement of the farm workers were performed; the ox and pigs were fully recovered. Biosecurity measures and vaccination are recommended for control and prevention of FMD.

Key Words: *FMD, Ox, Pigs, Treatment, Vesicular Eruptions*

Introduction

Foot and mouth disease (FMD) is an acute, infectious viral disease for animals, and is one of the most rapidly spreading diseases worldwide (Mushayabasa and Tapedzesa, 2015). According to the World Organization for Animal Health (OIE), FMD was recognized as the first viral infection of animal and an important transboundary disease (OIE, 2022). It is highly contagious disease of cloven-hooved animals, affecting cattle, pigs, goats, deer, sheep, and certain wild animals, including buffaloes (Alexandersen *et al.*, 2002; Cokcaliskan *et al.*, 2016). Despite this, the products and productivity of livestock are still very less due to the impacts of economically important diseases of animals, such as FMD (Sulayeman *et al.*, 2018). It is caused by a non-enveloped RNA virus within the family *Picornaviridae* and genus *Aphthovirus*. It has seven distinct serotypes, A, O, C, SAT-1, SAT-2, SAT-3, and Asia-1(OIE, 2021). It is first recorded in Ethiopia in 1957 and is endemic with almost five out of seven serotypes prevailing so far (O, A, C, SAT-1, and SAT-2) (Ayelet *et al.*, 2009; Negussie *et al.*, 2011; Jemberu *et al.*, 2016; Woldemariyam *et al.*, 2022).

The FMD virus can be discovered in the excretions and secretions of infected animals, including their saliva, milk, urine, semen, and expired air (Ringa and Bauch, 2014). Furthermore, the virus is airborne and can also be transmitted through indirect and direct contact over 60km overland and 300km by sea (Lubroth, 2002; Ringa and Bauch, 2014; Paton *et al.*, 2018). Animals infected with FMD exhibit signs of clinical illness after an incubation period of about 2 to 14 days (Mushayabasa and Tapedzesa, 2015). Generally, cattle and pig mainly get infection through inhalation and ingestion, respectively (Yadav *et al.*, 2019). Chronic carriers are described as animals with viral infections that have persisted for more than 28 days, with the virus typically persisting in the nasopharyngeal mucosa (Stenfeldt *et al.*, 2016; Stenfeldt and Arzt, 2020).

Clinical signs of the disease are high fever, Lameness, loss of appetite; vesicles and vesicular eruptions on the tongue, dental pad, gums, snoot, soft palate, nostrils, or muzzle that led to excess salivation and sudden death of young stock also be detected (Quinn *et al.*, 2005; Rweyemanu *et al.*, 2008; Fakhrul-Islam *et al.*, 2016; Windsor *et al.*, 2020). The diagnosis of FMD is made by combining history, clinical symptoms, and laboratory tests. FMDV can be

isolated on cell cultures, the viral nonstructural protein can be detected using ELISAs, and the presence of viral genomic material can be detected using PCR assays (OIE, 2012). Anti-NSP antibody testing is commonly utilized to distinguish infected animals from vaccinated animals in both FMD endemic areas (Brocchi *et al.*, 2006) and FMD-free countries (Barnett *et al.*, 2015).

Foot and mouth disease has no cure and requires control mechanisms to reduce its spread (Baipoledi *et al.*, 2004). Strategies such as immunization, restrictions on animals' movement, quarantine, culling, and education are used to reduce the spread of the infection (Kitching *et al.*, 2007; Ringa and Bauch, 2014). Economic losses due to decreased production of cattle in endemic regions and trade restrictions on disease-affected countries imposed by disease free countries are also a result of this (Knight-Jones *et al.*, 2016; Govindaraj *et al.*, 2021). “This case report describes the clinical signs and treatment outcomes of FMD case in an ox and pigs.”

Case descriptions

Case 1

A seven-year-old local breed ox from Bishoftu, Kebele 12, was presented to Professor Feseha Gebreab Memorial-VTH on December 1, 2022, with a history of refusing to feed and drink water, and drooling of saliva that started three days ago. The ox was managed extensively. Upon physical examination, the rectal body temperature was raised (40.8 °C), while other parameters were normal. The clinical findings were salivation, more than three ruptured circular lesions (erosions) on the upper gum, and the tongue (Figure 44). Based on the history and clinical findings, FMD was tentatively diagnosed.

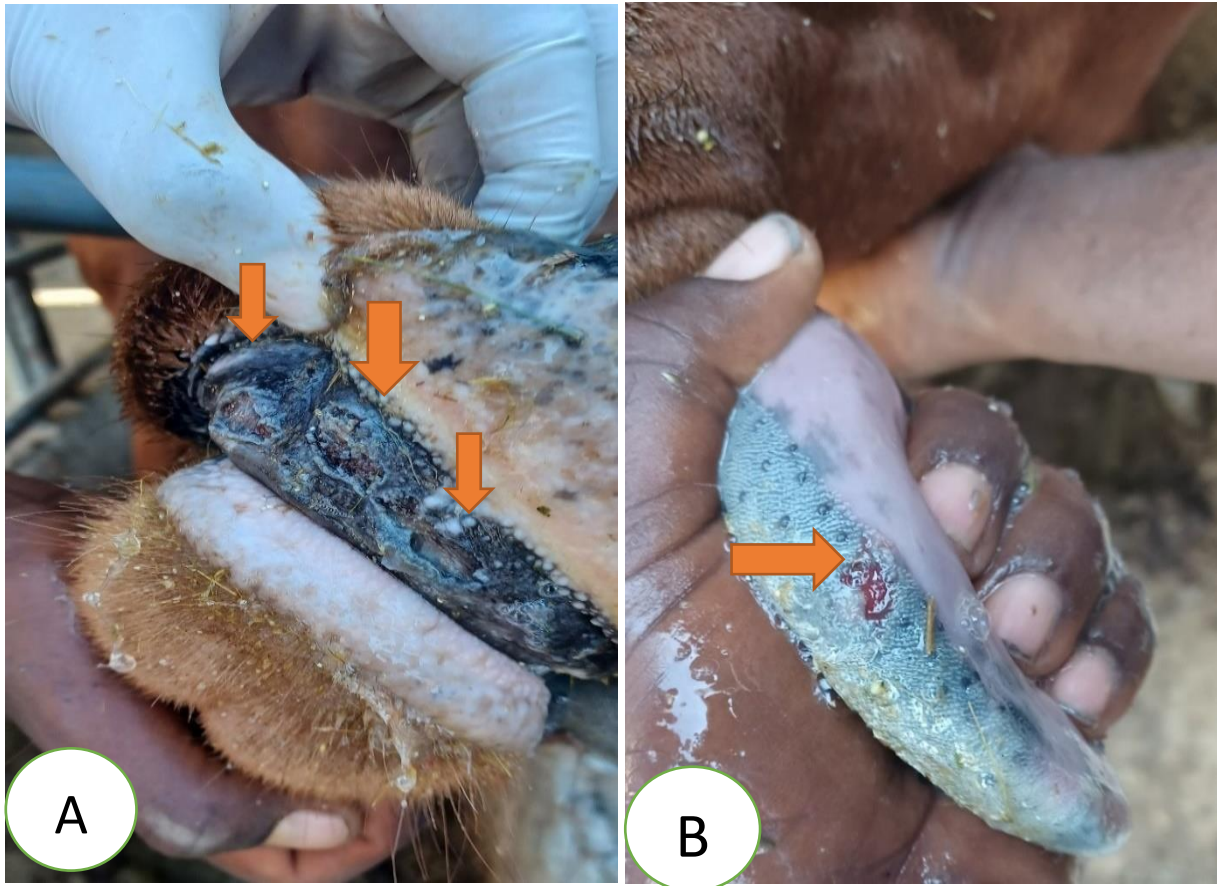


Figure 44: Ruptured circular lesions (erosions) on the gum (A) and tongue (B).

Case 2

Eight pigs were examined at Bishoftu on a privately owned farm on November 28, 2022, with a history of difficulty in movement and a lesion on the mouth and feet that started two days ago. Upon physical examination, the rectal body temperature was raised (40.5 °C), while other parameters were normal. Clinical findings were lameness, stamping of the feet, preferring to lie down, vesicular eruptions on the snout and feet, and an unruptured vesicle on the snout (Figure 45). Based on the history and clinical findings, FMD was tentatively confirmed.

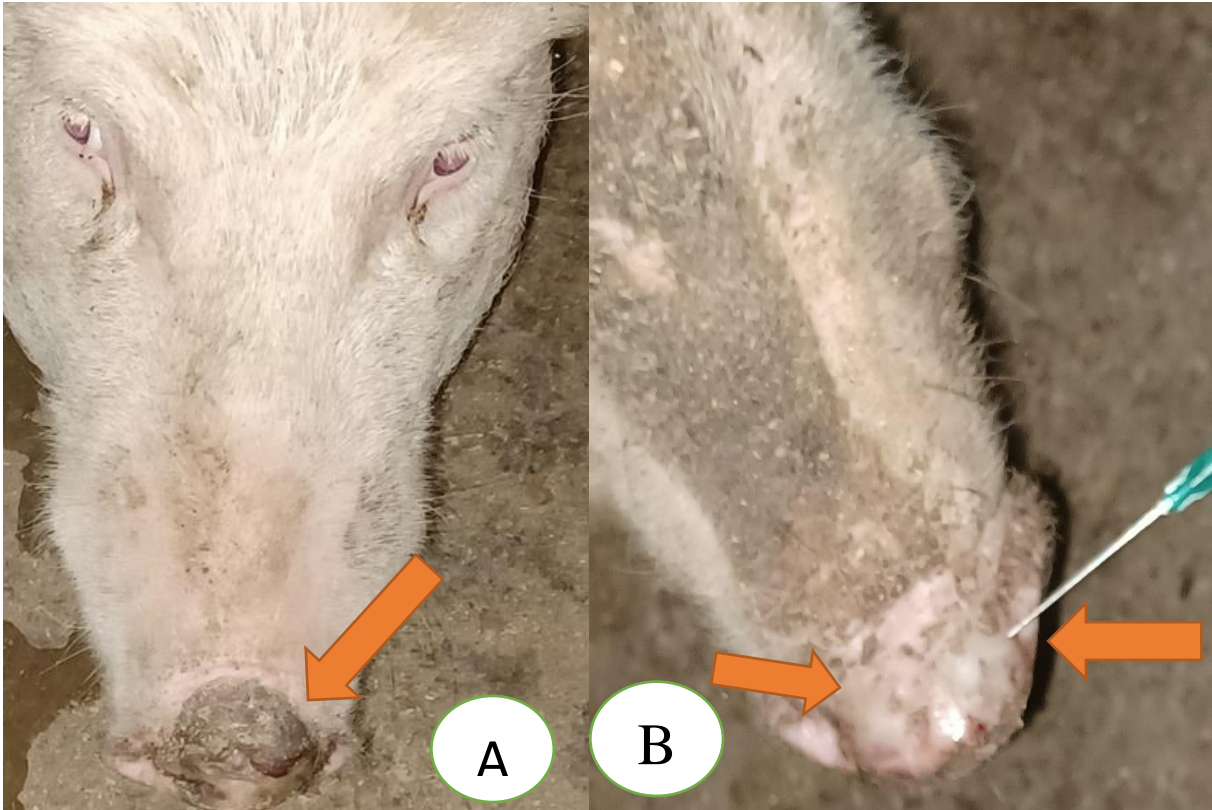


Figure 45: Indicating recently ruptured (A) and unruptured vesicle on the snout (B).

Case management and treatment outcome

The ox and pigs were treated with 10% oxytetracycline (Shanghai Thongren Pharmaceutical Co., Ltd., China) at a dose of 10 mg/kg for five days, IM, with application of tincture iodine on the eroded lesions of the ox gum daily till healing. Additionally, the 2% citric acid solution was used for disinfecting the pig farm where the case was observed. Besides the treatment management, the owners were also advised to segregate and nurse the infected animals and restrict the movement of the farm workers until the pigs fully recovered. The rectal body temperatures of the ox and pigs returned to the normal range post-treatment. One month later, the ox and pigs were fully recovered, and the vascular lesions disappeared (Figure 46).



Figure 46: Indicating disappeared FMD lesions of ox with scars and fully recovered pigs after three weeks of the treatment period.

Discussion

Foot and Mouth Disease is an acute, extremely contagious, and highly communicable viral disease of all cloven-hooved animals and is caused by the genus *Aphthovirus*. In the history of the present case report, it was indicated that the ox was managed extensively, which means the chance of exposure to the infection is very high due to a poor management system and consequently a longer exposure time. Support the earlier findings by Hasan *et al.* (2016), which suggests that older cattle may be more susceptible due to malnutrition, lowered immunity, poor management, and a longer exposure duration.

The clinical symptoms (high fever, foaming saliva, etc.) and vesicular eruptions (tongue, dental pad, gums) seen in the ox of this case report are consistent with the studies of Quinn *et al.* (2005), Rweyemanu *et al.* (2008), Fakhurul-Islam *et al.* (2016), and Windsor *et al.* (2020). The clinical symptoms of high fever, salivation, lameness, stamping of the feet, preference for lying down, and unruptured vesicles on the snout and vesicular eruptions (snout and interdigital cleft, etc.) seen in the pigs of this case report are consistent with the works of Ding *et al.* (2013) and Fukai *et al.* (2022).

As there is no specific treatment for this viral infection. Fakhurul-Islam *et al.* (2016) reported that Sulphonamide, amoxicillin, and sulfadimidine injections were found to be more effective in controlling secondary bacterial infections and healing FMD lesions. Sorwar *et al.* (2016) reported that cattle fed with zinc sulfate have more antiviral activity, and cattle fed with green treated with caustic soda found to have some activity against the FMD virus. 10% oxytetracycline (0.2 mg/kg/day) in combination with application of tincture iodine on the vesicular eruption lesions daily until healing was used to treat clinical FMD in this case report. The ox and the pigs successfully responded to this treatment and fully recovered. These findings are supported by the findings of Radostits *et al.* (2007) and Windsor *et al.* (2020). 10% oxytetracycline was used to prevent secondary bacterial infection, and tincture iodine was applied to produce soothing and coating effects on lesions and antiseptic action. Additionally, 2% of the citric acid solution was used for disinfecting the pig farm in order to minimize the spread of FMD around infected farms. Foot and mouth disease is one of the most economically

significant and endemic diseases in our country, Ethiopia. The disease is curable and preventable if we take proper steps in due time.

Limitations

Further laboratory confirmation was not performed for these cases because of lack of specific kits for the virus hence the animals were treated based on clinical sign and other indications

Acknowledgement

I am grateful to Dr. Taye, Dr. Mehari, the owners of the ox and the manager of the pig farm for their valuable information during the follow up.

3.5. Case Reports on Equine

3.1.5. Epizootic lymphangitis case in horse

Abstract

Epizootic lymphangitis is a chronic disease of horses that is caused by the fungus *Histoplasma capsulatum* var. *farciminosum*. This case report describes the clinical signs, laboratory findings, and treatment outcomes of an epizootic lymphangitis case in a horse. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. A seven years old local breed male horse from Bishoftu was presented to the CVMA, SPANA open-air clinic with a history of nodular lesions on the shoulder area that started one month ago. Rectal temperature, heart rate, and respiration rate were measured and found to be within the normal range. Observed clinical signs were multiple nodular lesions with pus discharge on the right pre-scapular region. Pus sample was collected and lemon-shaped yeast with a halo (unstained capsule-like) structure that appeared individually or in groups either free or intracellularly phagocytized within the macrophages were observed, which was indicative of *Histoplasma capsulatum* var. *farciminosum*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as Epizootic lymphangitis. The horse was treated with topical application of 15% zinc oxide (20 mg/kg) for two successive months with an interval of 15 days. The improvement was noted two months after treatment and fully recovered. Early identification of cases is critical for successful therapy. Treating early cases, avoiding contamination, restricting movement of positive cases, and euthanasia for advanced ones are recommended. Furthermore, research towards the development of vaccines and/or effective drugs should be encouraged for this disease.

Key word: *Giemsa's stain, Histoplasma capsulatum* var. *farciminosum, Horse, zinc oxide*

Introduction

Epizootic lymphangitis is also known as Equine Histoplasmosis, is one of the infectious diseases posing huge socioeconomic and welfare concerns on horses worldwide (Abdela *et al.*, 2021). It is a chronic, contagious disease of Equidae and can infect humans, characterized clinically by a spreading, suppurative, ulcerative pyogranulomatous dermatitis and lymphangitis (AL and Muhsen, 2016; Adedokun *et al.*, 2020). It was first described in (1906) by Darling among the workers of the Panama Canal (Owaid, 2018). It is caused by *Histoplasma capsulatum var. farciminosum*, a dimorphic fungus and filamentous (mycelial or mold states) which can live independently in soil, making eradication difficult (Amenia and Siyoum, 2002; Owaid, 2018). It is more common in tropical and subtropical regions than in temperate zones including Ethiopia (Alsaad *et al.*, 2016; Duguma *et al.*, 2021).

The source of the *H. capsulatum var. farciminosum* can be the skin lesions, nasal and ocular exudates of infected animals or the soil (Asfaw and Fentahun, 2020). The mode of transmission is not well established. Nevertheless, direct contact with infective materials through injured skin or through cutaneous abrasions is the most common mode of infection (AL and Muhsen, 2016). However, the role of inanimate objects (harness and other implements) and fly vectors via broken skin cannot be ruled out (Abdela *et al.*, 2021; Duguma *et al.*, 2021). Ticks are also thought to be important in the spread of this agent (Radostitis *et al.*, 2007). It is believed that flies of the *Musca* or *Stomoxys spp.* are responsible for spreading the conjunctival form of diseases (AL and Muhsen, 2016).

Epizootic lymphangitis has four forms: ocular, cutaneous, respiratory, and asymptomatic carriers (Scantlebury and Reed, 2009; Seid *et al.*, 2019). The cutaneous form is the most common, with multifocal pyogranulomatous subcutaneous nodules that develop throughout the lymphatic system and consolidate to produce cord-like lesions (Ameni, 2006). The respiratory form is classically characterized by pyogranulomatous lesions within the nasal mucosa and lung parenchyma, with potential for multisystemic pathology (Endebu and Roger, 2003; Scantlebury *et al.*, 2016). Mixed clinical manifestations are possible and may indicate various disease development phases. Chronic illness causes significant debilitation and progressive lameness,

which may be the result of multisystemic involvement (Meselu *et al.*, 2018). The clinical examination of the lesions, microscopic analysis of the yeast form of HCF in pus, serological tests, and skin hypersensitivity testing are used to make the diagnosis of EPL (Scantlebury *et al.*, 2015).

Many treatments have been tried largely without success, but parenteral iodides and amphotericin B have been reported as effective (Ameni, 2006; Jones, 2006; Hadush *et al.*, 2008). Treatment options such as intravenous sodium iodide, oral potassium iodide, and surgical excision of lesions are restricted due to the possibility of clinical symptoms returning months later (Seid *et al.*, 2019). There is no readily available commercial vaccine for prevention, but an attenuated vaccine and a killed formalized vaccine have been used for its control in some endemic areas of west Asia (Abdisa, 2017). To control the disease culling infected equids and applying strict biosecurity measures are recommended to prevent spread of the infectious agent (Scantlebury and Reed, 2009; Duguma *et al.*, 2021). “This case report describes clinical signs, laboratory findings and the treatment outcomes of Epizootic lymphangitis case in horse”.

Case description

A seven years old local breed horse from Bishoftu was presented to the SPANA open air clinic on November 7, 2022 with a history of nodular lesions on the shoulder area that started one month ago. Upon physical examination, the horse had a heart rate of 56 bpm, a respiratory rate of 22 bpm, and a rectal temperature of 37.2 °C. Observed clinical signs were multiple nodular lesions with pus discharge on the right pre-scapular region (Figure 47). No other nodules were seen in other parts of the horse ‘s body. Based on the history and clinical findings, lists of differential diagnoses were made, including epizootic lymphangitis and ulcerative lymphangitis, tentatively.



Figure 47: Indicating nodular lesion with pus discharge on the right pre-scapular region.

Laboratory investigations and findings

The pus sample was aspirated aseptically by sterile needles and syringes from an unruptured nodular swelling and immediately submitted to the veterinary clinical pathology laboratory of Addis Ababa University College of Veterinary Medicine, Bishoftu, for smear preparation to identify the causative agent. More than four thin smears were prepared and stained by Giemsa's stain, which revealed leucocytes, neutrophils, and gram-positive lemon-shaped yeast with a halo (unstained capsule-like) structure that appeared individually or in groups either free or intracellularly phagocytized within the macrophages under a 100x binocular microscope (Figure 48). It was confirmed that the yeast was *Histoplasma capsulatum*. Finally, it was concluded that based on the history, clinical examination, and laboratory findings, the final diagnosis was Epizootic lymphangitis

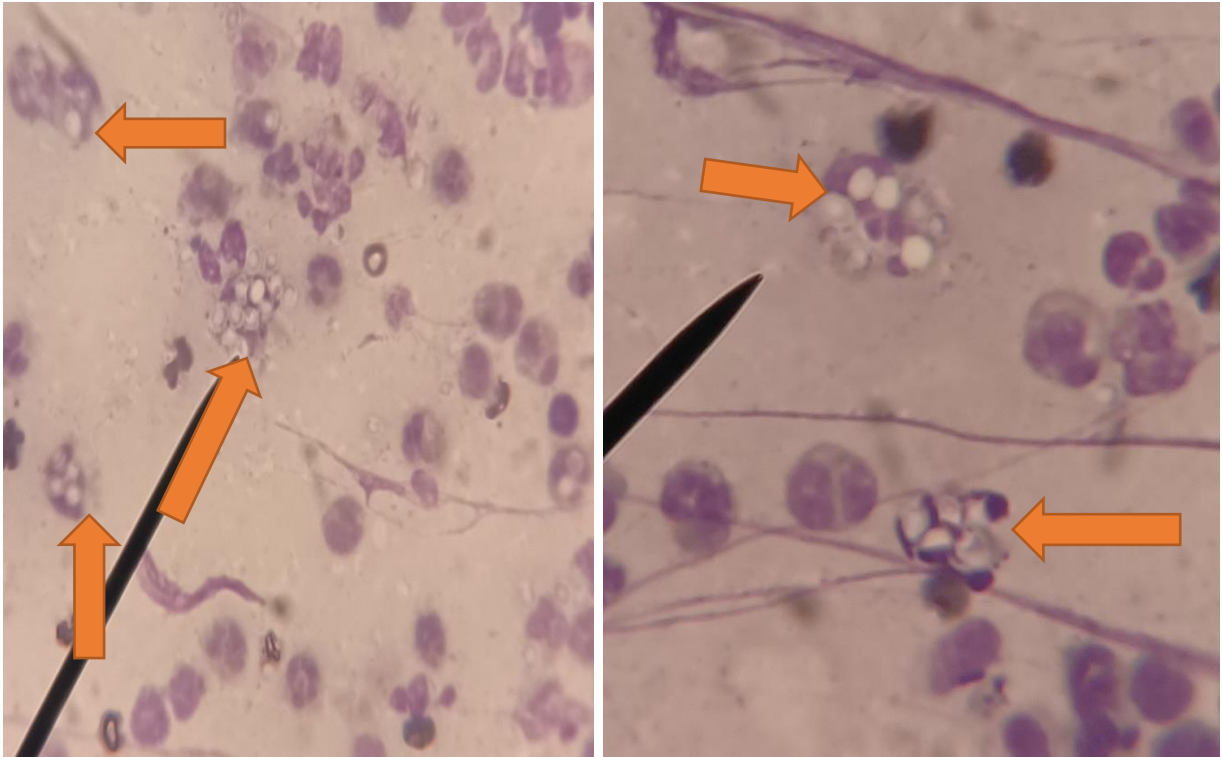


Figure 48: A lemon shaped and unstained capsule-like yeast of *Histoplasma capsulatum*.

Case management and treatment outcome

The horse was treated by shaving the area of infection; all nodules were incised, the pus drained, and the nodular lesions were packed topically with 15% zinc oxide (Ethiopian Pharmaceuticals Manufacturing) at a dose of 20 mg/kg for two successive months with an interval of 15 days. Besides the drug treatment, the owner was also advised to segregate and nurse the horse at home until he fully recovered. After two months of post-treatment, no new nodules emerged. All the nodular lesions had disappeared, and there was hair growth on the infection area (Figure 49).



Figure 49: Indicating when application of zinc oxide on nodular lesions (A) and recovered horse after two months of the post treatment (B).

Discussion

Epizootic lymphangitis is a chronic disease of horses that is caused by the fungus *Histoplasma capsulatum* var. *farcinosum*. The horse in this case study was diagnosed based on their history, clinical observation, and microscopic examination of the smear. Clinical signs exhibited by the horse in the present case study were multiple nodular lesions with pus discharge on the right pre-scapular region. These clinical findings are similar to those reported earlier by Ameni and Siyoum (2002), Ameni (2006), and Scantlebury *et al.* (2016). EL was observed to affect any part of the body.

In the present case study, direct microscopic examination of Giemsa-stained smears of pus revealed leucocytes, neutrophils, and gram-positive lemon-shaped yeast with a halo (unstained capsule-like) structure that appeared individually or in groups either free or intracellularly phagocytized within the macrophages. Similar Giemsa-stained microscopic examination results

were previously reported in horses by Scantlebury *et al.* (2016), Meselu *et al.* (2018), and Abdela *et al.* (2021).

Early identification and intensive follow-up are critical for successful therapy (Getachew, 2004; Getachew *et al.*, 2007; Mekonnen *et al.*, 2012; Scantlebury *et al.*, 2015). The horse in this case report was successfully treated with topical application of 15% zinc oxide (20 mg/kg) for two successive months with an interval of 15 days. However, the disease has not had an effective treatment so far (Ameni, 2006). The trial of zinc oxide and other treatments by SPANA is showing better results in the treatment of early cases of the Epizootic lymphangitis. Focus should be placed on disease control methods like euthanasia for advanced cases, as therapy is expensive and ineffective (Seid *et al.*, 2019; Duguma *et al.*, 2021). Early identification of cases is critical for successful therapy and furthermore, research towards the development of vaccines and/or effective drugs should be encouraged.

Acknowledgement

I am grateful to Dr. Tilaye, all staff of SPANA and the owner of the horse for his valuable information during the follow up.

3.5.2. Obstructive colic by *Parascaris equorum* in Horse

Abstract

Obstructive colic is considered one of the major disease states in horses, and it is an important cause of death in these species of animals. *Parascaris equorum* is important cosmopolitan nematode parasite causing colic in horses. This case report describes a clinical sign, laboratory findings and consequences of un-dewormed adult horse of obstructive colic case. History, clinical examination, and laboratory diagnosis are routine diagnostic procedures for this disease. The local breed horse of eight years old was presented to the CVMA, SPANA open air clinic with the history of rolling on the ground, being reluctant to move, and a cessation of urination and defecation that started last night before admission. The horse had not received any prophylactic anthelmintic treatment for the last three years. The parameters are slightly increased and Clinical signs during diagnosis include: getting up and lying down, rolling on the ground, flank watching, sweating, pawing the ground, and grunting sound. The bladder was not distended; urinates and defecates immediately upon rectal palpation. A fecal sample was taken, examined with the McMaster flotation method, and it was positive for *Parascaris equorum* eggs. Twelve eggs were counted from both chambers and multiplied by 50 (12 x50), and 600 eggs per gram of feces were found. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as obstructive colic. The horse was treated with Flunixin meglumine (1.1 mg/kg) I/V for stat to reduce pain and Ivermectin (0.2 mg/kg) PO, but the animal collapsed and died after twelve hours of symptomatic treatment. Effective deworming sessions twice or three times a year are recommended as prophylactic measures.

Key word: *Colic, Horse, McMaster, Parascaris equorum.*

Introduction

Gastrointestinal parasites are a major health problem for horses and are responsible for significant economic losses in livestock farming systems throughout the world (Mathewos *et al.*, 2021). Horses are most frequently infected by nematodes, gastrointestinal *strongyles*, *Parascaris equorum*, *P. univalens* and *Oxyuris equi*, and less frequent by cestodes, *Anoplocephala magna* and *Anoplocephala perfoliata*, and protozoa *Eimeria leuckarti*, are considered one of the parasitic diseases that have a significant impact on equine health, performance and production (Lyons and Tolliver, 2004; Laugier *et al.*, 2012; Bundina and Engashev, 2015; Dashinimaev, 2018).

P. equorum has a direct life cycle, where the development to the infective L3 larva occurs within an egg and can be completed in 9-14 days under optimum conditions (Nielsen, 2016). Horses get the infection via contaminated feed with infected eggs disseminated in the environment (Fritzen *et al.*, 2010). After ingestion, embryonated eggs hatch in the horse's small intestine, penetrate the intestinal mucosa, migrate to the liver and lungs, then migrate to the respiratory tree to the pharynx, and finally develop to mature adult worms within the duodenum and jejunum (Vendrame, 2018). The prepatent period is between 75-115 days (Nielsen, 2016). *Parascaris spp* infection can cause nasal discharge and coughing during larval migration, and adult parasites in the small intestine can cause inflammation and occasionally may cause colic, obstruction, intussusception or, rarely, perforation of the gut (Reinemeyer, 2009). Less serious effects include weight loss and ill thrift due to decreased food absorption (Cribb *et al.*, 2006; Nielsen *et al.*, 2014). Necrotising enteritis, septic peritonitis and intestinal rupture can also be seen on necropsy of parasitized horses (Upjohn *et al.*, 2010).

Colic is the most significant condition that veterinarians confront and a common problem in horse practice (Radostise *et al.*, 2007). There are two main divisions: gastrointestinal and non-gastrointestinal, with gastrointestinal colic being the most prevalent of the two. It can be brought on by a variety of situations, from harmless spasmodic colic to fatal strangulating blockage, and the obstruction colic is frequently brought on by feed, sand, or intestinal parasite impaction. The ileum, cecum, and pelvic flexure of the large colon are the most often affected areas (Singh *et*

al., 2019). Diagnosis of GIT infections of horses is based on clinical signs, fecal examination (McMaster egg counting flotation and sedimentation methods) and postmortem findings (Reinemeyer and Nielsen, 2014; Hautala *et al.*, 2019; Boelow *et al.*, 2022).

The anthelmintic used for *Parascaris spp* infection in horses are benzimidazoles (e.g., fenbendazole and oxfendazole); macrocyclic lactones (MLs; e.g., abamectin, ivermectin and moxidectin); and tetrahydropyrimidines (e.g., morantel and pyrantel) (Bellaw *et al.*, 2018; Raza *et al.*, 2019). The finest control of equine endoparasitism relies on use of integrated helminth control system including reduction of environmental contamination, grazing management and using prophylaxis (Kaplan and Nielsen, 2010; Mathews, 2014). “This case report describes the clinical signs, laboratory findings and consequences of un-dewormed adult horse of obstructive colic case”.

Case history

An eight-year-old local breed horse was presented to AAU-CVMA-SPANNA on December 14, 2022, with a history of laying down to the back, rolling on the ground, being reluctant to move, and a cessation of urination and defecation that started last night before admission. The horse was managed extensively and had been dewormed before three years. Upon physical examination, the horse had a heart rate of 120 bpm, a respiratory rate of 40 breaths per minute, and a rectal temperature of 39.9 °C. On clinical observation, getting up and lying down, rolling on the ground, flank watching, sweating, pawing the ground, and grunting sound were observed. The bladder was not distended; it urinates and defecates immediately during rectal palpation (Figure 50). Based on the history and clinical findings, obstructive colic was tentatively diagnosed.



Figure 50: Indicating horse with symptom of colic (smelling of the abdominal region and pawing of ground).

Laboratory investigation and findings

A fecal sample was collected directly from the rectum and immediately processed at the veterinary parasitology laboratory of SPANA. Both the sedimentation and floatation methods were applied, but the sedimentation method result was negative, but from the floated sample, 0.5 ml was transferred to the McMaster slide, and it was positive for *Parascaris equorum* eggs. Twelve eggs were counted from both chambers, and the average was multiplied by 50 (12x50), and 600 eggs per gram of feces were observed, which was highly infested by the parasite (Figure 51). Therefore, on the basis of clinical symptoms and laboratory feces, the disease condition was confirmed as obstructive colic due to *Parascaris equorum*.

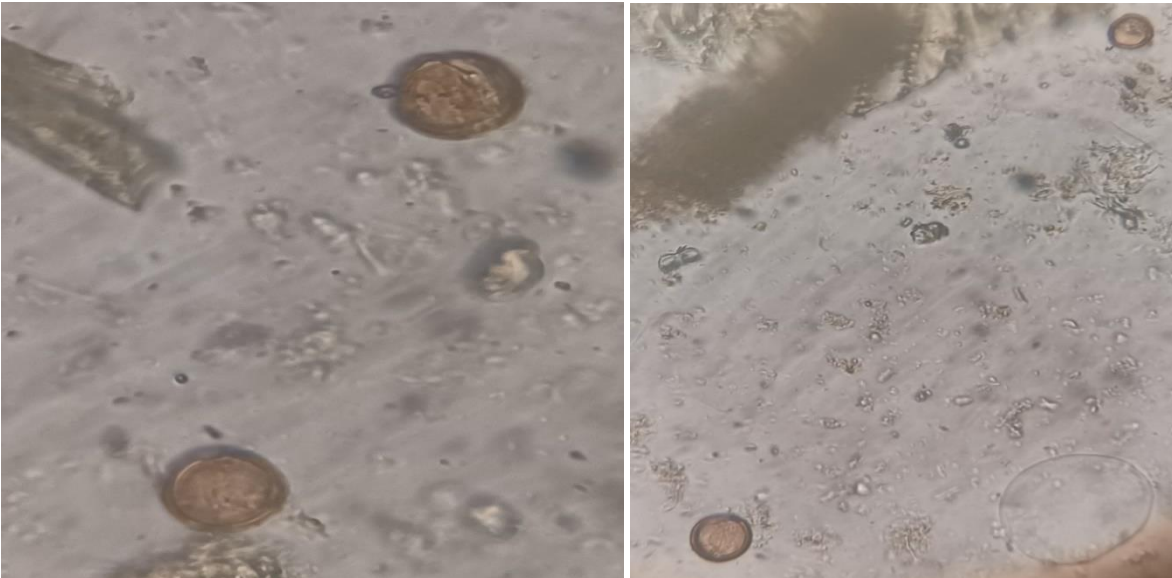


Figure 51: Indicating *Parascaris equorum* egg under 40X.

Case management and treatment outcome

The horse was treated with Flunixin meglumine (1.1 mg/kg) I/V for stat to reduce pain and Ivermectin (Sheyang Sunvictotor Pharmaceutical Co., Ltd., China) at a dose of 0.2 mg/kg PO. The horse was getting relief from severe sweating and severe abdominal pain after the IV injection of a strong analgesic (Flunixin) but after 12 hours post-treatment, the horse collapsed in the middle of the night and did not respond to the treatment (owner reported).

Discussion

Small intestinal obstructions have been reported to occur spontaneously in young horses and occasionally in adult horses with large burdens of *P. equorum*. The presence of adult ascarids in the gastrointestinal tract alters intestinal motility and may contribute to the occurrence of these obstructions (Cribb *et al.*, 2006). The history of this case study indicated that the horse had not been dewormed for the last three consecutive years, and the horse's feeding practice was free grazing, so the horse had a high chance of ingesting large amounts of gastrointestinal parasite eggs and larvae, which resulted in parasitic infection in the susceptible horse.

In the present case report, the observed clinical signs are flank watching, stretching, pawing at the ground, rolling on the ground, sweating, and getting up and lying down. Similar observations were made by Rabuffo *et al.* (2009), Scantlebury *et al.* (2011), and Tatz *et al.* (2012). The fecal sample examined with the McMaster flotation method was positive for *Parascaris equorum* eggs. Twelve eggs were counted from both chambers, then the average was multiplied by 50 (12x50), and 600 eggs per gram of feces were counted, which was highly infested by this parasite. This examination technique was previously supported by Dissanayake *et al.* (2017), and Hautala *et al.* (2019). The diagnosis of this case report indicated that the horse was highly infested by the *Parascaris equorum* parasite, and on the basis of clinical symptoms and fecal examination, the condition was confirmed as the parasite-induced obstructive colic.

In the current case report, flunixin meglumine (1.1 mg/kg, SIV) and ivermectin (0.2 mg/kg, PO) were used to control the colic and deworm the horse, but the horse did not respond to the treatment and collapsed 12 hours later. This is similar to the previous treatment results of Boersema *et al.* (2002), Robertson and Sanchez (2010), and Sakhaee *et al.* (2011). Severe cases did not respond to Flunixin and required surgical intervention (Ryu *et al.*, 2004; Singh *et al.*, 2019), which was confirmed by the post-mortem findings of some cases that died following severe colic attacks. In this case, the owner's economic constraints prevented him from performing a laparotomy and limited his therapeutic options. Death was thought to result from colic from a heavy worm burden in the gastrointestinal tract, and to do a postmortem, the owner passed the cadaver outside of his premises and had it ingested by the hyaena.

In conclusion, continuous monitoring and routine deworming programs in horses lower the incidence of parasitic colic. So, effective deworming sessions twice or three times a year should be recommended as prophylactic measures.

Acknowledgement

I am grateful to Mr. Tibebu and all SPANA staff for their contribution, and again the owner of the farm for his valuable information during the follow up.

3.6. Case Reports on Canine

3.6.1. *Canine parvovirus infection in puppy*

Abstract

Canine parvoviral infection is caused by *canine parvovirus* (CPV 1 and CPV 2) belonging to the family *Parvoviridae*, which is one of the most dreadful diseases that commonly infects puppies at 1-6 months of age. The present case study reports the case of canine parvovirus infection in a puppy and its treatment outcome. A 4 months old puppy from Bishoftu was presented at Professor Feseha Gebreab Memorial-VTH with a history of bloody diarrhea, loss of appetite, and vomiting that started two days ago. On physical and clinical examination, the puppy was suffering from foul-smelling, bloody diarrhea, vomiting, dehydration, and a subnormal temperature. Based on the history and clinical signs, it was tentatively diagnosed as a canine parvovirus infection. The puppy was immediately treated with fluid therapy of Ringers lactate with 40% dextrose (10 ml/kg) for 2 days, I/V. Antibiotic: Sulfamethoxazole-Trimethoprim (1 ml/16 kg/day) for 5 days, I/M. Anti-inflammatory: Dexamethasone (0.2 mg/kg/day) for 2 days, I/V. Antiemetic: Metoclopramide (0.2 mg/kg) for 2 days, I/V. Appetizer: Vitamin B-complex (3 ml) for 2 days, I/V. Treatment was carried out for 5 days, and the puppy was fully recovered. To prevent the reoccurrence of the disease, vaccination and good hygienic practices in kennels, including proper disinfection of all exposed surfaces and personnel, are highly recommended.

Keywords: *Diagnosis, Parvovirus infection, Puppy, Treatment.*

Introduction

Canine parvoviral infection is a highly contagious and fatal disease of dogs and other carnivores caused by canine parvovirus (CPV) (Chen *et al.*, 2019). CPV is a small, non-enveloped virus with single-stranded DNA, and has sustained pandemic circulation in dogs for more than 40 years (Tagorti, 2018; Voorhees *et al.*, 2019). It has two types, CPV-1 and CPV-2, and three subtypes, CPV-2a, CPV-2b and CPV-2c, are distributed worldwide (Decaro *et al.*, 2007; Cotmore *et al.*, 2014). Canine parvovirus (CPV) causes haemorrhagic gastroenteritis, dehydration, immune suppression and death in dogs under 6 months of age. Adults can also be affected (Tattersall *et al.*, 2005; Allison *et al.*, 2014; Altman *et al.*, 2017; Mylonakis *et al.*, 2016). Doberman, Rottweiler and German shepherd dogs are more susceptible to Parvovirus infection than other breeds (Nandi and Kumar, 2010). The disease has high morbidity and mortality in the canine population and the predisposing factors for parvoviral infection in puppies are lack of protective immunity, co-infection with other pathogens, overcrowded, breed, unsanitary and stressful environmental conditions (Khare *et al.*, 2020; Kelman *et al.*, 2020).

Canine parvoviral infection can be transmitted to dogs through direct or indirect transmissions, such as contact with contaminated faeces and contaminated fomites, as well as through insect and rodent vectors (Hoelzer *et al.*, 2008). It needs the host for replication and binds itself to the host cell with double-stranded ends of the genome (Hasan *et al.*, 2016). After 3-7 days, the disease can be characterized by an enteric form that includes fever, anorexia, vomiting, mucoid to hemorrhagic diarrhea, and dehydration and hypovolemic shock (Markovich *et al.*, 2012). Dehydration and hypovolemic shock often develop quickly due to a large number of protein(s) and fluid loss from the gastrointestinal tract (Goddard and Leisewitz, 2010).

Clinically, the disease manifests either as cardiac or enteric form. The enteric form is an acute onset of lethargy, anorexia, fever, vomiting and diarrhoea, which may contain mucus or blood and foul smell (Khare *et al.*, 2020; Suvethika and Kumar, 2021). The cardiac form is only seen in neonates infected in utero with respiratory or cardiovascular failure manifestations and is uncommon due to effective immunizations protecting puppies during their early period of life (Schatzberg *et al.*, 2003; Shima *et al.*, 2015). The diagnosis of CPV infection can be made based

on clinical signs (Nandi and Kumar, 2010), and detection of CPV in the faeces of affected dogs, serology, necropsy and histopathology (Sykes and Greene, 2013; Desario *et al.*, 2005). Other methods of detection include electron microscopy, viral isolation, fecal hemagglutination, latex agglutination, and PCR (Decaro and Buonavoglia, 2012; Terzungwe *et al.*, 2018; Hasib *et al.*, 2021).

The infection can be managed by application of proper antibiotics, restoration of the electrolyte and fluid balance, and vaccination (Dongre *et al.*, 2015; Poude *et al.*, 2020). Modified live vaccines are used for immunization at the age of 45 days with booster dose annually and there after 3 years. Inactivated vaccines are preferred in pregnant and colostrum deprived puppies (Odueko, 2019). “The aim of the present case report is to frame a diagnostic and treatment protocol based on the signs and symptoms of parvovirus infection in puppy below 6 months of age”.

Case description

The local breed puppy around 4 months of age from Bishoftu, Kebele 12, was presented at Professor Feseha Gebreab Memorial-VTH of Addis Ababa University on February 4, 2023, with a history of bloody diarrhea, loss of appetite, and vomiting that started two days ago. The puppy had no previous vaccination or treatment history. The physical examination revealed that the rectal body temperature of the puppy was 40 °C, while the respiratory rate and heart rate were 28 breaths per minute and 104 beats per minute, respectively. On clinical examination, the puppy was suffering from foul-smelling, bloody diarrhea, vomiting, and dehydration (Figure 52). It was tentatively spotted as canine parvovirus disease (CPV) based on the age, history, and clinical findings.



Figure 52: Indicating a puppy with bloody diarrhea.

Laboratory investigation

The fecal examination of the dog was done to check whether there was a parasitic infestation or not. No parasitic involvement was observed in the fecal examination. For further confirmation of the diagnosis, there was no facility to detect the virus. According to the history of no vaccination and clinical findings (bloody diarrhea, vomiting, and dehydration), it appeared to be a case of canine parvovirus (CPV).

Case management and treatment outcome

As there is no treatment for any viral infection, the treatment for canine parvo infection is mostly supportive and symptomatic during the course of the disease. So, symptomatic and supportive therapy was used as a line of treatment. Fluid therapy: Ringer Lactate (Addis Pharmaceutica Factory PLC) with 40% Dextrose a 10 ml /kg I/V for 2 days to correct hydration status. Antibiotic: Sulphamethoxazole-Trimethoprim (Chengdu Qiankun Veterinary Pharmaceutical Co., Ltd., China) at a dose of 1 ml/16 kg/day for the subsequent five days, IM. Anti-inflammatory: Dexamethasone (Sokar Healthcare Pvt. Ltd., Gujarat, India) at 0.2mg/kg/day for

2-day, I/M. Antiemetic: Metoclopramide (Leben Laboratories Pvt. Ltd) at a dose of 0.2 mg/kg for 2 days, I/V and Vitamin B-complex (Shandong Shenglu Pharmaceutical Co., Ltd) 3 ml for 2 days, I/V. The puppy owner was instructed strictly to monitor the puppy's feed and water and to ensure proper disinfection of all exposed surfaces and personnel. On the second day of therapy, vomiting was stopped while the diarrhea was still there. On the fifth day of therapy, the bloody diarrhea was changed, and the puppy recovered (Figure 53).



Figure 53: Indicating fully recovered puppy with normal faeces after one month of post treatment.

Discussion

Canine parvovirus causes a highly contagious and fatal disease, developing into acute hemorrhagic enteritis and myocarditis in dogs (Miranda and Thompson, 2016). Based on the history and clinical findings, the current case of puppy was tentatively diagnosed as canine parvovirus disease. In addition, the age of the dog and the abundance of similar cases were also considered. The puppy in the present study showed similar clinical manifestations indicated in various literature, including anorexia, depression, vomiting, and profuse hemorrhagic diarrhea (Tagorti, 2018; Khare *et al.*, 2020; Gerlach *et al.*, 2020; Suvethika and Kumar, 2021).

Based on the nature and severity of the condition, the pup was treated with antibiotics (Sulfamethoxazole-Trimethoprim at 1 ml/16 kg/day), anti-inflammatory drugs (dexamethasone at 0.2 mg/kg/day), antiemetic drugs (Metoclopramide at 0.2 mg/kg), an appetizer (Vitamin B-complex at 3 ml/day), and fluid therapy (Ringer's lactate with 40% Dextrose at 10 ml/kg). The medications showed significant improvements, and the treated pup made an uneventful recovery on the fifth day of treatment and returned to normal life in 6–8 days. The treatment outcomes of the present case report are similar to the report of Munibullah *et al.* (2017), Rishikesavan *et al.* (2021), and Suvethika and Kumar (2021). The highest rate of canine parvovirus incidence in young pups may be due to viral magnetism for quick multiplying intestinal crypt cells with the highest mitotic catalog due to alternation in bacterial flora (Deka *et al.*, 2013). This infection can be controlled by providing good nutrition and a clean environment, reducing overcrowding, and vaccinating the dogs as per schedule (Odueko, 2019).

In conclusion, the type of disease, duration of time, and frequency of therapy determine the survival rate of canines with parvovirus infection. The main method of preventing the disease's recurrence is vaccination, and appropriate hygiene procedures in kennels, such as thorough staff and surface cleanliness, are strongly advised.

Limitations

Further laboratory confirmation was not performed for this case because of lack of specific kits for the virus hence the pup was treated based on clinical sign and other indications.

Acknowledgement

I am grateful to Mr. Daraje Gudeta. Mr. Hayimanot for their contribution and again the owner of the animal for his valuable information during the follow up

3.6.2. *Canine transmissible venereal tumor in bitch*

Abstract

The canine transmissible venereal tumor has been common in veterinary clinics and hospitals, mainly in populations of stray dogs in the active sexual phase. This case report describes the clinical signs, cytological findings, and treatment outcomes of a CTVT case in a dog. A 5-year-old local breed bitch from Bishoftu was presented to Professor Feseha Gebreab Memorial-VTH with a history of abnormal growth in the genital organ, bloody vulvar discharge, and licking of the genital area that started 6 months ago. Rectal temperature, heart rate, and respiration rate were measured and found to be within the normal range. Clinical observations were a cauliflower-like mass protruding from the vulva, licking of the vulvar area, an unpleasant odor, and bloody vulvar discharge. Impression smears from the mass was stained with modified Giemsa stain, and cytological examination revealed a predominance of round cells with abundant pale cytoplasm, distinct, punctate cytoplasmic vacuoles, an increased nuclear to cytoplasmic ratio, and some inflammatory cells (75% lymphocytes and 25% macrophages) were observed, which was indicative of TVT cells. Based on the history, clinical signs, and cytological findings, the case was diagnosed as CTVT. The bitch was treated by surgical removal of the mass, along with Ivermectin (0.2 mg/kg) subcutaneously, and application of tincture iodine on the wound daily until healed. The bitch was followed for 5 months; tumor recurrence was not observed, and the wound was well healed. Stray dog management and neutering of these dogs are recommended to decrease sexual activity among dogs in the locality, thus further preventing the spread of the disease.

Keywords: *Bitch, Cytology, Ivermectin, Transmissible venereal tumor.*

Introduction

A canine transmissible venereal tumor (TVT), also known as canine transmissible sarcoma, has been reported in many regions of the world (Goldschmidt M., 2002; Kabuusu *et al.*, 2010). Due to the uncontrolled stray dog population and the inadequate use of effective treatments, it is thought to be endemic in many tropical and subtropical countries with free-roaming or stray dog breeding populations, allowing for continuing local proliferation. Sexually mature dogs (more than two years old) are most commonly affected (Das and Das, 2000; Abeka, 2019). Novinsky first reported it in 1876, demonstrating that by inoculating a vulnerable host with tumor cells, the tumor might be transplanted from one host to another (Mello Martins *et al.*, 2005). The increased number of stray dogs, age, and immunological state may be predisposing factors for the genital and extragenital occurrence of TVT, which may be contributory reasons for the unusual metastasis to distant sites (Gurel *et al.*, 2003; Park *et al.*, 2006; Chikweto *et al.*, 2013).

CTVT is a naturally occurring contagious venereal tumor of dogs, and the disease is primarily observed on the mucosal surface of the external genitalia of male and female dogs. The most frequent method of transmission is venereal, but it can also spread to other sites and be transmitted to other dogs by licking, biting, or direct contact with the tumor (Kabuusu *et al.*, 2010; Ostrander *et al.*, 2016; Hiblu *et al.*, 2019). Rarely, they may also be discovered in the peritoneum, the lips, the oral mucosa, the liver, the spleen, the kidney, the lung, and the muscles. Metastasis of this tumor is uncommon; susceptible groups for tumor metastasis include puppies and immunocompromised dogs (Pereira *et al.*, 2000). (Abedin, 2020) reported that spontaneous regression of CTVT may occur, and recovered dogs can acquire humoral and cellular immunity, which prevents subsequent tumors.

Clinical presentation of a TVT: In females, the tumor frequently protrudes through the vaginal lips and can be seen in the vestibule of the vagina. They are usually shown as being friable, red to flesh-colored, and cauliflower-like (Abedin, 2020).

Canine TVT is diagnosed based on clinical examination (anatomical location) and environmental history and confirmed by laboratory tests supported by cytology, histopathology,

immunohistochemical staining, and the polymerase chain reaction technique (Park *et al.*, 2006). Round to oval in form, mitotic patterns, chromatin clumping, and one or two conspicuous nucleoli can all be observed in the cytological picture of TVT cells (Gonzalez *et al.*, 2000). The most notable cytological observation is the presence of several visible cytoplasmic vacuoles (Tella *et al.*, 2004; Abedin, 2020). There is a lower nuclear to cytoplasmic ratio with distinct vacuoles that can be helpful in distinguishing this tumor from mast cell tumor, lymphoma, plasmacytoma, histiocytoma, and other tumors because the vacuolated cytoplasm is found only in the cytology of canine TVT (Mukaratirwa and Gruys, 2003; Park *et al.*, 2006; Simoni and Knoll, 2008; Santiago-Flores *et al.*, 2012).

Several treatment regimens both invasive and non-invasive including surgery, immunotherapy, radiotherapy, biotherapy and chemotherapy have been applied for correction of TVT. Chemotherapy has been proved to be the most effective and practical therapy, with vincristine sulfate being the most frequently used drug (Abedin, 2020). Measures that are helping to control the transmission are: Managing the number of free-roaming dogs, maintaining strict spay and the effective treatment of clinical cases (Abeka, 2019). “This case report describes the clinical sign, cytological finding and treatment outcomes of CTVT case in bitch”.

Case description

A 5-year-old local breed bitch from Bishoftu was presented to Professor Feseha Gebreab Memorial-VTH of Addis Ababa University on November 7, 2022, with a history of abnormal growth in the genital organ, bloody vulvar discharge, and licking of the genital area, starting 6 months ago. Upon physical examination, the bitch's heart rate, respiratory rate, and rectal temperature were in the normal range. Clinical observations were a cauliflower-like mass protruding from the vulva, licking of the vulvar area, an unpleasant odor, and bloody vulvar discharge (Figure 54). It was tentatively spotted as a canine transmissible venereal tumor based on the history and clinical findings.



Figure 54: Indicating a fragile vestibular mass (A cauliflower like mass protruding from the vulva).

Laboratory investigations and findings

A surgically removed mass sample was submitted and processed at the veterinary clinical pathology laboratory of Addis Ababa University College of Veterinary Medicine, Bishoftu. More than eight smears were prepared by the impression method and stained with modified Giemsa stain. Cytological examination revealed a predominance of round cells with abundant pale cytoplasm, distinct, punctate cytoplasmic vacuoles, an increased nuclear-to-cytoplasmic ratio, and some inflammatory cells (75% lymphocytes and 25% macrophages) under oil immersion magnification (Figure 55). Finally, it was concluded based on the history, clinical signs, and cytological findings that the definitive diagnosis was CTVT.

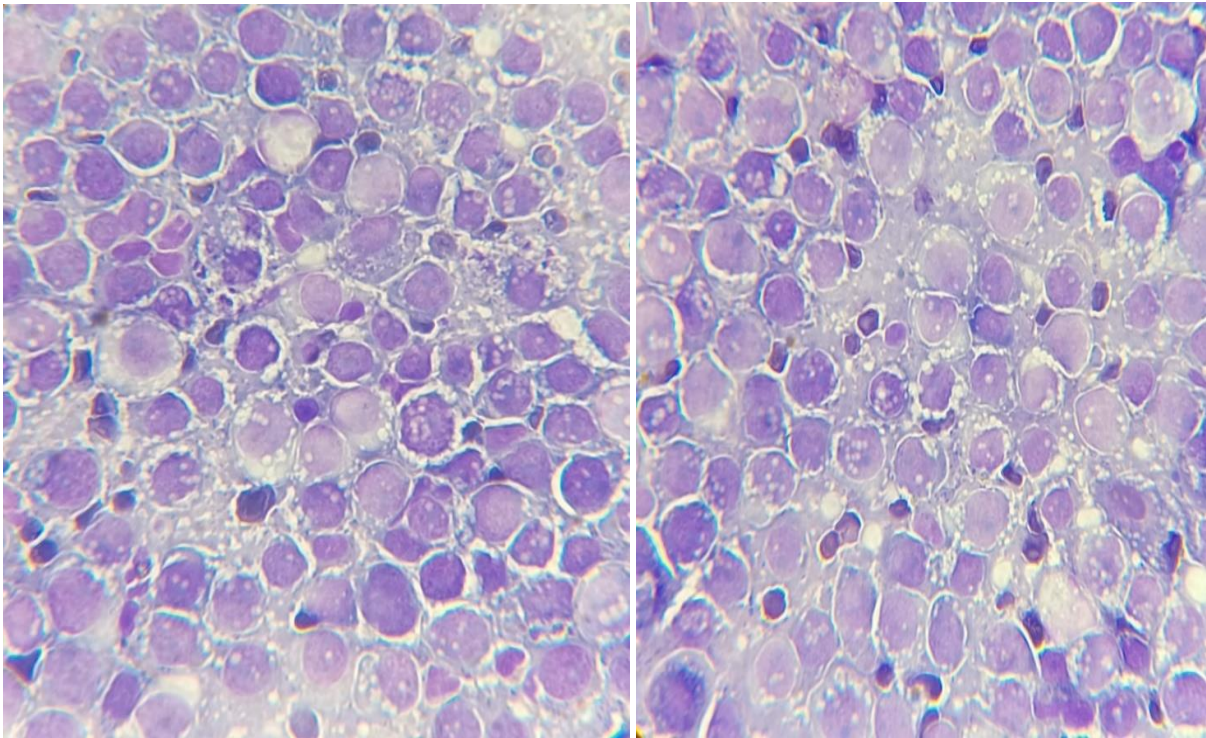


Figure 55: TVT cells, lymphocytoid standard (round cells, little cytoplasm and high nucleus: cytoplasm ratio) and multiple vacuoles in the cytoplasm.

Case management and treatment outcome

Because we do not have effective chemotherapy for this case in our hospital, we discussed it with our surgical team colleagues on the first day of admission and argued for the surgical excision of the tumor by the surgical team, which was followed by the clinical medicine team. After complete surgical removal of the tumor, we follow the case by administering a double shot (within a two-week interval) of ivermectin (Sheyang Sunvictor Pharmaceutical Co., Ltd., China) at a dose of 0.2 mg/kg subcutaneously and applying a tincture of iodine to the wound daily till healing. Besides the treatment, the owner advised managing the bitch intensively and maintaining the hygiene of the bitch and bedding environment. The bitch was followed for 5 months, and post-treatment, tumor recurrence was not observed, the wound was well healed, and the bitch was pregnant after 5 months of the follow-up period (Figure 56).

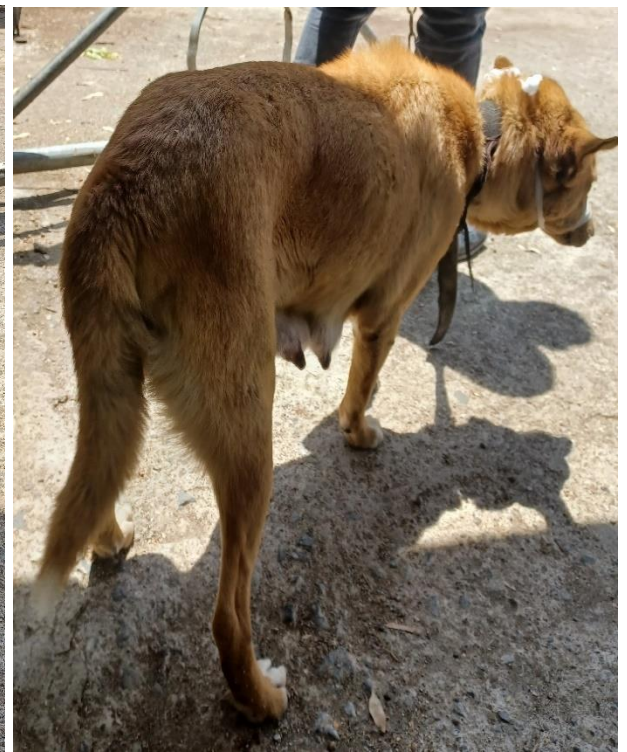
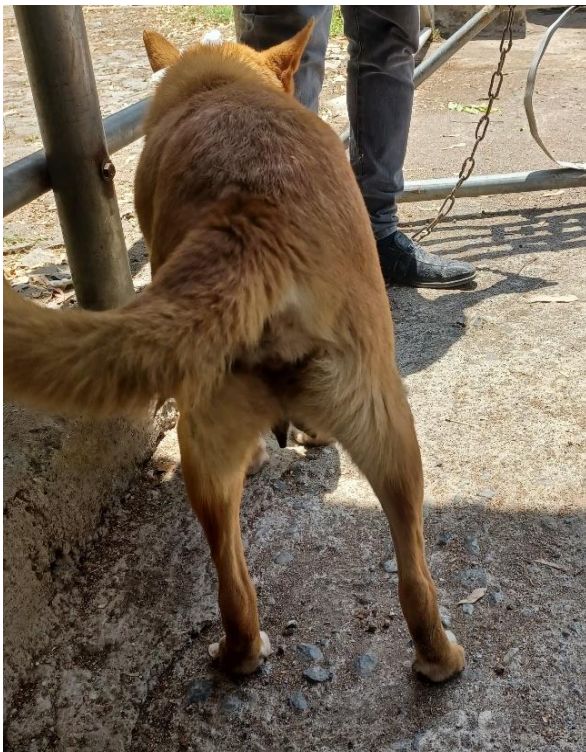


Figure 56: Well healed wound after five month of post treatment

Discussion

CTVT is a tumor that primarily affects stray dogs between the ages of three and eight, or during their active sexual cycle. The majority of transmission occurs during coitus, and the pathogenesis is thought to be cell transfers from sick to healthy canines. Although it can arise in various extragenital areas, the tumor is frequently seen in the genital areas (Stockmann *et al.*, 2011). The bitch in this case study was diagnosed based on the history, clinical observation and microscopic examination of the impression smears. Clinical signs exhibited by the bitch in the present case study were a cauliflower-like mass protruding from the vulva, licking of the vulvar area, an unpleasant odor and bloody vulvar discharge. These clinical findings are similar to those reported earlier by Hoque (2002), Antonov (2015), and Hiblu *et al.* (2019).

In the present case study, to confirm the diagnosis, more than eight impression smears stained with modified Giemsa stain and cytological examination revealed a predominance of round cells with abundant pale cytoplasm, distinct, punctate cytoplasmic vacuoles, an increased nuclear-to-cytoplasmic ratio and some inflammatory cells (75% lymphocytes and 25% macrophages). Similar cytological examination results were previously reported by Gonzalez *et al.* (2000), Park *et al.* (2006), Santiago-Flores *et al.* (2012), Chikweto *et al.* (2013), Milo and Snead (2014), and Gupta *et al.* (2022).

In the current case study, the bitch was successfully treated by surgical removal of the tumor, administration of ivermectin (0.2 mg/kg) subcutaneously, and application of a tincture of iodine on the wound daily until healing. The bitch was followed for 5 months, and post-treatment, tumor recurrence was not observed; the wound was well healed and gradually recovered, which is in agreement with a case reported by Lapa *et al.* (2012), Kumar *et al.* (2014), Lopes *et al.* (2015), and Bulhosa *et al.* (2020), who report surgical removal of the mass with subcutaneous administration of ivermectin as the best treatment for TVT cases in dogs. Ivermectin has been proposed to be a therapeutic agent as an anticancer drug (Jiang *et al.*, 2019; Markowska *et al.*, 2019). Surgery has been extensively used for the treatment of small, localized TVTs, but the recurrence rate can be as high as 50–68% in cases of large invasive tumors (Martins *et al.*, 2005; Abedin, 2020). Tella *et al.* (2004), Pansawut *et al.* (2012), and Hiblu *et al.* (2019) suggested that

intravenous administration of vincristine sulfate was very effective in achieving complete remission of CTVT in dogs. This case report suggests surgical removal of the tumor combined with administration of Ivermectin and tincture of iodine was the best treatment for a TVT case in dogs.

In conclusion, surgical removal of the mass combined with the administration of Ivermectin and application tincture of iodine successfully treat the CTVT case as shown in this case. Stray dogs' management and neutering these dogs may also be beneficial to decrease sexual activity among dogs in the locality, thus further preventing the spread of the disease.

Acknowledgement

I am grateful to Dr. Cheru Telila, Dr. Tilaye, Ms. Adisu Wakuma and the owner of the animal for their contribution during diagnosis, treatment and the follow up periods.

3.7. Case Reports on Poultry

3.7.1. *Infectious coryza in chickens*

Abstract

Infectious coryza, caused by *A. paragallinarum*, is an acute respiratory disease of poultry that can result in substantial morbidity, mortality, and economic losses. This case describes the clinical signs, laboratory diagnosis, and treatment outcome of an infectious coryza case in 10 isolated chickens. A phone call was received from a small poultry farm in Bishoftu with the history of decreased feed and water consumption, facial swelling, and nasal or lacrimal discharge, starting three days ago. Clinical observations were depression, facial swelling, conjunctivitis, and unilateral caseous exudates in the infraorbital sinuses. The exudate samples were collected, and a culture on 5% sheep blood agar without the feeder culture of *Staphylococcus aureus* was performed. Growths of small, dewdrop-like colonies and gram-negative, short rod-shaped bacilli bacteria arranged as single or paired bacteria were observed. In biochemical tests: it was negative for MR, Indole, V-P, H₂S production and catalase tests, and positive in TSI and citrate tests which was indicative of *A. paragallinarum*. Based on history, clinical diagnosis, and laboratory findings, the case was diagnosed as Infectious coryza. The chickens were treated by Gentamycin sulfate (one drop in each affected eye twice per day) and 10% Enrofloxacin (1 ml per 1 liter of drinking water) both for five days, and the chickens were successfully cured at the end of the therapy. Although early intervention with an appropriate drug could cure the infectious coryza case. Strict biosecurity and vaccination are recommended to overcome the problem.

Keywords: *Chickens, Biochemical test, Gram- negative, Infectious coryza.*

Introduction

Respiratory diseases have emerged as a great challenge to poultry industry and remains a serious problem in many parts of the world (El-Naenaey *et al.*, 2021). One of the respiratory diseases affecting chickens, infectious coryza, is caused by the bacteria *A. paragallinarum* and affects the upper respiratory system, including the nasal passages, infraorbital sinuses, and paranasal sinuses (Ali *et al.*, 2013). It is mainly observed in pullets and layers, and occasionally broilers (McMullin, 2006). It has major economic effects due to decreased egg production, slowed growth, and increased mortality (Xu *et al.*, 2019). *A. paragallinarum*, previously known as *Haemophilus paragallinarum*, a disease first recognized as a distinct entity in the late 1920's (Blackall and Soriano, 2020).

A. paragallinarum is gram-negative, capsulated, nonmotile, and composed of short rods or coccobacilli that measure 1 to 3 µm in length and 0.4 to 0.5 µm in width (Garcia *et al.*, 2004; Yehia *et al.*, 2023). The incubation period is 1 to 3 days, with the duration being 14 days in the infected individual bird (Akter *et al.*, 2013). Transmission occurs through direct bird-to-bird contact, inhalation of aerosols, coughing into the air, or ingestion of contaminated feed and water (Blackall and Soriano, 2020). Chronically infected birds or recovered healthy birds act as reservoirs of infection, making the disease endemic in an area (Chandravathi *et al.*, 2021).

The clinical signs associated with infectious coryza include nasal discharge, facial swelling, lacrimation, conjunctivitis, diarrhea, decreased feed and water consumption, retarded growth in younger chickens, an increased number of culls, and reduced egg production (Guo *et al.*, 2022; Badr *et al.*, 2022). Postmortem findings include catarrhal sinusitis and tracheitis (Byarugaba *et al.*, 2007). The conventional diagnosis of infectious coryza is based on clinical signs, demonstration of satellite colonies by cultural examination, and confirmation by biochemical tests (Han *et al.*, 2016). Hemagglutination-inhibition testing and PCR tests are the best alternative tools for easy and rapid confirmatory diagnosis (Chandravathi *et al.*, 2021; Badr *et al.*, 2022; Khan *et al.*, 2022).

The *A. paragallinarum* isolates are sensitive against erythromycin, gentamycin, lincomycin, neomycin, spectinomycin, oxytetracycline, Tylosine, ciprofloxacin and azithromycin (Deshmukh *et al.*, 2015; SaadEldin *et al.*, 2021). However, high levels of antimicrobial resistance to neomycin, streptomycin, tetracycline, doxycycline, ampicillin, cephalixin and erythromycin have been reported (Han *et al.*, 2016; Guo *et al.*, 2022). The prevention and control of IC depends on hygienic measures, proper management, balanced food rations, biosecurity measures, antibiotics, and proper vaccination programs are critical for prevention and control this infection (Yehia *et al.*, 2023). “This case describes clinical signs, laboratory diagnosis and treatment outcome of Infectious coryza case in isolated 10 chickens”.

Case description

A phone call was received from a small poultry farm at Bishoftu on February 17, 2023, with the history of 10 chickens decreased feed and water consumption, facial swelling, and closed eyes that started three days ago. The owner mentioned that he had 1000 bred chickens, and the chickens were bought from Alema Farms within one month. On clinical examination of the chickens, there were signs of depression, facial swelling, conjunctivitis, and unilateral caseous exudates in the infraorbital sinuses (Figure 57). Based on the history and clinical findings, infectious coryza was tentatively diagnosed.

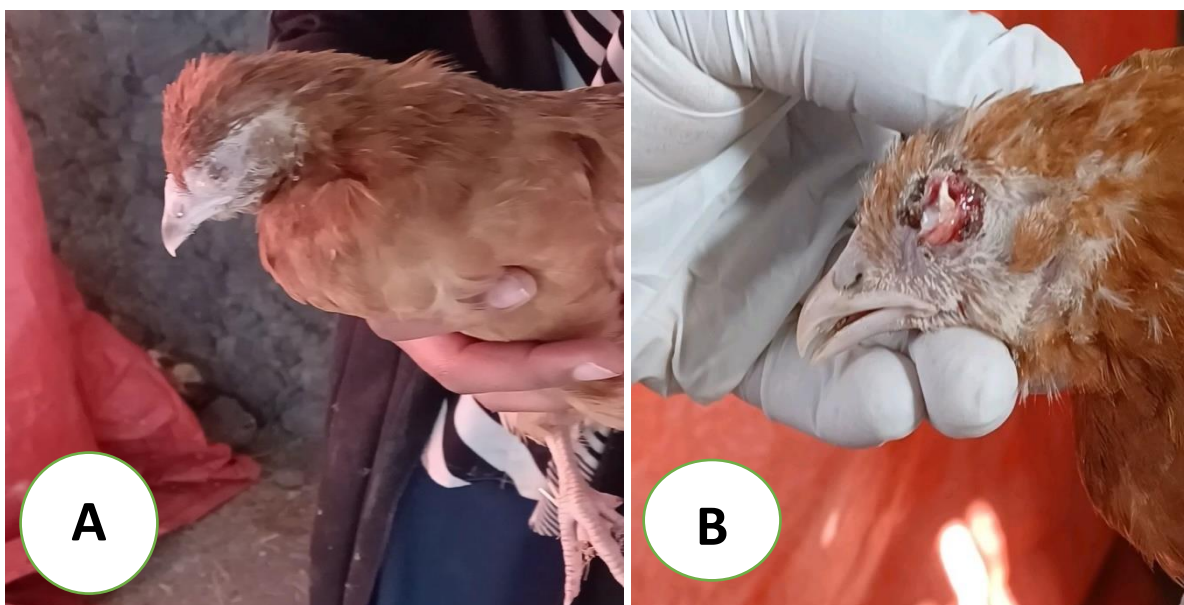




Figure 57: Indicating facial swelling (A), conjunctivitis (B), and caseous exudates in infraorbital sinuses (C).

Laboratory investigation and findings

The exudate samples from infraorbital sinuses were collected aseptically by using sterile swap, cultured on 5% sheep blood agar without the feeder culture of *Staphylococcus aureus*, and incubated anaerobically (candle jar method) at 37°C for 24 hours. The growth of small, dewdrop-like colonies was observed (Appendix) and stained by Gram's stain, which revealed gram-negative, short rod-shaped bacilli bacteria arranged as single or paired were observed under a 100x binocular microscope (figure 58). In biochemical tests, the bacteria revealed negative reactions in the MR test (i.e., absence of red color), Indole test (i.e., absence of pink color ring), V-P test (i.e., no color change), H₂S production (i.e., absence of black coloration at TSI slant), and catalase tests (i.e., production of no bubble). Also, the bacteria revealed positive reactions in TSI (i.e., color changed into yellow) and citrate (i.e., presence of color change) (figure 59). Finally, based on the history, clinical, and laboratory findings that the bacterium was *A. paragallinarum*, and the final diagnosis was infectious coryza.

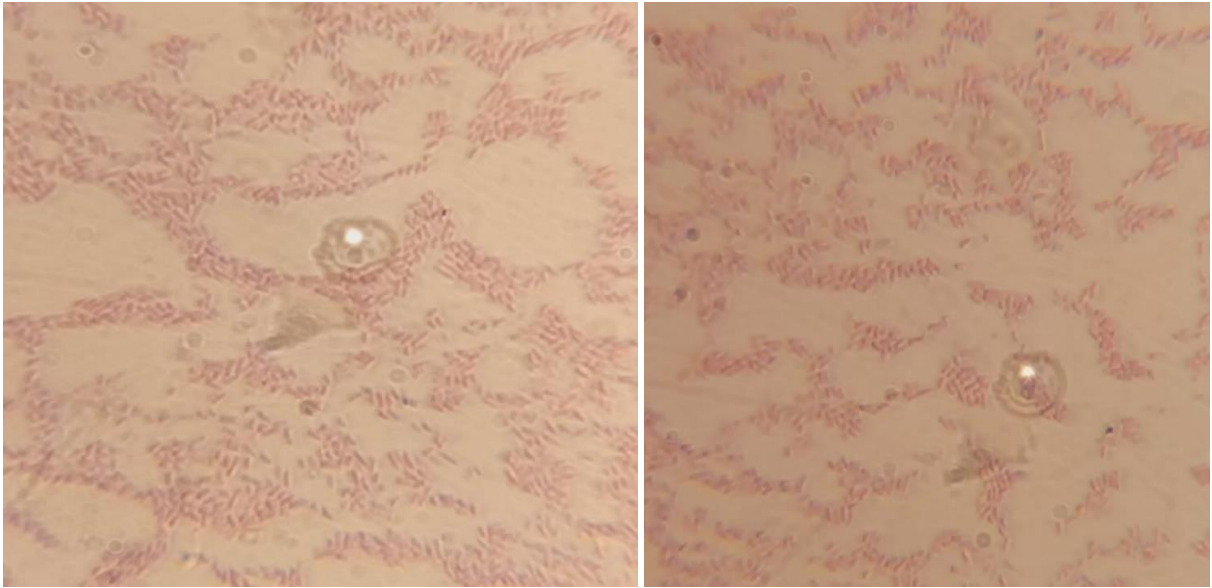


Figure 58: Gram- negative, short rod-shaped bacilli arranged as single or paired (*A. paragallinarum*).

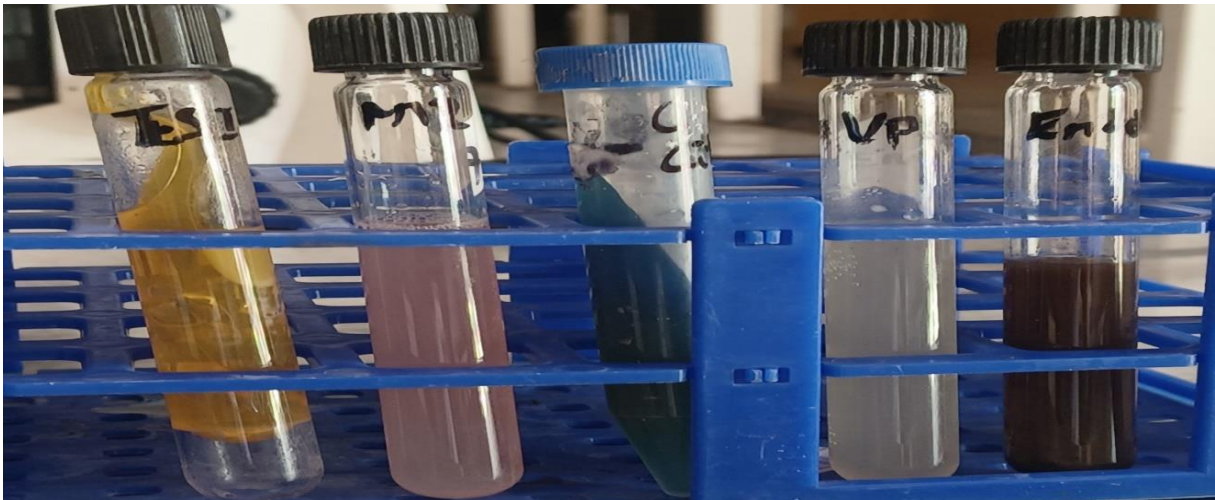


Figure 59: Indicating biochemical tests, Negative reaction in (MR test, Indole test, V-P test, H₂S Production at TSI slant, and catalase tests) and positive reaction in (TSI and citrate).

Case management and treatment outcome

The chickens were treated with Gentamycin sulfate ophthalmic solution (Axa Parenterals Ltd.) at a dose of one drop in each affected eye twice per day for five days and Enrofloxacin 10% (Ashish Life Science Pvt. Ltd.) at 1 ml per 1 liter of drinking water for five days. After the end of the treatment, the chickens in the isolation pen were bright and had good feed intake. After two weeks, all the chickens were fully recovered (Figure 60).



Figure 60: Indicating fully recovered chickens after two weeks of post treatment.

Discussion

Infectious coryza is a highly infectious poultry disease caused by *A. paragallinarum* and is seen in many countries all over the world, including Ethiopia (Dereja and Hailemichael, 2017). The current case was asserted as infectious coryza based on the history, clinical findings and laboratory result (bacteriology examinations). In the present case report, the prominent clinical signs observed are decreased feed and water consumption, open mouth breathing, sneezing, facial swelling, conjunctivitis, and unilateral caseous exudates seen in the paranasal and infraorbital sinuses. Similar clinical observations were made by Welchman *et al.* (2010), Rajurkar *et al.* (2010), Dwivedi *et al.* (2018), and Chandravathi *et al.* (2021).

In the present case report, the sample was incubated anaerobically (candle jar method) at 37°C for 24 hours on 5% sheep blood agar without the feeder culture of *Staphylococcus aureus* and did not show any satellitism; small, dewdrop-like colonies were observed. The growth characteristics indicated that the organism might be *A. paragallinarum*. Similar to the findings of Quinn *et al.* (2011), Priya *et al.* (2012), Akter *et al.* (2014), Crispo *et al.* (2019), and Anne *et al.* (2022). In Gram's staining, the morphology of the isolated bacteria exhibited gram-negative, short rods arranged in singles or pairs, which is similar to the findings of Ali *et al.* (2013), and Akter *et al.* (2016). In biochemical tests, the bacteria revealed negative reactions in the MR test, Indole test, V-P test, and catalase test, which is similar to the findings of Kaur *et al.* (2004), Akter *et al.* (2013), and Beiranvand *et al.* (2022), and the bacteria revealed positive reactions in the TSI and citrate tests within 24 hours of incubation, which is similar to the findings of Kaur *et al.* (2004), Khatun (2016), and Fauziah *et al.* (2021).

The present cases were successfully treated with gentamicin sulfate (one drop in each affected eye twice per day) for five successive days, coupled with Enrofloxacin 10% (1 ml per 1 liter of drinking water) for five days. The improvement was noted 72 hours post-treatment and fully recovered, which is in agreement with a case reported by Akhtar *et al.* (2001). In conclusion, the chickens were successfully treated with gentamicin sulfate and 10% enrofloxacin, which was the most effective antibiotic for respiratory disease in chickens. To prevent the reoccurrence of the infection in carrier chickens, it is advisable to vaccinate them with an inactivated coryza vaccine. However, further examinations are needed to isolate and characterize the locally prevalent serotypes of *A. paragallinarum*, and research work will also extend to the production of a vaccine to control infectious coryza in chickens in Ethiopia.

Acknowledgement

I am grateful to Dr. Mehari, Dr. Simret, Mis. Bilsuma and Mis. Tesfanesh for their contribution and again the owner of the farm for his valuable information during the follow up.

3.7.2. Newcastle disease in layers hens

Abstract

Newcastle disease (ND) is a contagious viral disease of birds, and chickens are highly susceptible to infection with the Newcastle disease virus. This case report describes the clinical findings, postmortem findings, and laboratory PCR results of Newcastle disease in layer hens. A phone call was received from a small poultry farm, Bishoftu, with a history of six chickens died with twisted necks and another six chickens changed the color of their feces to green, and depressed that started four days ago. Clinical signs were severe depression, swollen eyelids, paralyzed wings and legs, greenish diarrhea, dyspnea, sneezing, and rales. Post-mortem inspection showed hemorrhagic intestine and trachea, and pin-point hemorrhagic ulcers at the proventriculus glands and cecal tonsils. Cloacal and tracheal swabs were collected, PCR test was performed at the animal health institute (Sebeta) to detect NDV, and it was positive. Based on the history, clinical diagnosis, postmortem findings, and laboratory PCR results, the case was diagnosed as Newcastle. To protect against the secondary bacterial complication, oxytetracycline powder (20 mg/kg) with feed was given continuously for 5 days. After two days of therapy, the isolated layer hens died and the others recovered. Early isolation of infected chickens, adequate ventilation in the poultry house, and proper biosecurity are recommended to minimize deaths and prevent the spread of the disease.

Key word: *Chicken, Newcastle, PCR, Post mortem*

Introduction

Newcastle disease (ND) is a serious and commonly fatal viral poultry disease that causes economic losses to commercial poultry farmers over the world (Qin *et al.*, 2008; Rezaeianzadeh *et al.*, 2010; Diel *et al.*, 2012; Ashraf and Shah, 2014). The Newcastle disease virus (NDV) is an enveloped virus of *paramyxoviridae* with a negative-sense, single-stranded, nonsegmented RNA genome (Adi *et al.*, 2010; Worku *et al.*, 2022). The nucleocapsid protein (NP), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin-neuraminidase (HN), and RNA-dependent RNA polymerase (L) proteins are among the at least six key genes found in the genome (Perozo *et al.*, 2012; Radwan *et al.*, 2013). Based on their pathogenicity in chickens, the strains are categorized as highly virulent (velogenic), moderate (mesogenic), or avirulent (lentogenic) (East *et al.*, 2006).

The ND virus can be transmitted directly from diseased to healthy birds through oral and respiratory routes (Sharif *et al.*, 2014). Rodents, insects, dogs, fleas, and scavenging animals can mechanically spread contaminated feces (Ullah *et al.*, 2004). The virus strain that causes ND in chickens determines its pathogenicity, but other factors such as dose, mode of administration, age of the bird, and environmental factors also play a role (Jaganathan *et al.*, 2015). In general, a chicken's susceptibility to the illness increases with age (Wakamatsu *et al.*, 2006).

Clinical signs seen in birds infected with Newcastle disease vary widely (Worku *et al.*, 2022). The respiratory system can cause rales, coughing, sneezing, and gasping, while the neurological system can cause tremors, paralyzed wings and legs, twisted necks, circling, clonic spasms, and occasionally total paralysis (Nabila *et al.*, 2014; Messai *et al.*, 2019). Other common symptoms include greenish diarrhea, depression and inappetence, a partial or complete decrease in egg production, and a rise in the number of eggs with abnormal shapes (Okwor *et al.*, 2011; Abolnik, 2017). The ND lesions were dehydrated and dull with some neurological symptoms, such as hemorrhagic tracheitis and pin-point hemorrhage in the proventriculus glands' tips and caecal tonsils (Aldous and Alexander, 2010; Das *et al.*, 2018). The following diseases have clinical symptoms with Newcastle disease: fowl cholera, highly pathogenic avian influenza, laryngitis,

diphtheritic form of the (Abdisa and Tagesu, 2017). Diagnosis of NDV is based on history, clinical signs, postmortem findings and lesions, but laboratory confirmation must be done (Aliyu *et al.*, 2015; Das *et al.*, 2018). Isolation of virus and serological diagnostics such as HI Test, ELISA and real time PCR confirm the presence of NDV (Ashraf and Shah, 2014; Getabalew *et al.*, 2019).

The treatment cost of Newcastle disease in poultry flocks increases economic losses and the egg quality of ND affected flocks is decreased due to the use of antibiotics (Sharif *et al.*, 2014). Vaccination is the most successful tool for prevention of ND, and good biosecurity measures are essential to prevent Newcastle disease in poultry flocks (Aldous and Alexander, 2010; Meher *et al.*, 2020). Biosecurity measures include minimizing stress on birds, quarantine measures, vectors control, proper management, killing of ND virus, rearing of disease-free poultry flocks, zoning of outbreak area, proper disposal of dead birds, and regular surveillance of carrier birds (Roy, 2012; Apopo *et al.*, 2020). “This case report describes the clinical finding, postmortem finding and laboratory PCR result of Newcastle disease in layers hens”.

Case description

A phone call was received from a small poultry farm, Bishoftu, on February 17, 2023, with the history of six chickens who died with twisted necks; six of the chickens changed the color of their feces into green diarrhea; and six of the chickens were depressed that started four days ago. The owner mentioned that he had 1000 chickens, and the chickens were bought from one of his friends' farms within three months. A farm visit indicated inadequate ventilation in the poultry house and very poor structural and operational biosecurity. Clinical signs seen were severe depression, swollen eyelids, paralyzed wings and legs, greenish diarrhea, dyspnea, sneezing, and rales (figure 61). Based on the history and clinical findings, lists of differential diagnoses were made, including Newcastle disease, acute fowl cholera, poisoning, and Marek's disease, which were tentatively diagnosed.



Figure 61: Indicating isolated chickens with greenish diarrhea (A) and paralyzed wing and leg chicken (B).

Post mortem findings

Post-mortem inspection showed hemorrhagic intestine and trachea, pin-point hemorrhages, ulcers at the proventriculus glands, and cecal tonsils (Figure 62).

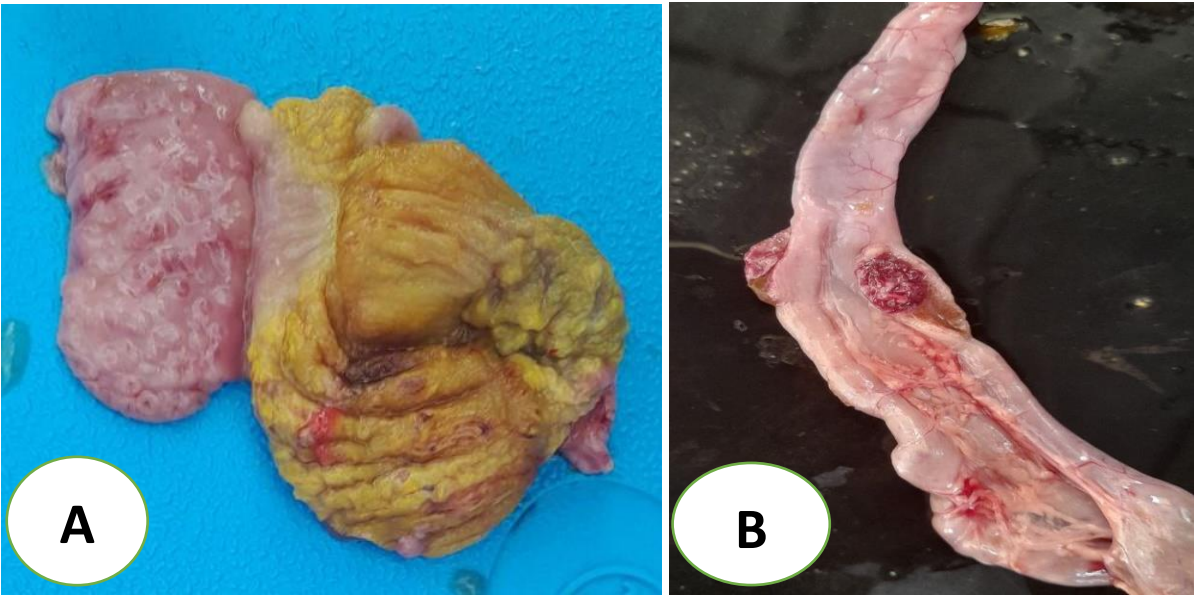




Figure 62: Indicating pin point hemorrhages ulcers at the proventriculus glands(A) and cecal tonsils (B) haemorrhagic intestine (C) and trachea (D).

Laboratory investigation and findings

Cloacal and tracheal swap samples were collected from five live chickens using sterile swabs, and immediately the swabs were put into virus transport media and given to the Animal Health Institute, Sebeta, for confirmatory diagnosis. PCR was used to detect NDV from the homogenized swap samples obtained from the cloacal and tracheal swabs taken directly using the pair of primers and probes' targeting the M gene that were specific to the virulent strains of NDV, which showed that out of 5 pooling samples, all samples were positive in PCR (Figure 63). Finally, it was concluded based on the history, clinical findings, postmortem findings, and laboratory PCR results that the final diagnosis was Newcastle.

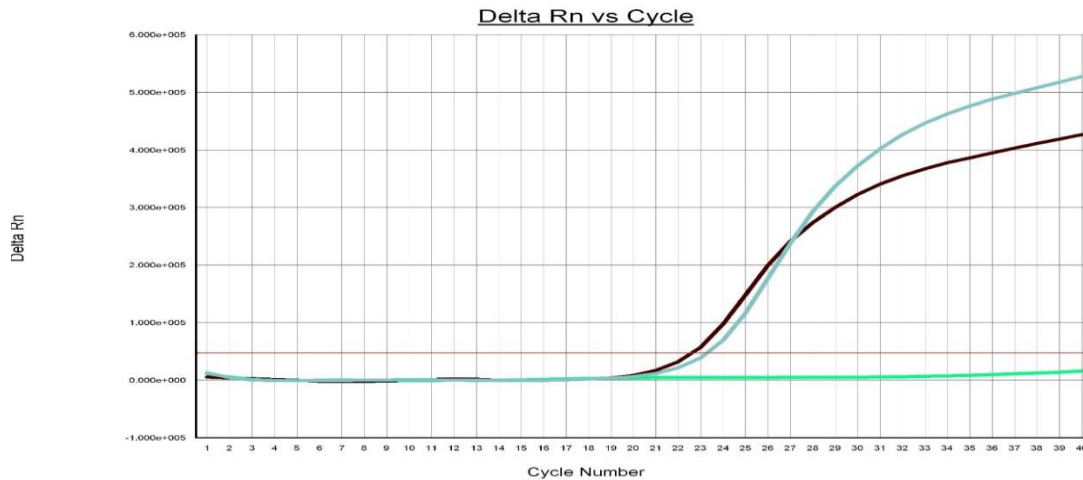


Figure 63: Indicating 5 outbreak samples standard curve (Ct value 23.103).

Case management and treatment

To protect against the secondary bacterial complication, oxytetracycline powder 20% (Ashish Life Science Pvt. Ltd.) at 20 mg/kg with feed was given continuously for 5 days. Beside the treatment, the owner was advised to isolate the depressed chicken, provide adequate ventilation in the poultry house, and practice proper biosecurity. After two days of therapy, the isolated layer hens died and the others recovered (Figure 64).



Figure 64: Indicating healthy layers hens after the outbreak of the infection.

Discussion

Newcastle disease (NCD) is a contagious viral disease of chickens. The current case was asserted as Newcastle based on the history, clinical findings, post-mortem findings, and laboratory PCR result. The major clinical signs observed in this case report were severe depression, swollen eyelids, paralyzed wings and legs, greenish diarrhea, dyspnea, sneezing, and rales at ante-mortem. This is in agreement with reports by Lawal *et al.* (2015), Abdu *et al.* (2004), and Mariappan *et al.* (2018), who confirmed similar signs as tentative in the diagnosis of ND in infected birds. The post-mortem findings shown were haemorrhagic intestine and trachea, and pin-point haemorrhagic ulcers at the proventriculus glands and cecal tonsils. Similar observations were made with Ratih *et al.* (2017), Mariappan *et al.* (2018), Ahmed and Odisho (2018), and Mostaree *et al.* (2021).

In the present case report, PCR was used to detect NDV from the homogenized swap samples of affected chickens obtained from the cloacal and tracheal swabs taken directly using the pair of primers and probes' targeting the M gene that were specific to the virulent strains of NDV, which showed that out of 5 pooling samples, all samples were positive in PCR; these results were in line with those mentioned by Garcia (2016), Dhaygude *et al.* (2017), Shadood and Najem (2021), and Villalobos-Agüero *et al.* (2022).

In the present case study, to protect against the secondary bacterial complication, the chickens were treated with oxytetracycline powder at 20% (20 mg/kg) with feed, given continuously for 5 days, but the isolated layer hens were died and the others were recovered. Inconclusion, Early isolation of infected chickens, adequate ventilation in the poultry house, and proper biosecurity are recommended for the prevention of the disease.

Acknowledgement

I am grateful to Dr Mehari, Dr Hana and the owner of the farm for his valuable information during the follow up.

4. CONCLUSION AND RECOMMENDATIONS

This case study illustrated different diseases including bacteria, virus, fungus, protozoa, metabolic, parasitic and tumor cases of animals examined at Professor Feseha Gebreab Memorial-VTH and SPANA, AAU, CVMA, and private farms in Bishoftu. The study also showed that application of thorough clinical case handling protocols, rigorous and prompt treatment with rational drugs and thorough follow-up has a paramount role in reducing the impact of diseases on the animals, the owner and the country in general. Besides, antimicrobial therapy coupled with supportive therapy could minimize the animal suffering and hence facilitate recovery. However, drugs used in this study were not supported with sensitivity tests which could have been helpful to select the most effective drugs. Some diseases require due follow-up as inpatient and some require imaging facilities, for better care and diagnosis, respectively.

Based on the above conclusions the following recommendations are forwarded;

- ✚ Confirmatory diagnosis through proper sample collection is essential for effective treatment to maintaining animal welfare and limiting the risk of disease transmission to other animals and humans,
- ✚ Post treatment follow-up record is essential in animal health facilities and in private animal farms for obtaining information about status of diseased animals and treatment responses.
- ✚ Animal owners and farm attendants require awareness-raising training on early admission of diseased animals to veterinary clinics or consulting professionals in addition to the successful implementation of veterinary advice, and
- ✚ Research towards the development of vaccines and/or effective drugs should be encouraged for those economically important disease that have no vaccines and/or effective drugs in Ethiopia.

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6. APPENDICES

Appendix 1: Gram-staining procedure

- ❖ Place slide with heat fixed smear on staining tray.
- ❖ Gently flood smear with crystal violet and let stand for 1 minute.
- ❖ Tilt the slide slightly and gently rinses with tap water or distilled water using a wash bottle.
- ❖ Gently flood the smear with Gram 's iodine and let stand for 1 minute.
- ❖ Tilt the slide slightly and gently rinses with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
- ❖ Decolorize using 95% ethyl alcohol or acetone. Tilt the slide slightly and apply the alcohol drop by drop for 5 to 10 seconds until the alcohol runs almost clear. Be careful not to over-decolorize.
- ❖ Immediately rinse with water.
- ❖ Gently flood with safranin to counter-stain and let stand for 45 seconds.
- ❖ Tilt the slide slightly and gently rinses with tap water or distilled water using a wash bottle.
- ❖ Blot dry the slide with bibulous paper.
- ❖ View the smear using microscope under oil-immersion.

Appendix 2: Giemsa staining procedure

- ❖ One drop blood was placed near one end of clean, dry and grease-free glass slide.
- ❖ Spreader slide inclined about 45° was pushed horizontally from the one end to another and get thin smear with clear tail.
- ❖ The resulting film was dried rapidly by waving it in the air, and was fixed with absolute methanol for 2 minutes
- ❖ Flood the smear with Giemsa stain and keep it submerged for 30 seconds
- ❖ Flood the smear with clean water
- ❖ Allow to dry before examination

- ❖ Examine under oil immersion on the microscope using high power to identify blood cells and blood parasites

Appendix 3: Slide-Catalase test procedure

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. Place a drop of 3% H₂O₂ in the glass slide.
3. Observe for the evolution of oxygen bubbles.

Appendix 4: Fecal Flotation procedure

1. Three grams of feces was added to 42 ml of magnesium sulfate solution in a graduated cylinder.
2. The contents were then mixed thoroughly using a glass rod, and were poured through a tea strainer into another beaker.
3. The filtrate was then filled to 10ml test tube and centrifuged by 1200 rpm for 5 minutes
4. The cover-slip was putted on the top of the test tube thoroughly and kept for 10 min in the test tube rack.
5. The cover-slip was carefully lifted and placed on glass-slide and viewed under a compound microscope X10 objective lens.

Appendix 5: PCR result of ND (Ct Value = 23.103)


Applied Bio-systems 7500 Real-Time Polymerase Chain Reaction (RT-PCR) thermocycler were used for amplification of all NDV isolates. A primer probe combination from a conserved region of the M gene:

- APMVI F M+4100 (Forward): 5'-AGT GAT GTG CTC GGA CCT TC-3'
- APMV-I R M- 4220 (Reverse): 5'-CCT GAG GAG AGG CATTG CTA-3'
- APMV-1M+4169 (Probe): 5'-FAM TTCTCT AGC AGT GGG ACA GCC TGC

Appendix 6: Case paper

Bishoftu

CVMA/PATIENT CARD


ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE
PROFESSOR FESEHA G/AB VETERINARY TEACHING HOSPITAL

Date _____

Case No: _____ Herd size: _____

Name of the Hospital/Clinic: VTH Donkey Sanctuary SPANA Field _____

Owner's Name _____ Address Town _____ Village _____ House No _____

Occupation _____ Phone No _____

Animal detail and identification

Species: Bovine Ovine Caprine Feline Canine Swine Equine Poultry

Breed _____ Sex _____ Male Female Age _____ Name if any _____

CASE HISTORY

CLINICAL FINDINGS

Body Temp _____ c° Respiration rate _____ Breaths/min Heart rate _____ Beat/min

Ruminal motility _____ /min Gut sound _____ VMM: Normal pale Congested Jaundice

Cyanotic CRT: _____ Body condition: _____ Weight _____ Superficial Ln: PF _____ PS _____

MND _____ RPH _____ Other _____

Description of the Case/Clinical Findings

Organ or system affected: Nervous Musculoskeletal Respiratory Circulatory

Digestive Urogenital Integuments Other _____

SAMPLE TAKEN

Feces Blood skin scraping Nasal swab vaginal swab Ruminal content

Urine Other _____

Differential Diagnosis List: _____

Laboratory Result: _____

Tentative Diagnosis: _____

Final/Definitive Diagnosis: _____

1000

OBSERVATION AND TREATMENT

Prognosis: _____

Clinical work to be performed: Surgical _____

Medical _____

Gynecology/Obstetric _____

Follow up /Quarantine _____

Medical and supportive treatment administered

1. _____

2. _____

3. _____

4. _____

Outcome

2nd day _____

3rd day _____

4th day _____

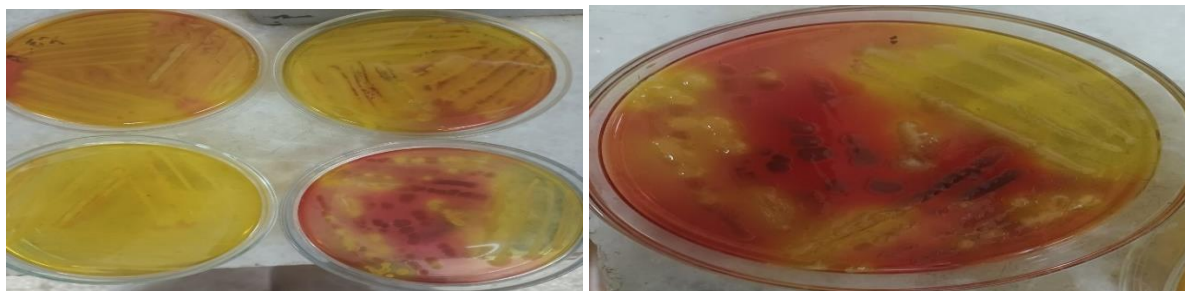
Description of final outcome (weeks or months post-treatment):

Date: _____

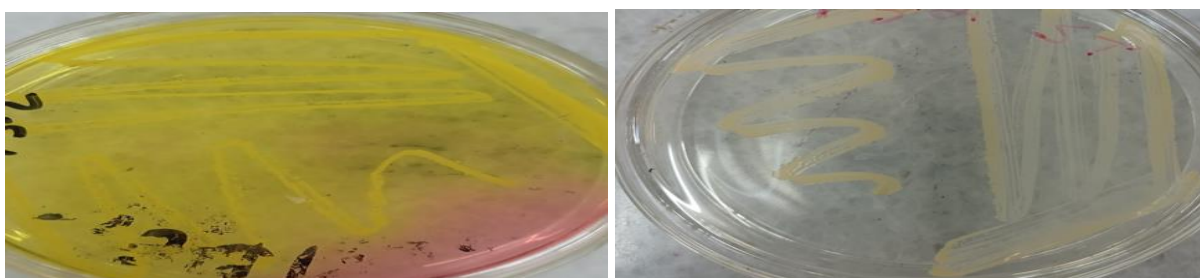
Name of Staff In Charge: _____ Signature _____

Student In Charge: _____ Signature _____

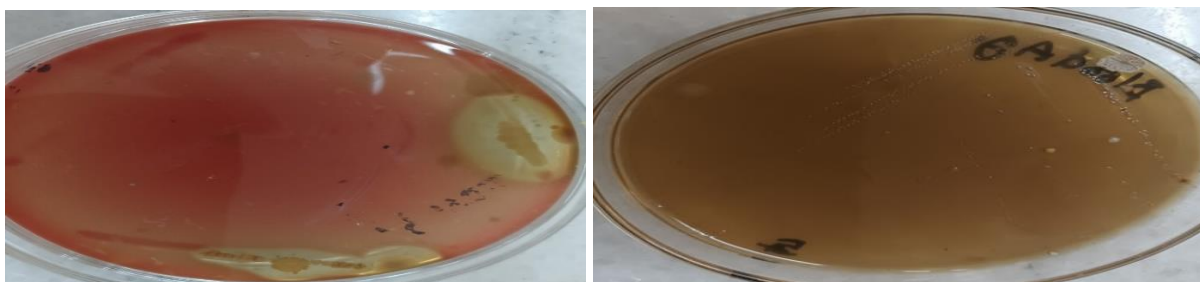
Appendix 7: Laboratory works (bacteriology)



Indicating black with wide range of red zone of *Salmonella spp* of bacteria on XLD agar



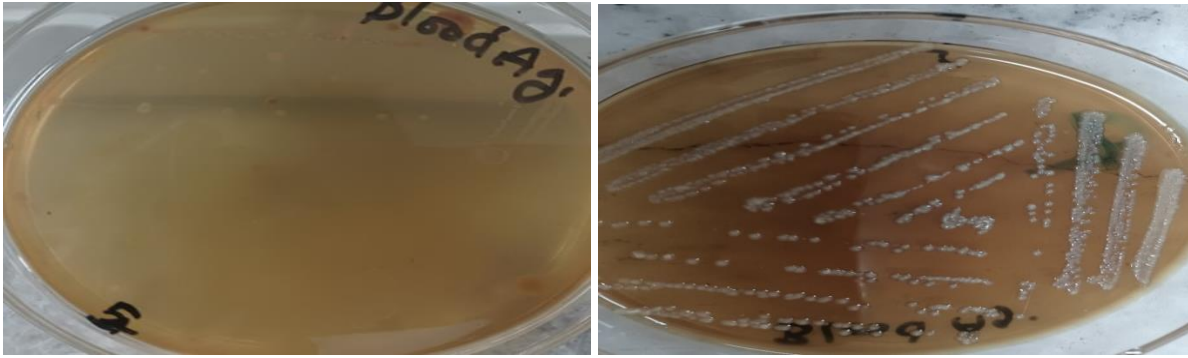
Indicating golden yellow colonies of *S. aureus* on mannitol salt agar and Nutrient agar.



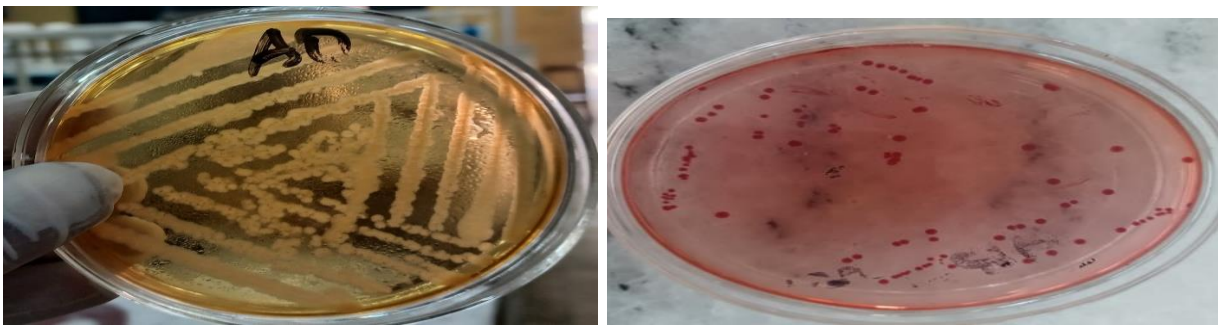
Indicating Growth of small colony with wide range haemolysis (*Moraxella spp*) and small dewdrop-like colonies (*A. paragallinarum*) on blood agar



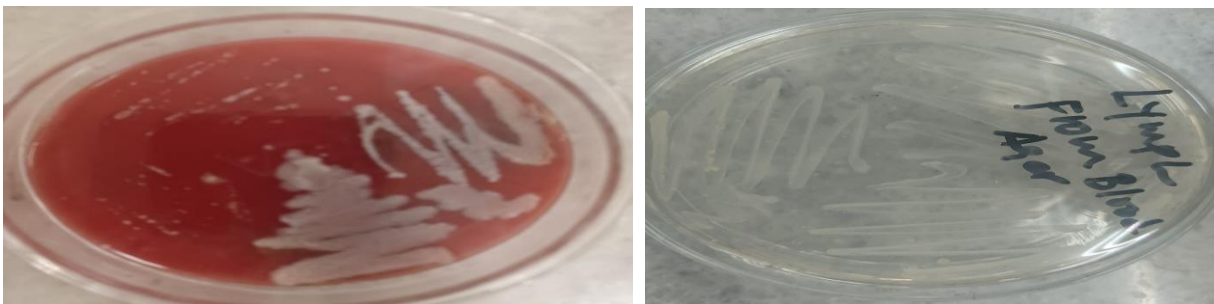
Indicating growths of small, white, dry with narrow hemolysis (*C. Perferenges*) on blood agar



Indicating growths of small, white, dry with narrow hemolysis (*C. chauvoei*) on blood agar



Colonies of the *A. lignieresii* on MacConkey agar



Indicating *C. pseudotuberculosis* colony on Blood agar and Nutrient agar (Ulcalerative lymphangitis)



Indicating biochemical testes with their reagent



Indicating dispensing of media on Petridis and preparation of anaerobic jar



Indicating crushing the faeces by pestle and mortem and centrifuging the fecal sample for flotation examination



Indicating Giemsa-stained smear preparation

Appendix 8: Clinic works



Indicating preparation and administration of Diminazen Di acetate solution



Indicating Physical examination of the body parameters



Indicating clinical examination of the oral cavity and the body wound management



Indicating surgically removal of the tumor and wound management





Indicating clinical examination, site preparation and topical application of zinc oxide



Indicating post mortem examination



Indicating clinical examination of the eye and eye drop treatment



Indicating sample taking by sterile swab from the eye



Indicating drenching of Indigestion powder solution and intravenous rehydration of the cow



Indicating intravenous administration of calcium borogluconate



Indicating wound management