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CLINICAL CASE STUDIES ON MAJOR DISEASES OF VETERINARY IMPORTANCE AT ANIMAL HEALTH FACILITIES IN BISHOFTU TOWN, ETHIOPIA

MVSc THESIS

BY

TSEDALE TESHOME

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CLINICAL CASE STUDIES ON MAJOR DISEASES OF ANIMALS PRESENTED TO ANIMAL HEALTH FACILITIES IN BISHOFTU TOWN, ETHIOPIA

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

By

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CVMA, Bishoftu

CLINICAL CASE STUDIES ON MAJOR DISEASES OF ANIMALS PRESENTED TO ANIMAL HEALTH FACILITIES IN BISHOFTU TOWN, ETHIOPIA

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STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been accordingly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced MSc degree at Addis Ababa University, College of Veterinary Medicine, and is deposited at the College library to be made available to borrowers under the rules of the library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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ABBREVIATIONS

ACP	Acepromazine
AHS	African Horse Sickness
CCBP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia.
CMT	California Mastitis Test
CPV	Canine parvovirus infection
CVMA	College of Veterinary Medicine and Agriculture
CSA	Central Statistics Agency
EDTA	Ethylenediaminetetraacetic acid
EZL	Epizootic Lymphangitis
FAD	Flea allergy dermatitis
FMD	Foot and Mouth Disease
FAO	Food And Agriculture Organization
GDP	Growth and Development Plan
IBD	Infectious bursal disease
OIE	World Organisation for Animal Health
PPR	Peste de Petits Ruminants
SCM	Sub-clinical mastitis
SPANA	Society for the Protection of Animals Abroad
TAT	Tetanus antitoxin
UNDP	United Nations Development Program

ABSTRACT

An estimated 2.5 billion people depend on agriculture for their livelihood, and 1.3 billion people are employed by it. Livestock make up about 30% of the income from agriculture in poor nations like Ethiopia. Ethiopia has a significant quantity of livestock; however, the industry is characterized by low output since a variety of diseases are common. Therefore, the goal of this case study was to compile a clinical case report on the major disease of veterinary importance at Fisseha Gebreab memorial Veterinary Teaching hospital of CVMA, Bishoftu, Ethiopia from September 2022 to June 2023. Twenty cases were studied and compiled following the scientific case publishing format where abstract, introduction, case description, tentative diagnosis, laboratory diagnosis and result, treatment, and outcome of the disease were briefly recorded. The findings of each case were finally discussed concerning the findings in other literature and scientific publications. The twenty compiled case studies include ten cases on ruminants, two cases on equines, three cases on poultry, three cases on canines, and one case on pigs. Of a total of twenty cases, nine cases (47.6%) were caused by bacteria, six cases (28.6%) were caused by parasitic agents, four cases (19.05%) by viral particles, and one case (4.7%) were caused by a fungal pathogen. Therefore, clinical diagnosis complimented by laboratory examination is recommended to initiate effective treatment and control measures that safeguard the health and welfare of the treated animal and reduces the spread of infection to other animals or, in the case of zoonotic disease, to humans.

Keywords: *case study, case description, treatment and outcome, discussion*

1. INTRODUCTION

Around the world, agriculture is thought to support 2.5 billion people and employ 1.3 billion people (World Bank, 2007). Livestock makes up about 30% of the income from agriculture in underdeveloped nations like Ethiopia (World Bank, 2009). With over 70 million cattle, 42.9 million sheep, 52.5 million goats, 2.15 million horses, 10.80 million donkeys, 0.38 million mules, 8.1 million camels, and 57 million poultry Ethiopia has the largest livestock in Africa (CSA, 2021). The majority of Ethiopians depend heavily on livestock as a source of meat and milk, draught power for agricultural production, and a source of money for farmers. Additionally, livestock helps to significantly increase the national GDP and supplies the nation with foreign currency (Ayele *et al.,* 2015). About 65% of Ethiopian families directly depend on the economic contribution of livestock and their products, which accounts for about 20% of the country's overall GDP and 45% of its agricultural GDP (FAO, 2011).

Cattle diseases are one of the most significant factors affecting cattle productivity and production (Ayele *et al.,* 2015). The extensive occurrence of a wide variety of illnesses and parasites in all of the country's agroecological zones results in direct economic losses due to excessive animal mortality, and it also affects the animals' low reproductive and productive performance. According to estimates, the yearly livestock mortality rates for cattle, sheep, and goats are 8–10%, 14–16%, and 11–13%, respectively (Asresie and Zemedu, 2015). Numerous bacterial, viral, protozoal, endoparasitic, and ectoparasitic diseases of livestock are often seen throughout the nation and are responsible for significant economic losses. At the national level, the effects are equally catastrophic because the existence of the diseases has led to a severe trade embargo, which results in annual losses of hundreds of millions of birr (UNDP, 1994; Abdeta *et al.,* 2015).

Animal diseases can be divided into infectious and non-infectious conditions; pathogens such as viruses, protozoa, parasites, bacteria, and fungi are the main causes of infectious diseases, while conditions caused by conditions other than agents are non-infectious (Jana and Ghosh, 2013). An analysis of 1966 agricultural animals admitted to the university of

Gondar veterinary clinic from 2007 to 2009 revealed that more than 75% of them had bacterial, parasitic, or viral infections, whereas only 4.2% had metabolic problems (Alemu & Zegeye, 2011) Viral diseases affecting the nation's livestock industry include a foot-and-mouth condition (FMD), Capri pox virus diseases (sheep, goat, and lumpy skin disease), PPR (Peste de petits ruminants), contagious ecthyma, AHS (African Horse Sickness), and rabies are the most common viral diseases affecting the country's livestock sector.

In contrast, anthrax, blackleg, CBPP (contagious bovine pleuropneumonia), CCPP (contagious caprine pleuropneumonia), pasteurellosis, IBK (Infectious Bovine Keratitis), Brucellosis, clinical mastitis, dermatophilosis, actinobacillosis, actinomycosis, foot rot and abscess are among consistently reportable bacterial diseases (UNDP, 1994). Asmare *et al.,* (2016) The findings of this paper add to the mounting evidence of the prevalence of many gastro-intestinal tracts (GIT) parasites in cattle and their negative effects on Ethiopian production and productivity. There have been sporadic reports of tick-borne hemoparastic disorders such as Babesiosis, Theleriosis, Anaplasmosis, and Ehrlichiosis (heart water) in various parts of the nation (Nejash, 2016). The subsequent interventions are not under the accepted procedures, even though the majority of these disorders are curable and treatable.

One of the countries where general animal services have been negatively impacted by insufficient and poorly maintained infrastructure and a lack of specialists is Ethiopia (Jibat *et al.,* 2015). Due to a lack of laboratory space and financial restrictions, the approach used to provide this animal health service at various animal health centers is based on a tentative diagnosis that lacks accuracy in the disease's diagnosis (Beyene *et al.,* 2018). Lack of access to skilled veterinary services, which leads to improper diagnosis and treatment of endemic diseases, particularly those affecting cattle, exacerbates the impact of the diseases. Therefore, the case study aimed at identifying and reporting the occurrence of major domestic animal diseases in Bishoftu to the college and the community.

Therefore, the objective of this case study was: -

To handle and identify major diseases of veterinary importance by clinical and laboratory diagnosis, with a focus on recommending diagnosis, treatment, and an overall management approach at animal health facilities in Bishoftu town, Ethiopia.

2. MATERIALS AND METHODS

2.1. Study area and Study Animals

The clinical study was conducted at VTH and SPANA animal health facilities of Addis Ababa University College of Veterinary Medicine, Bishoftu from September 2022 to June 2023. Bishoftu town is located in East Shoa Zone of the Oromia Regional 47.9 km to south east of Addis Ababa. It is located at latitude and longitude of 10° 35' 0" N and 35° 48' 0" E respectively. The area lies at an altitude of 1850 meters above sea level and experiences the mean annual rainfall, maximum and monthly temperatures range between 801.3mm, 25.5 0 C, 23.7 in July & 27.70 C in May, respectively. Moreover, Bishoftu and its surroundings have variable and yet representative agro-ecologies of the country. As the Bishoftu town is well known in animal production and medication center, many animals specious visit the teaching hospital and different community services and campaigns take place in this town. Cattle, small ruminants, poultry, equines and pet animals are the major livestock species reared in the area.

2.2. Study Design and Case Handling Protocols

A standard clinical case observation was performed. This clinical study was undertaken at animal health facilities in Bishoftu towns, Central Ethiopia to report clinical diseases and disorders in animals during the study period from September 2022 to June 2023. A detailed report of species, breed, age, and sex and a detailed description of the symptoms, signs, diagnosis, treatment, and follow-up of an individual patient animal will be done. Different species of animals (bovine, equine, ovine, caprine, canine, swine and poultry) of different age and sex were included in the study. Clinical Examination of the individual animal or groups of animals was beginning with history. History is then followed by performing a complete clinical examination. Based on the information obtained during the clinical examination and owner complaint, tentative diagnoses of the cases were asserted and empiric therapy was started by using clinical case handling protocol (Jana and Ghosh, 2013) (Annex1). The appropriate sample was taken, carefully

labeled (label including the type of specimen, date of sampling, preservative used, identification of animals and name of the owner) and transported to the laboratory in the appropriate transporting medium. The sample was processed in the laboratory based on the laboratory procedure requested to diagnose the disease. Then the final diagnosis of the disease was set based on the laboratory result combined with the history and clinical findings of the case. If the already initiated empiric therapy was not a drug of choice for treatment of the case it was discontinued and new treatment initiated but if the empiric therapy was a drug of choice, the treatment continued to the end. Response to treatment and follow-up of the outcomes was complete through phone communication to the owners and also by home visit. The information for all the cases was first obtained using the standard clinical case recording format of Addis Ababa University (Annex2).

The next step is advising the owner how to care for the diseased animal for example isolating the diseased animal for the prevention of disease transmission, care associated with the drug like withdrawal period of the drug and so on. Finally, follow-up was take place until recovery or death takes place.

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4. COMPILED CASE REPORTS

A total of 20 major diseases of veterinary importance involving 20 individual animals were compiled during the clinical case study period. Species of animals included in this case report were 10 ruminants, 4 canines, 3 poultry, 2 equine and 1 pig. From a total of 20 treated animals 19 (90.05%) recovered from their disease while 1 animal (9.05) died of its disease (Table 1). From a total of 20 cases, 9 cases (47.6%) were caused by bacteria, 6 cases (28.6%) were caused by parasitic agents, 4 cases (19.05%) by viral particles and 1 case (4.7%) were caused by a fungal pathogen (Table 2).

Table 1: Total number of animals examined and treatment outcome

Animal	**Total**	**Recovered**	**Died**
Ruminants	10	10	0
Equine	2	2	0
Canine	4	3	1
Poultry	3	3	0
Swine	1	1	0
Total (in percentage)	**20**	**19(90.5%)**	**1(9.5%)**

Table 2: Cause of diseases encountered during the study in percentage

Diseases	**Number of affected animals**	**Percentages (%)**
Bacteria	9	47.6%
Virus	4	19.05%
Parasites	6	28.6%
Fungal	1	4.7%
Total	20	100%

4.1. Case Reports on Bovine

4.1.1 Actinobacillosis in dairy cow

Abstract

Actinobacillosis is an infectious, chronic, and generally, non-fatal disease caused by *Actinobacillus lignieresii*. This case report summarizes the case of Actinobacillosis in a cross breed cow from one of the dairy farms found in Bishoftu, Ethiopia, with a history of off-feeding for 2-3 days and difficulty mastication due to swelling of the mouth part. A week ago, swellings were seen on the right mandible and started to grow slowly, and the swellings started to discharge pus. On clinical examination, the rectal body temperature was 38.1 ℃ with 40 breaths per minute respiratory rate and a 56 beats per-minute pulse rate. The cow had bilateral swelling of the face and an abscess in the ventral part of the mandible. The Gram stain of the granules, taken from the pus, revealed Gram-negative rod bacteria. The pus was surgically drained and flushed with 2% tincture iodine for seven days, and the animal was also treated with systemic antibiotics. The swelling started to regress slowly, and on the day of the visit, it changed to very small fibrous tissue. When compared with other literature findings, the present treatment approach, which is a local infiltration of iodine, took less time to heal than systemic administration. Therefore, further case studies should be done on the benefits of using iodine tinctures locally rather than systemic iodides.

Keywords*: Actinobacillosis; Gram stain, Antibiotic and Iodide*

Introduction

Actinobacillosis is an infectious, chronic, generally non-fatal bacterial disease of cattle caused by the bacteria *Actinobacillus lignieresii*. The disease is most commonly found in dairy cattle and is characterized by localized tissue necrosis, lymphadenopathy, and abscess formation in the lymph nodes, lungs, and other organs (Bundy, D., Fyock, T., and Holmberg, D. 2013). An organism that lives commensally in the mouth and rumen of sheep and cattle is the cause of the disease. The classic form of the disease in cattle causes the tongue to enlarge and harden, earning it the nickname "wooden tongue." A gram-negative, aerobic rod that is a typical resident of ruminants' digestive systems is the cause. The oropharynx and rumen of cattle and sheep typically contain the causative agent. The opportunistic infection infects cows, buffalo, sheep, goats, and horses, causing chronic pyogranulomatous lesions of the soft tissues of the head and neck areas (Aziz *et al.,* 2019).

Common symptoms of bovine actinobacillosis include pyogranulomatous glossitis (wooden tongue). It has been deemed atypical or cutaneous when other tissues, typically the skin or lymph nodes, are involved (Caffarena *et al.,* 2018). Skin lesions, respiratory system lesions, lymph node lesions, and widespread lymph node and organ lesions are its defining features. A clinical condition known as the "hippo-like face" is caused by diffuse lesions on the lips, palate, pharynx, nasal pits, and face (Scheid *et al.,* 2020).

The presence of several hard, yellowish nodules surrounded by fibrous tissue, as well as the isolation and identification of the responsible organism, are lesions that are indicative of a wooden tongue (Caffarena *et al.,* 2018). Treatment for Woody Tongue is typically effective and easy to follow. Intravenous (IV) sodium iodide solution is administered, and it is repeated after 7–10 days. Given that the organism is a bacteria, antibiotics may be useful. Drugs including ceftiofur, ampicillin, penicillin, florfenicol, and tetracyclines are effective to most strains of *A. lignieresii* (John Maas, 2009). Thus, the present case report described Actinobacillosis in dairy cows.

Case description

A phone call from one of the dairy farm in Bishoftu with a history of off-feeding for 2-3 days and difficulty with mastication due to swelling of the mouth part of one lactating cow was received. Before a week, swellings were seen on the right mandible and started to grow slowly, and the swellings started to discharge pus. On clinical examination of the body parameters, the rectal temperature was 380 °C, respiration was 40 breaths per minute, and the pulse rate was 56 beats per minute. All measured parameters were in normal range, but the cow had bilateral swelling of the face and an abscess at the ventral part of the mandible region, as indicated in the figure below. The animal also presented with slight nasal discharge and slightly swollen sub-mandibular lymph nodes. Based on the history and clinical findings, differential diagnoses were set as: oral foreign bodies such as a bone or stick, broken teeth or jaw, or a grass seed abscess affecting lymph nodes of the throat; actinobacillosis; and actinomycosis.

Figure 1: Swellings on the left mandible of Actinobacillosis

Laboratory finding

A sample of pus was collected and placed on a microscopic slide and allowed for air dry. After 30 minutes Gram staining was done according to the procedure (annex3) to the dried sample on slide. A drop of oil immersion was added and the prepared sample was observed under 100x oil objective lens of binocular microscope. The result revealed Gram-negative, rod-shaped bacteria as indicated on figure below.

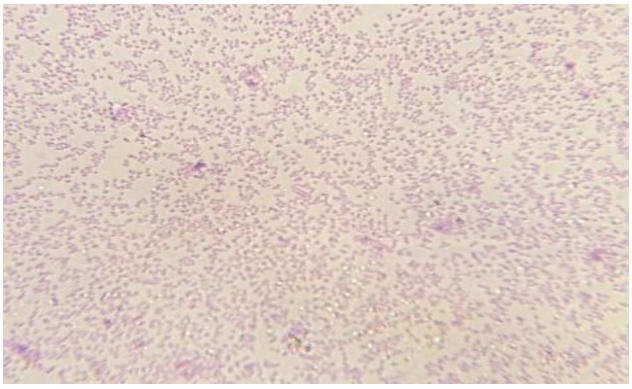

Figure 2: Actinobacillus; Gram negative, rod-shaped bacteria

Treatment and outcome

Following all aseptic techniques, the swellings were incised and the pus from the swellings was removed and the pouches were flushed and packed with gauze that was socked with 2% tincture iodine. The flushing and packing with socked gauze continued for seven days with systemic injection of Pen-strip (Intrchemie veterinary drug manufacture in Netherands) 10mg/kg, IM, for five days and the owner advised to make all feed stuffs free from foreign bodies; separate the sick animal from the herd and keeping the hygiene of the farm (any surfaces that come into contact with an affected animal should be disinfected) . He also advised to feed the diseased cow a soften feed. There was no pus from the pouches after the end of treatment. After one month, when the cow was visited the swelling regressed in noticeable way and it was changed to fibrous tissue as indicated on figure 3.

Figure 3: A cow after recovery from Actinobacillosis.

Discussion

Actinobacillosis is an infectious, chronic, bacterial and generally non-fatal disease. Even if the fatality rate of the disease is less it is a common disease of dairy cows that can cause severe clinical signs and economic losses. It is caused by bacteria called *Actinobacillus bovis,* a Gram-negative aerobic rod-shaped organism which is a normal inhabitant of the gastrointestinal tract and lower respiratory tract of ruminant and it can also be found in other organs, including the heart, kidneys, and reproductive organs when a disease condition is occurred. As indicated by many authors the classical form of this disease in cattle results in swelling and hardening of tongue which is often referred as wooden tongue (Lizano, S., Barrios, A. M., & Grant, I. R. 2012).

The clinical signs observed in this case; slowly growing firm swellings, discharging of pus are all similar findings with the clinical signs of the disease written on literatures Lizano, S., Barrios, A. M., & Grant, I. R. 2012, Caffarena *et al.*, 2018. In literatures, treatment of bovine Actinobacillosis includes oral or intravenous (IV) dosing of iodides and/or antibiotics such as ceftiofur, ampicillin, penicillin, florfenicol, and tetracyclines

but with variable results. And oral or IV dosing of iodides needs longer period of administration (John Maas, 2009). But in this case study treatment with penstrip (combination of penicillin and streptomycin) and local infiltration of the swellings with 2 % tincture iodine took only seven days. When this approach is compared to the conventional treatment approach in different literatures, this approach took lesser time. This might be because of high bioavailability of the drug when it is used in local administration than the systemic one. So, further case studies should have to be done on the benefits of use of local tincture iodine rather than systemic iodides.

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4.1.2. Case report on Blackleg in ox

Abstract

Blackleg is an infectious bacterial disease of cattle and, rarely, other ruminants. It is caused by *Clostridium chauvoei*, which is an anaerobic, gram-positive, motile, rod-shaped bacillus. Blackleg is one of the oldest known diseases affecting cattle and is an endemic disease in both developed and developing countries. This case report summarizes an incident of blackleg that occurred in a three-year-old ox that was brought to the Feseha Gebereab Memorial Veterinary Teaching Hospital of AAU-CVMA. The ox was brought with a chief complaint of loss of appetite and lameness. The physical examination revealed that the ox was febrile, with a rectal body temperature of 41.0 °C and increased respiratory and heart rates of 56 breaths per minute and 100 beats per minute, respectively. On the clinical examination, swelling on the left hind limb and subcutaneous emphysema were observed over the shoulders and hind quarters of the ox. Gram staining of the smear indicated gram-positive rod bacteria. The case was treated successfully with Procaine penicillin G at a dosage of 20,000 IU and dexamethasone at a dose of 0.2 mg/kg, IM. Although early intervention with an appropriate drug could cure the animal, the better option to control the disease would be to get the animals vaccinated against blackleg.

Keywords: *Blackleg, anaerobic bacteria, Ox and Procaine penicillin*.

Introduction

A bacterial infection that affects mostly cattle and infrequently other ruminants causes blackleg. This disease is brought on by *Clostridium chauvoei*, a gram-positive, anaerobic, motile, rod-shaped bacillus bacterium that survives in soil as hardy spores. Blackleg is an acute or subacute condition, although it can also be persistent. Animals between the ages of 6 and 24 months are particularly susceptible, and neither vaccined nor well-nourished animals are immune to the disease (N, Gedefa & Gedefa, 2021).

One of the earliest known cattle diseases, blackleg is endemic in both developed and developing nations and is a well-known source of loss for cattle farmers financially. The majority of blackleg cases take place in the warm months, following soil excavation, or during very heavy annual rains that can reveal and activate dormant spores (Ziech *et al.*, 2018).

Infected pastures contain *C. chauvoei*, which when consumed undergo one or more cycles of reproduction in the colon before being absorbed through the intestinal mucosa and/or being expelled in feces. The spores are transported to several tissues after absorption, including skeletal and heart muscle. Although lymphatics might potentially contribute to spore dispersal, hematogenous transport is thought to account for the majority of it. Once inside the muscle, *C. chauvoei* spores are phagocytized by local macrophages and can persist in their cytoplasm for extended periods of time without harming the host (Stratton, J. 2015). However, those dormant spores germinate, multiply, and release toxins that result in the clinical symptoms and lesions of blackleg when anaerobic circumstances are produced in regions where the spores are present, most frequently due to trauma and concomitant bleeding and necrosis (Tagesu *et al.*, 2019).

Blackleg is a disease that affects ruminant populations all over the world. It results in considerable losses in livestock output due to lesions that are predominantly characterized as myonecrosis and have a high mortality rate, usually in animals that are in good health and between the ages of 6 months and 2 years. Although the fast onset of

clinical indications that may include lethargy, unwillingness to move, recumbency, edema, and crepitus have been observed, it is believed that animals contract *C. chauvoei* from spores consumed from contaminated soil (Nampanya *et al.,* 2019).

Blackleg is often diagnosed based on the clinical history, symptoms, and gross and histologic alterations; the presence of *C. chauvoei* in the afflicted tissues is necessary for a definitive diagnosis. This can be done by immunodetection techniques like FAT and IHC, PCR, and/or culture. The availability of particular tests at nearby diagnostic laboratories frequently influences test selection decisions. Gas gangrene, also known as malignant edema, clostridial cellulitis, and occasionally even myositis, which are all linked to contaminated wounds, is the main differential diagnosis for blackleg (N, Gedefa & Gedefa, 2021).

If the animal is not morbid, treatment with penicillin and surgical debridement of the lesion, including fasciotomy, is advised. Due to the extensiveness of the lesions, recovery rates are low. Large doses (44,000 IU/kg BW) should be given, starting with intravenous administration of crystalline penicillin and then longer-acting medications. The fast course means that treatment for clostridial myositis is rarely effective. In addition to supportive care, vigorous surgical debridement to allow aeration and antimicrobials (drug of choice: procaine penicillin) around afflicted tissues may be beneficial (Tagesu *et al.,* 2019).

The technique of vaccination, which is a crucial part of the health management of many cattle-producing companies, can prevent blackleg. Annual or biannual boosters shots are advised. The neuraminidase of *C. chauvoei* is another promising candidate for vaccine manufacturing. This antigen is anticipated to have a strengthened protective effect against blackleg when combined with one or more polypeptides made from the toxins generated by *C. chauvoei* (Abreu *et al*., 2017). Thus, the present case report described Blackleg on ox.

Case description

An owner presented a two-year-old ox to Fisseha Gebreab Memorial Veterinary Teaching Hospital of AAU, CVMA, on October 10, 2022. The owner complained that the ox had lost its appetite for a day and that it was very difficult for the ox to walk. Upon physical examination, the ox was febrile with a rectal body temperature of 41.0 °C and had increased respiratory and heart rates of 56 breaths per minute and 100 beats per minute, respectively. There was swelling and subcutaneous emphysema on the shoulders and left hind quarter of the ox as indicated in Figure 4. Therefore, based on the history, clinical findings, and laboratory results, a black leg was established.

Figure 4: Clinical examination of the swelling area; Blackleg case.

Laboratory finding

The sample was collected from the swelling part and cultured on blood agar anaerobically in an anaerobic jar for 48 hours in an incubator at 370c, then sub-cultured on nutrient agar and incubated 370c for 24hrs then prepared for the smear. The prepared smear was stained with the Gram staining procedure (annex3) and a drop of immersion oil was added to the slide and observed under a 100X binocular microscope. Gram-positive and rod-shaped bacteria were observed, as indicated in the figure below. Therefore, based on the history, clinical findings, and laboratory findings, black leg was established as a definitive diagnosis.

Figure 5: *C. chauvoei**;*** Gram-positive and rod-shaped bacteria

Case management and treatment outcome

Procaine penicillin G (EPHARM, Addis Ababa, Ethiopia) was used to treat the bull for four straight days at a dosage 22,000 IU/kg BW. I added dexamethasone at a dose of 0.2 mg/kg on the second day despite the fact that the rectal body temperature was unchanged (40.5^0C) from the first day. However, rectal body temperature and heart rates returned to normal on the third day of treatment (39.0C and 72beats/min, respectively). The bull was completely recovered after therapy as indicated figure 6.

Figure 6: The status of the ox after four days treatment to Blackleg.

Discussion

Blackleg is an infectious bacterial disease of cattle and rarely of other ruminants. It is endemic in Ethiopia and cause overwhelming economic loss in small holder farmers which is associated with death, losses in production and reduction in working capacity of farm animals. The current case was asserted as black leg based on the history, clinical findings and laboratory result which is similar with marked lameness with pronounced swelling of the affected limbs, marked depression, and high pulse rate high (100-120/min) temperature (41^0C), emphysema and crepitation of affected heavy muscles as specified in literatures Ziech *et al.*, (Ziech *et al*., 2018).

The present case was treated successfully with procaine penicillin 22,000 IU/kg BW for four successive days coupled with dexamethasone (0.2mg/kg/day) to reduce pain and fever which is in agreement with Tagesu *et al*., (Tagesu *et al*., 2019). In the cattle-producing industries, black leg leads to significant losses in terms of lost output and animal mortality. Although early treatment with the right medication could cure the animal, vaccination against black leg would be a preferable alternative to control the disease.

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4.1.3. Foot-and-Mouth Disease in Ox

Abstract

Foot-and-Mouth Disease (FMD) is a highly contagious viral infection of cloven-hoofed animals such as cows, sheep, and pigs. It is caused by a virus belonging to the genus *Aphthovirus*. This case report provides a detailed clinical case report of FMD in ox. The case report includes the ox's clinical signs, diagnosis, treatment, and outcome of treatment. The ox was brought from Dire district with a complaint of lameness and reduced appetite and examined at Feseha Gebereab Memorial Veterinary Teaching Hospital of AAU-CVMA. The ox was febrile, with a rectal body temperature of 40.90 °C and 64 beats per minute and 36 breaths per minute heart and respiratory rates, respectively. There were lesions on the tongue, some parts of the mouth, and the hooves. Additionally, lameness, swelling of both prescapular and pre-femoral lymph nodes, and excessive salivation were appreciated. Foot-and-mouth disease (FMD) was suspected based on the history of an outbreak in the area and typical clinical features. The ox was treated with both antibiotics and anti-inflammatory drugs. Additionally, I have flushed the wound in the mouth using ascetic acid. Two weeks after treatment, the animal returns to normal physiology, but the sloughed area doesn't take this long to heal. Although foot-and-mouth disease (FMD) has low mortality rates, it spreads quickly, resulting in severe economic consequences. Hence, priority must be given to preventing the disease through vaccination.

Keywords*: Antibiotics, Foot-and-Mouth Disease and Ox*

Introduction

Foot and Mouth Disease (FMD) is a highly contagious viral disease that affects cloven-hoofed animals, such as cattle, pigs, sheep, goats, and deer,). The FMD virus, which is which belongs to the family Picornaviridae family and has seven unique serotypes (O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3), is the culprit behind the disease (Tolawak & Pal, 2022). The FMD virus is renowned for both its rapid animal population proliferation and its environmental endurance. FMD outbreaks can significantly damage the livestock industry's economy and society, as well as the world's trade (Grubman and Baxt, 2004).
According to Maree *et al*., (2014), FMD in cattle is distinguished by fever, vesicular lesions on the mouth, tongue, feet, and teats, decreased milk production, and weight loss. Direct contact with sick animals or their secretions can spread the disease, as can contact with contaminated equipment, clothing, and other objects. It is challenging to prevent the spread of the diseases since the virus can persist in the environment for several days (Aftosa, 2021).

Due to decreased milk production, weight growth, and meat quality brought on by FMD outbreaks in cattle, can result in significant economic losses (Knight-Jones and Rushton, 2013). Additionally, communities that depend on cattle production for their way of life may experience societal consequences as a result of the disease (Knight-Jones *et al*., 2016).
Clinical symptoms, such as the presence of vesicular lesions on the mouth, tongue, feet, and teats, are the primary criteria used to diagnose FMD in cattle. However, a number of different diseases can exhibit comparable clinical symptoms, so a laboratory diagnosis is necessary to identify the disease. Serology, antigen detection, and viral isolation are all parts of laboratory diagnostics. Viral isolation is the gold standard for diagnosing FMD, but because of the virus's meticulous nature and biosecurity issues, it can be difficult. To control secondary bacterial infections, treatment combines supportive care, such as fluids, with the administration of antibiotics (Maree *et al*., 2014).

In order to prevent and control FMD in cattle and other susceptible animals, vaccination and strict biosecurity measures are primarily used (Grubman and Baxt, 2004). For each of the seven FMD virus serotypes, there is a vaccine; however, depending on the viral strain and the time of vaccination, each vaccine's efficacy differs. As part of biosecurity procedures, contaminated animals are quarantined, contaminated equipment and trucks are cleaned and contaminated persons and animals are banned from entering and leaving impacted areas (Tadesse *et al*., 2020). Therefore this case report describes about FMD in Ox and its treatment option. And finally suggest the best way to prevent and control the disease at the farm level.

Case description

On October 28, 2022, a local breed ox was examined at the Feseha Gebereab Memorial Veterinary Teaching Hospital of AAU-CVMA from Dire district due to a complaint of lameness. The complaints also noted a decrease in feeding intake. The animals were not vaccinated and were kept in common grazing areas with other herds. Upon physical examination, the ox was febrile, with a rectal body temperature of 40.9 OC and 64 beats per minute and 36 breaths per minute heart and respiratory rates, respectively. There was a lesion on the tongue and some parts of the mouth; as indicated on figure 7, there was lameness and swelling of both the prescapular and pre-femoral lymph nodes. The symptomatic diagnosis was established as foot-and-mouth disease (FMD) based on the history, clinical findings, and eruption of similar cases in other areas.

Figure 7: Lesion of FMD infected tongue indicated by the arrow

Treatment and outcome

The treatment of the ox was aimed at reducing secondary bacterial complication to the animal. Hence, 10% Oxytetracycline (Shanghai Thongren Pharmaceutical Co., Ltd, China) at 10mg/kg/day for five successive days and dexamethasone at a dose of 0.2mg/kg for three consecutive days were administered. The lesion was also washed with acetic acid. The owner was also advised to segregate and nurse the animal at home until fully recovered. The ox recovered and was able to return to its normal activities within two weeks as indicated on figure. But the lesions had healed within a week. This might be because of the local effect of ascetic acid.

Figure 8: FMD affected ox after recovery

Discussion

Foot-and-Mouth Disease (FMD) is a highly contagious viral disease that affects cloven-hooved animals, such as cattle, sheep, goats, pigs, and deer. FMD is highly contagious and can spread quickly between animals. The disease has the potential to cause significant economic losses due to reduced productivity and the cost of controlling outbreaks (Grubman and Baxt, 2004). In this paper, we will discuss the clinical signs, diagnosis, treatment, and outcome of treatment of FMD in accordance with other published papers.

The diagnosis of FMD in cattle is primarily based on clinical signs, such as the presence of vesicular lesions on the mouth, tongue, feet, and teats. However, several other diseases can present with similar clinical signs, making laboratory diagnosis essential to confirm the disease. Laboratory diagnosis includes virus isolation, antigen detection, and serology. The gold standard for FMD diagnosis is virus isolation, although it can be challenging due to the virus's fastidious nature and biosecurity concerns (Maree *et al*., 2014). The clinical signs and diagnosis of the current case report which was observed to have a lesion on tongue, some parts of mouth and hooves besides, there was lameness, swelling of both prescapular and prefemoral lymph nodes and excessive salivation. The symptomatic diagnosis was established as Foot-and-mouth disease (FMD) based on the history, clinical findings in agreement with the published report on Maree *et al*., (Maree *et al*., 2014).

Treatment of FMD is largely supportive and may include antibiotics to prevent secondary bacterial infections, fluid therapy, and anti-inflammatory medications. Vaccination may also be used to prevent or reduce the severity of disease. The outcome of treatment of FMD is largely dependent on the severity of the disease and the timely diagnosis and treatment The control of FMD in cattle and other susceptible animals relies on vaccination and strict biosecurity measures include quarantine of infected animals, disinfection of equipment and vehicles, and restricting the movement of animals and people in and out of affected areas (Grubman and Baxt, 2004). The ox was treated

systematically with antibiotic (10% Oxytetracycline) which was in agreement with (Ding & Kinnucan, 2011)

In conclusion, FMD is a highly contagious viral disease that affects cloven-hooved animals. Clinical signs may include fever, lethargy, and drooling, and diagnostic testing may be necessary for confirmation. Treatment of FMD is largely supportive and may include antibiotics, and anti-inflammatory medications. The outcome of treatment is largely dependent on the severity of the disease and the timely diagnosis and treatment. Vaccination may reduce the severity of the disease and decrease the risk of mortality. Finally, the involvement of stakeholders, including farmers, veterinarians, and policymakers, is crucial in implementing biosecurity measures to prevent the spread of the disease (Knight-Jones *et al*., 2016).

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4.1.4. Colibacillosis in Calf

Abstract

Colibacillosis is a diarrheal disease caused by a gram-negative bacterium called *E. coli*. Calf diarrhea is a frequent cause of pre-weaning morbidity and mortality. One of the most serious clinical disorders in newborns is diarrhea in the first few months of life. A report was received from one of the dairy farm in Bishotu, Ethiopia, on January 5, 2023. The 5-day-old calf was reared in an intensive farming system and had diarrhea for the previous 3 days. The calf was strained when defecating, and occasionally blood is visible in its feces. On physical examination, the calf showed signs of diarrhea, overall weakness, dehydration, and a rectal body temperature of 39 °C with 36 breaths per minute and 64 beats per minute respiratory and heart rates, respectively. Bacteriological culture of fresh fecal samples was done, and the isolation has typical characteristics of E. coli. Additionally, parasitological examination for Eimeria oocytes was done, and the result was negative. Therefore, based on the age of the animal, the clinical findings, and the laboratory results, the case was diagnosed as colibacillosis. Hence, Trimethoprim Sulfadiazine 30mg/kg was administered intramuscularly (IM) for 5 days. The calf was successfully recovered at the end of the treatment course. The disease can be prevented with proper farm hygiene and controlled by early diagnosis, prompt treatment, and the provision of adequate colostrum at the right time.

Keywords: *Calf, Colibacillosis, E.coli and Trimethoprim Sulfadiazine*

Introduction

Colibacillosis is a diarrheal disease caused by the Gram-negative bacteria, *E.coli*. In cattle operations, calf diarrhea is a frequent cause of pre-weaning morbidity and mortality. One of the most serious clinical disorders in newborns is diarrhea in the first few months of life. In young calves, diarrhea is a syndrome with a complex etiology that results in mortality and indirect economic losses due to poor growth (Ngeleka *et al.*, 2019). Despite the fact that there are various enteropathogens that can cause neonatal calf diarrhea, E. coli, Rotavirus, Corona virus, Clostridium perfringens, Salmonella, and Cryptosporidium are the most widespread disease in most places. The most frequent primary causes of calf diarrhea and septicemia are E. coli strains (Gebregiorgis & Tessema, 2016b).

Diarrhea due to E. coli is one of the most common diseases of newborn calves in affected calves, diarrhea typically begins within 36–72 hours of birth, and affected calves die within 2-3 days. Some calves die several hours after appearing healthy and free of diarrhea The acute form is characterized by progressive dehydration and death, sometimes in as few as 12hrs. In the sub-acute form, diarrhea may persist for several days and characterized by a rise in temperature, weakness and lack of appetite which results in malnutrition and emaciation (Tarekegn, 2017).

Diarrheagenic *E. coli* can also result in extra-intestinal infections in both humans and animals of almost all species of warm-blooded vertebraes. All of these microorganisms cause the host they infect to excrete E. coli, which aids in the infection's ability to spread to more hosts. (Starr, 2014). The diagnosis is depended on an accurate history, clinical signs, and the isolation and characterization of the E. coli. Strong antibiotics, intravenous fluids (IVF), and non-steroidal anti-inflammatory medicines (NSAID) are required for the treatment of colibacillosis (Habte, 2022).

Enteric colibacillosis has been successfully treated with parenteral antibiotics such oxytetracycline and sulfachlorpyridazine. Amoxicillin with clavulanic acid is likely to be

the antibiotic of choice, along with rehydration and NSAIDs, as the majority of pathogenic E. Coli have shown resistance to ampicillin. It has been demonstrated that using strict sanitary procedures to maintain a clean environment, providing enough colostrum, and immunizing calves are effective ways to prevent and control bovine Colibacillosis.(Jesse Abdullah, 2016). Thus, the present case report describes the diagnosis of colibacillosis in a calf.

Case description

A report was received from one of the dairy farm in Bishotu, Ethiopia, on January 5, 2023. The owner mentioned that the 5-day-old calf was reared in an intensive farming system and had diarrhea for the last 3 days. The calf strains during defecation, and sometimes blood is seen with its feces. The calf loses interest in taking milk. During my farm visit, the farm hygiene was not good. On physical examination there was diarrhea, weakness, dehydrated and depression as indicated on figure 9.The rectal body temperature of the calf was 39.8 oC, 36 breaths per minute, and the respiratory rate and heart rate were 64 beats per minute. The hair coat was rough and the conjunctiva was pale. The feces, taken for a sample, had little blood stains.

Figure 9: Photograph showing the presentation of the Calf affected by colibacillosis on first day.

Laboratory findings

A fecal sample was collected from the calf and the sample was aseptically collected in a sterile stool pot and sent to microbiology and parasitology laboratory of Addis Ababa University College of veterinary medicine and agriculture. The fecal sample was checked for Eimera in the parasitology laboratory and it was negative during floatation technique. Sample was enriched with Tryptone soya broth for 24 hours in microbiology laboratory. For the isolation and identification of *E. coli* the sample was cultured on MacConkey (MC) agar medium as indicated in figure10 (A) and Eosin Methylene blue (B) agar medium which was used as a selective medium for *E.coli* and metallic sheath characteristics of *E.coli* was observed on Eosin Methyelene blue (EMB) as indicated below in figure10. Based on the history obtained, clinical signs observed and laboratory finding the case was finally diagnosed as colibacillosis.

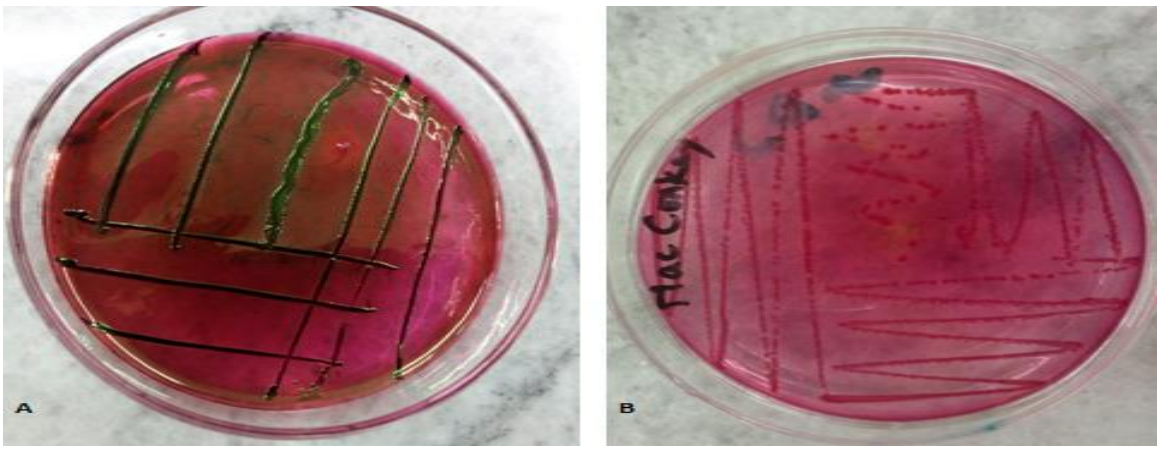

Figure 10: Photograph showing *E coli* on EMB agar (A) and on MacConkey agar (B)

Case management and its treatment outcome in calf

As a treatment antibiotic Trimethoprim Sulfadiazine 30mg/kg (Hebei Yuanheng Pharmaceutical Co., Ltd, China) administered intramuscularly (IM) for 5 days. The calf was constantly followed during the treatment for 5 days and responded well to the

treatments indicated on figure 11(A and B). The diarrhea subsided, and the calf recovered completely. The owner was advised to give new-borns enough colostrum right away.

Figure 11: Photograph showing the presentation of the Calf (A) and after recovery (B) from colibacillosis

Discussion

Colibacillosis, an infection with *Escherichia coli (E. coli)* is a major cause of mortality among young calves. In this case report, based on history, clinical sign and laboratory findings the case was finally diagnosed as colibacillosis which is in line with the case reported by Jesse Abdullah, (Jesse Abdullah, 2016). Bacteria, viral and protozoa are few pathogens those may cause calf scour (Tedla & Degefa, 2017) and the current report agreed with these principle as the cause was bacteria pathogen. In this case report, the isolated pathogen was bacteria specifically *E. coli* which caused colibacillosis in a calf. The primary therapeutic plan for animals with collibacillosis is usually vigorous antimicrobial therapy to reduce morbidity and mortality (Wininger *et al.*, 2020).

In the present case report, sulfadiazine was used as the antibiotic of choice and the calf responded well to the treatment which is in agreement with the treatment given by Jesse *et al*.,. (2016). The disease can be prevented with proper farm hygiene and can be control by early diagnosis, prompt treatment and give enough colostrum right away for new born calf to prevent great economic losses in the ruminant industry.

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4.1.5 Haemonchosis in calf

Abstract

Haemonchosis is a disease of the gastrointestinal tract of calves caused by the parasite *Haemonchus contortus*, commonly known as the barber pole worm. This paper is a case report on a calf with haemonchosis. The calf was a one year-old female calf from a dairy farm with a history of anorexia, swelling under the neck before two-day weight loss, and dark, firm feces voiding before one day, and the calf had not been treated before. Upon physical examination, the calf was found to be weak, dehydrated, capillary refill time was around 10 seconds, depressed, pale conjunctiva mucous membrane, and swelling under the neck. The temperature of the calf was 37.5 °C, with a heart rate of 96 beats per minute and a respiratory rate of 22 breaths per minute. The simple floatation parasitological technique revealed eggs of Haemonchus. The calf was treated with ivermactin (200 g/kg) and multivitamin (1 ml/10 kg) as supportive therapy. Treatment was successful in eliminating the parasites, and the calf's condition improved significantly. The calf was monitored closely for the next two weeks, and her condition improved significantly with no further signs of hemorrhage. This case report demonstrates the importance of an accurate diagnosis and appropriate treatment for haemonchosis in cattle. Early diagnosis and treatment can help reduce the risk of complications and improve the animal's outcome. This case also highlights the importance of closely monitoring animals for signs of improvement after treatment.

Keywords*:* *Calf, Ellipsed eggs, Floatation technique, Haemonchosis, Ivermactin and Multivitamin*

Introduction

Haemonchosis, which affects the digestive tract of calves, is brought on by the parasite *Haemonchus contortus*, also known as the barber pole worm. It can result in significant financial losses for producers and is a significant cause of morbidity and mortality in young calves. The disease is most usually found in areas with warm, humid climates and spreads through contact with sick animals or contaminated pastures. *Haemonchus contortus* is the primary parasite that causes haemonchosis in calves, while other nematode species may potentially be present (Arsenopoulos *et al*., 2021). When a female infected with H. contortus produces eggs in a calf's gut, the life cycle of the parasite begins. The larvae from these eggs develop into adult worms in the abomasum after hatching into larvae and traveling through the animal's body till that location. These adult worms subsequently lay eggs, which the animal excretes in its feces and which can contaminate the surrounding area. The cycle is then completed when a different calf consumes the eggs (Abdo *et al*., 2017).

Depending on the severity of the infection, haemonchosis in calves manifests differently clinically. Anorexia, weight loss, and pale mucous membranes may be symptoms in mild cases. In more severe cases, anemia, jaundice, diarrhea, and even death are possible outcomes. Haemonchosis is normally diagnosed based on the clinical symptoms, medical history, and physical examination of the animal. H. contortus can be found on fecal samples using laboratory techniques as well (Afolayan & Olukosi, 2013).

The use of anthelmintic medications, such as ivermectin or fenbendazole, is necessary for the treatment of haemonchosis in calves. Usually, these medications are taken orally or subcutaneously. In order to aid the animal's recovery from the infection, additional supportive care practices including hydration treatment and nutritional support may be required (Souza, A. P. *et al*.,. 2016). This paper presents a clinical case report of a calf suffering from haemonchosis and discusses the clinical signs, diagnosis, treatment, and outcome of the disease.

Case description

There was a phone call for veterinary service from one of the dairy farm in Bishoftu on March 27, 2023, with a history of anorexia, swelling under the neck before two days, weight loss, and dark, firm feces voiding before one day. The calf had not been treated before, as the farm attendant complained. The owner also told me that his flock feeds both in the pasture and intensively. On clinical examination, the calf was found to be weak, dehydrated, capillary refill time was around 10 seconds, depressed, pale conjunctiva mucous membrane as indicated in figure 12B, and swelling under the neck as indicated in figure 12A. The parameter of the calf was 37.5 0C, heart rate 96 beat/min, respiratory rate 22 breath/min. Based on the clinical findings and history obtained, the case was tentatively diagnosed as haemonchosis, and its differential diagnosis is fasciolosis.

Figure 12: physical examination of haemonchosis affected sheep

Laboratory findings

Fresh Fecal sample was taken from the rectum of the calf and taken to veterinary parasitology laboratory of Addis Ababa University. Simple Floatation technique and sedimentation technique were performed. No egg was observed under 40xmicroscope in sedimentation but was in flotation, technique an ellipsed eggs of haemonchus was seen under microscope; as indicated figure as follow. Based on the history, clinical signs observed, and laboratory finding the case was finally diagnosed as Haemonchosis.

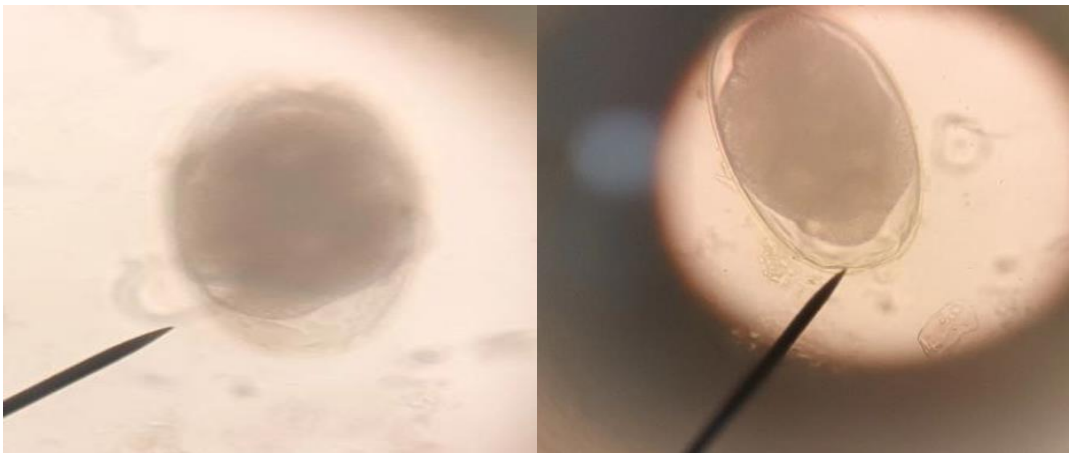

Figure 13: Ellipted eggs of haemonchus

Treatment and outcome of the case

The current case was treated with ivermectin 1ml/50kg and multi-vitamin 1ml/10kg as a supportive therapy since the calf was suffered from in-appetence and the owner also recommended deworming their animals. After two weeks of treatment, the calf had fully recovered as indicated on figure below.

Figure 14: Hemonchosis affected calf after recovery

Discussion

Haemonchosis is a common parasitic disease in cattle and can cause significant morbidity and mortality if left untreated (Amero, K. *et al*.,. 2015). The present cases of calf were diagnosed as Haemonchosis based on the clinical picture like bottle jaw and dark colour firm feces. The clinical signs of haemonchosis in calves depend on the severity of the infection. Mild infections may not show any signs, while more severe infections can cause anemia, depression, decreased appetite, weight loss, and bottle jaw, which is an accumulation of fluid around the lower jaw as indicated by Koehler, P. *et al*.,. (Koehler, P. *et al*.,. 2013). The diagnosis of haemonchosis in calves can be made based on clinical signs, as well as laboratory findings. The most common laboratory tests used to diagnose haemonchosis are fecal egg counts, packed cell volume (PCV) analysis, and blood smear analysis (Al-Deeb, M. *et al*.,. (2015). Similarly, the animals in the present study a clinical sign were anemia, depression, decreased appetite, weight loss, and bottle jaw and the diagnosis were based on clinical signs and laboratory findings.

Treatment of haemonchosis consists of anthelmintic drugs and supportive care (Näslund, J. 2017). In this case, a combination of ivermectin and mulitivitamin was used to successfully to treat haemonchosis in agreement with the report published on Näslund, J. (2017) and Amero, K. *et al*.,. 2015). In general, this case report demonstrates the

importance of an accurate diagnosis and appropriate treatment for haemonchosis in cattle. Early diagnosis and treatment can help reduce the risk of complications and improve the outcome for the animal. This case also highlights the importance of closely monitoring animals for signs of improvement after treatment.

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4.1.6 Sub clinical Mastitis

Abstract

Sub-clinical mastitis (SCM) remains an economically important condition for the dairy industry. SCM is one of the major causes of poor growth in young animals, reduced milk quantity, and undesirable changes in the milk's composition. This case report describes the clinical signs, diagnosis, treatment, and outcome of a case of two cows with reduced milk production. There was no gross change in milk quality, but the two cows were given on average three litters less per day for eight consecutive days. Up on clinical examination, the body temperature of the cows was in the normal. A general examination of the udder was done, but there were no abnormal swelling or inflammatory signs on the udders of all the two cows. The California Mastitis Test (CMT) was performed on all the quarters of each cow, but the result was positive on the left hind quarter of one cow. A milk sample from the affected quarter was taken with a sterile screw-top bottle to isolate the causative agent. The characteristics of the bacterial colony on blood agar, the characteristics of the Gram-stained bacteria, and again, the growth on selective Mannitol Salt agar confirmed the causative agent as *Staphylococcus aureus.* Pen-strip in a dose of 10 mg/kg was given for five consecutive days. Following the treatment of a previously CMT-positive cow, the test was repeated, and the results were negative. Milk production resumed following the successful treatment. It is often difficult to diagnose subclinical mastitis due to the lack of visible clinical signs, but it can be detected with a CMT. Treatment of *S. aureus*-caused SCM is not usually successful, but it was in this scenario. Finally, the application of in-farm mastitis prevention protocols was recommended.

Keywords: *California Mastitis Test, Cow, Staphylococcus aureus, Sub-clinical mastitis*

Introduction

Mastitis, which is defined as the inflammation of the mammary gland, is a frequent and economically significant disease affecting dairy cows worldwide (Radostits *et al*., 2007). The problem is caused by bacterial infection, which can arise as a result of environmental contamination or as a result of the cow's own immune response to the milking process (Hogeveen *et al*., 2011). Mastitis has a significant financial impact on milk producers because it reduces milk yield, lowers milk quality, increases culling rates, and increases veterinary and treatment costs (Barkema *et al*., 2015). For the dairy business, sub-clinical mastitis (SCM) continues to be a financially significant condition. SCM is one of the main causes of animal suffering since it results in decreased milk quality, poor growth in young animals, poor product hygiene, and unwanted changes in the composition of the milk (Shittu *et al*., 2012).

Depending on the area and type of farm, the documented incidence rates of mastitis range from 10% to 60%, according to Hogeveen *et al*.,. (2011). Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli are the most common causes of mastitis, according to Halasa *et al*., 2007. Mastitis-causing microbes can spread in a variety of ways, including through contaminated milking equipment, damaged teats, and poor hygiene practices (Hogeveen *et al*., 2011).

Clinical signs of mastitis in cows can vary depending on the severity of the infection. Generally, cows may show signs of inflammation in the affected mammary gland, including swelling and firmness. Other signs may include heat, redness, pain, and/or discharge. In some cases, cows may also show signs of systemic illness, such as fever, depression, and loss of appetite (Mastitis in cows is typically diagnosed based on a combination of physical examination, laboratory tests, and imaging studies. During the physical examination, the veterinarian will look for signs of inflammation and palpate the affected mammary gland to check for any firmness. Laboratory tests can be used to identify the cause of the infection, such as bacteria, viruses, or fungi. Imaging studies, such as ultrasound or radiography, may also be used to check for any abscesses or other

changes in the mammary gland (Cheng & Han, 2020). But sub clinical mastitis is has no clinical sign diagnosis only the history of decreased milk quality, poor growth in young animals, poor product hygiene, and unwanted changes in the composition of the milk and Laboratory tests (CMT test).

The goal of treatment for mastitis in cows is to reduce inflammation, eliminate the underlying cause of the infection, and prevent any further spread of the infection. Treatment typically involves antibiotics, anti-inflammatory drugs, and supportive care. Antibiotics can be used to eliminate the underlying cause of the infection and prevent any further spread of the infection. Anti-inflammatory drugs, such as non-steroidal anti-inflammatory drugs (NSAIDs), can be used to reduce inflammation. Supportive care may include fluids, electrolyte replacement, and nutritional support (Nguyen, T. T., & Nguyen, T. L. 2018).

Prevention and control of mastitis in dairy farms is based on a multifaceted approach, which includes milking management, hygiene, and herd health practices. Proper milking techniques, such as the use of pre-milking teat disinfection and post-milking teat dipping, can significantly reduce the risk of mastitis transmission (Hogeveen *et al*., 2011).

Case description

The dairy farm owner brought a cow to the Feseha Gebreab Memorial Teaching Hospital at CVMA, AAU, on December 5, 2022, in Bishoftu, Ethiopia. The owner of the farm mentioned that he has 16 cows kept in an intensive farming system, and among them, two cows have reduced milk production. There was no gross change in the milk quality. Two cows averaged three fewer litters per day over the course of eight days. Clinical examinations showed that the cows' body temperatures were normal. (38 ^{o}C and 38.5 ^{o}C).A general examination of the udder was done, but there were no abnormal swelling or inflammatory signs on the udders of all the two cows, as indicated in the figure below.

Figure 15: Mastitis affected cow during physical examination

Sample collection and laboratory finding

California Mastitis Test (CMT) was done for each quarter of individual cows where a four-well white plastic paddle was used; one well was used for each quarter of the cow to be tested. The foremilk was discarded, and then a little milk was drawn into each well. An equal volume of CMT test reagent was added and then the sample was slowly agitated (procedure as described by Radostitis *et al*., 2006). One quarter of the udder of only one cow showed positive reaction but the three quarters of this cow and all the four quarters of the one cow were negative on the test. The infected quarter did not have any gross abnormality; have equal size with other quarters and no fibrosis felt on palpation.

Figure 16: Positive CMT test result of hind left quarter

For isolation of the causative agent before treatment about 5 ml of milk was collected in a screwed bottle from the quarter that was positive on CMT and it was transported with cold chain to microbiology laboratory of college of Veterinary medicine, Bishoftu. The samples were kept at 4°C during transport to the laboratory and processed within 4 h. For analysis, the milk samples were incubated at 37°C for 30 min to detach bacteria from fat. The sample was vortexed for 5 min to achieve a homogeneous mixture. The samples were inoculated on blood agar plates and incubated under aerobic conditions at 37°C for 24 hrs. Colonies were golden in colour. Were observed on the blood agar. To identify the bacteria Gram staining was done.

For Gram staining; a drop of sterile water was placed on a clean labelled microscopic slide with a sterile cooled loop. The loop was again sterilized over Bunsen burner flame and allowed to cool. With the cooled loop a very small sample of a bacterial colony was picked up and gently stirred into the drop of water/saline on the slide to create an emulsion. The slide was allowed to air dry, and then fixed by passing it through Bunsen burner flame quickly 3 times. The sample was stained with Gram staining as the recommended protocol(Annex3).

A drop of oil immersion was added on the stained slide and it was observed under 100X objective lens of a binocular microscope. Gram positive bunch of Cocci were observed under the microscope as indicated on picture Staphylococcus species were suspected.
For further confirmation suspected colonies were sub-cultured on Mannitol salt agar plate and incubated aerobically for 24 hours. The colonies grew on Mannitol salt agar; yellow colonies with yellow zones. Colonies characteristics on Blood agar, characteristics of the bacteria on Gram staining and growth of the colonies on Mannitol salt confirmed the causative agent as Staphylococcus species. Based on clinical sign History and laboratory result the case was diagnosis as sub clinical mastitis caused by Staphylococcus aureus.

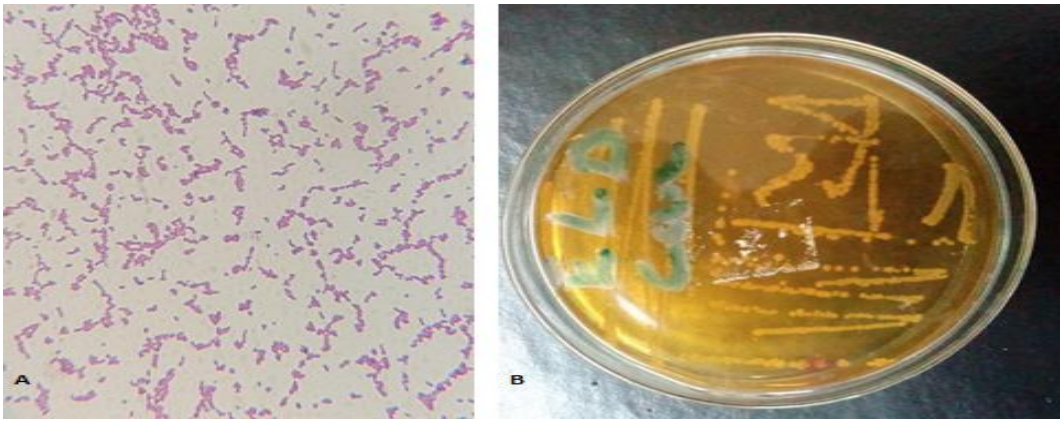

Figure 17: Laboratory result of sub-clinical mastitis affected cow: (A); Gram positive bunch of Cocci bacteria (B); The colonies grew on Mannitol salt agar

Treatment and outcome

The cow, that was positive for California mastitis test, was kept in isolated pen to reduce the risk of transmission of the case to other cows. The affected quarter was milked at the end. Pen-stripe 10mg/ kg body weight, through intramammary for five days was used. The outcome of treatment was positive, the infection can be eliminated and milk production was resuming. The cow, with mastitis was again rechecked with CMT test and the result was negative.

Figure 18: Negative CMT test result after treatment

Discussion

Mastitis is an inflammation of the mammary gland caused by bacterial infection. It is one of the most common diseases seen in dairy cows and can lead to significant economic losses for dairy farmers. Subclinical mastitis is an infection of the mammary gland without any clinical signs of the disease, and is the most common form of mastitis in cattle (Barkema *et al*., 2015). In this case report, we will discuss the clinical signs, diagnosis, treatment, and outcome of a case of subclinical mastitis in a dairy cow.

The pathogenesis of mastitis is influenced by a range of factors, including the cow's immune response, the milking process, and environmental conditions. Mastitis transmission can occur through various routes, such as contaminated milking equipment, teat injury, and poor hygiene practices. Subclinical mastitis can often be difficult to diagnose as there are no visible signs of the disease. The cow may continue to produce milk normally and show no signs of distress. However, the milk may contain abnormal amounts of white blood cells or bacteria (Hogeveen *et al*., 2011). The clinical sign of current case report was only reduced milk production in agreement with (Hogeveen *et al*., 2011).

The most common method of diagnosing subclinical mastitis is through a California Mastitis Test (CMT) or by examining a milk sample under a microscope. If the CMT is positive, further testing may be necessary to determine the cause of the infection. The diagnosis of current case was in line with the report published by Narváez-Semanate *et al*., (Narváez-Semanate *et al*., 2022).

Once subclinical mastitis has been diagnosed, it is important to treat the infection as soon as possible. The most common treatment for subclinical mastitis is the use of antibiotics. Antibiotics are usually given orally or intramammary. The cow affected quarter was milked at the end and treated with Pinstripe 10mg/ kg body weight, through intramammary for five days was used in agreement with the report that published in Cheng & Han, 2020 (Cheng & Han, 2020).

In Conclusion subclinical mastitis is a common infection of the mammary gland in dairy cows. It is often difficult to diagnose due to the lack of visible clinical signs, but can be detected with a CMT or by examining a milk sample under a microscope. Even if treatment of *S. aureus*-caused SCM is usually unsuccessful; in the current case the treatment shows a good result. And the owner also advised to use in-farm mastitis prevention protocols.

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4.1.7 *Parafilaria bovicola infestation in cow*

Abstract

Parafilariasis is a vector-borne parasitic infection of cattle and buffalo that result in painful and irritating cutaneous nodules worldwide. On October 25, 2022, a cow with a history of local bleeding was brought to Fisseha Gebreab Memorial Veterinary Teaching Hospital AAU, CVMA. The owner reported that he had tried cleansing the bleeding spots with salt and water, but the bleeding relapsed again after a few days of disappearing. Antibiotics were given, but they could not help it. On clinical examination, the body parameters were in the normal range. Giemsa staining of the blood from the bleeding points indicated eosinophilia. Sedimentation techniques on the sample from the bleeding points revealed larveted parafilaria parasite eggs. The case was treated with three times shots of ivermectin subcutaneously with an interval of 14 days; a one-time pour-on onto the lesion was done; and the focal lesions were washed with iodine. After two months, the focal bleeding stopped and the sites healed. This case report shows the presence of *Parafilaria bovicola* in Bishoftu and its treatment protocol. It remains to be determined whether the case sporadic or whether the parasite is becoming endemic in Bishoftu. Evolution towards an endemic situation needs to be investigated.

Keywords: *Cow, larvated egg, Ivermectine and Parafilaria*

Introduction

Parafilariasis, a parasite disease of cattle and buffalo, is transmitted by vectors and results in painful, itchy nodules on the skin. On the shoulder, neck, withers, groin, thoracic region, and ribs of the upper body, adult worms in the subcutaneous connective tissue produce lesions that resemble bruises. Inflammatory lesions and haemorrhagic nodules up to the size of little nuts are also caused by them (Alemayehu *et al*., 2013). The disease has long been known to have an impact on cow husbandry in Africa, Asia, and South America, claim Losson and Saegerman (2009). There have been reported examples of the syndrome in a number of European countries, including France, Romania, Bulgaria, Sweden, Ireland, and more recently the Netherlands (Pardon *et al*., 2010), Germany, Italy, and Belgium (Oehm *et al*., 2019a). There is one study in Ethiopia, and it found a prevalence of 20.42% overall in the Raya-Kobo district in the northeast (Alemayehu *et al*., 2013).

The only visible symptoms of infection in cattle are isolated cutaneous hemorrhages (bleeding patches), which may leak for many hours before clotting and drying in the matted hair of the coat. The incubation period is between 7 and 10 months. The female worm, which forms a minute nodule, pierces the skin, and oviposits in the blood trickling from the central lesion, is what produces bleeding patches. The parasite's microfilariae first larval stage is present in the small eggs (OIE, 2020).

Adult *P. bovicola* ovoviviparous females are found in these nodules, and they burst through the skin to discharge eggs and microfilariae (L1 larvae) into the serosanguinous fluid that is oozing from the location. After a lengthy prepatency period of seven to ten months, the L1 larvae are consumed by Musca spp. and grow into infectious L3 larvae that are transmitted to cattle and cause cutaneous bleedings (Hund *et al*., 2021). Infection happens when the vector, acting as the intermediate host, feeds once more on cattle. In these circumstances, the L3 larva can penetrate the dermis and travel to many parts of the body, including the subcutaneous and intermuscular tissue of the neck, rump, and other

areas. Female nematodes laid eggs that can be found in exudates or blood after developing adults from L3 larvae (Oehm *et al*., 2019b).

The seasonal bleeding spots are sometimes confused with those caused by thorns, wire, ticks, or biting insects. Fresh or dried blood should be mixed with water in a test tube and centrifuged for distinction. When the sediment is examined under a microscope, the distinctive eggs are discovered. The presence of many eosinophils in Giemsa-stained impression smears taken from carcass lesions allows one to distinguish them from bruising. Additionally, the damaged tissue emits a distinct, repulsive metallic odor.

Typically, just a few worms are found in small numbers in affected carcass that have been impacted, and they can be tricky to locate due to their color and the inflammatory reaction that goes along with them. To help parasites recuperate, affected tissues can be incubated in warm saline to facilitate the recovery of parasites. An ELISA for the detection of antibodies against *P. bovicola* is available (Veterinary Manual, 2018f). Injection of ivermectin group, subcutaneously with 0.2 mg/kg moxidectin has shown good effect in the treatment of Parafilariosis (Deprez *et al*., 2010). The current case report describes successful treatment of bovine Parafilariosis using ivermectin in cattle.

Case description

On October 25, 2022, a cow with a history of local bleedings brought to Feseha Gebereab Memorial Veterinary Teaching Hospital of AAU-CVMA. The owner told that he had tried cleansing the bleeding spots with salt and water but the bleeding relapsed again after a few days of break. On clinical examination the Cow's body condition score was normal, and apart from skin lesions, a clinical examination revealed no problems. The most prominent finding was the presence of multiple nodules around the bleeding site on the right side of shoulders as indicted on figures 19.

Figure 19: Parafilariosis case; cutaneous focal bleeding site

Laboratory Diagnosis and Result

About 4ml of oozed blood, from the bleeding sites, was collected in a sterile test tube. Immediately after collection of samples, small amount of blood was put on one end of microscopic slide and another spreader slide was kept at 45^0 with the blood sample. It was waited until the blood sample spread through the whole width of the spreader slide. While holding the spreader slide at the same angle, 45^0, it was pushed forward rapidly and smoothly. The smear was allowed to air dry for 5 minutes. The dried smear was stained with giemsa stain. A drop of oil immersion added on top of the smear and it was observed under 100x oil objective lens of binocular microscope and there were numerous eosinophils Figure 20.

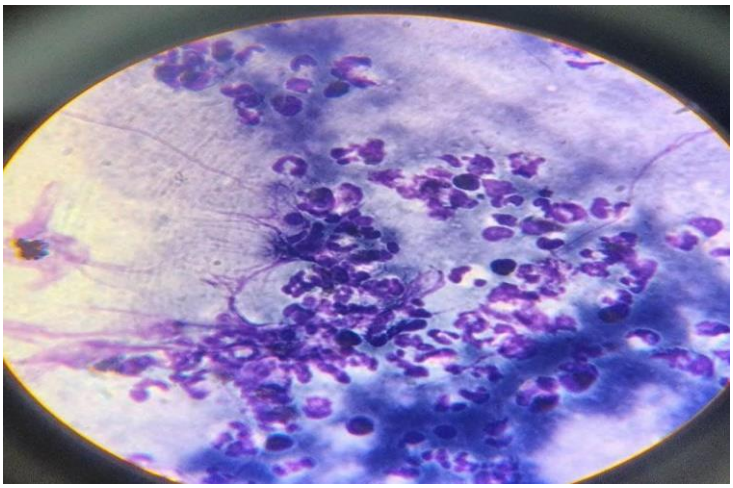

Figure 20: Numerous Eosinophils in the smear made from a drop of oozed blood in parafilariosis case

The test tube containing the blood sample was filled with water and centrifuged. The supernatant was slowly discarded off and the sediment, with a pipette was taken to the microscopic slide and covered with cover slip then it was examined under 40X objective lens of binocular microscope for the presence of a parasitic egg. The process was repeated until all the sediment was finished The. larvated egg(A) and Parafilaria parasite(B) was detected as indicated in (figure 3). Based on the history obtained, clinical signs observed and laboratory finding the case was finally diagnosed as bovine Parafilariosis

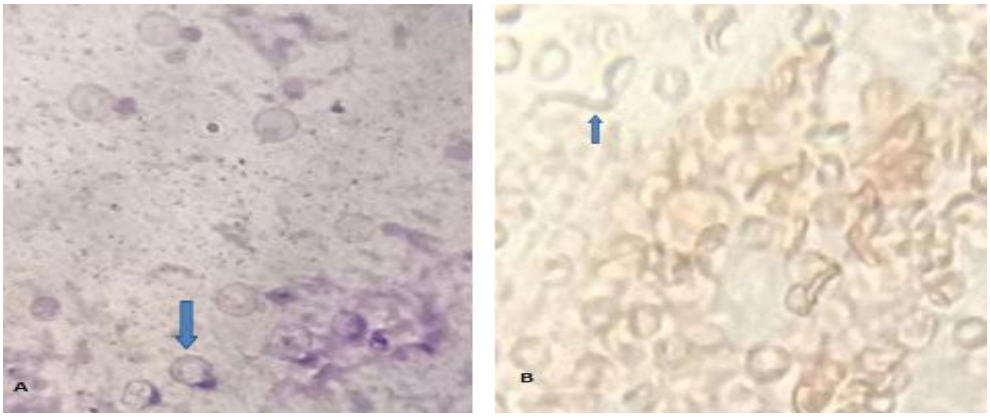

Figure 21: Parafilaria: (A) Shooing larvated egg and (B) Parafilaria Parasite

Treatment and Outcome of the case

Ivermectin 2ML/50kg (Sheyang Sunvictotor Pharmaceutical Co., Ltd. /China) was three times an interval of 14day S/c and one time pour on lesion were given and the local bleeding and the bleeding was washed off with water and iodine. After 14 days of treatment when the owner was come to VTH, the cutaneous wounds of the cow dried (Figure 22A). But after one month the local bleeding was reappeared as indicated figure 22B. Therefore, the second shoot was given subcutaneously and infiltrate on the local wound. Consequently after 1 month and 14 day the cow was visited at the farmer home, the cow was in a good health condition and the cutaneous wounds of the cow was dried as indicated on figure 22C. After a month the owner report for me by phone the local bleeding was never reappeared after the third treatment.

Figure 22: The outcome of parafilariosis treatment: (A); Recovered cutaneous focal bleeding on the right side of shoulder (day 14) (B); Relapsed cutaneous focal bleeding (one month) (C); Fully recover cutaneous focal bleeding

Discussion

Bovine parafilariosis is a seasonal vector-borne parasitic disease caused by the cutaneous filaria Parafilaria bovicola (Hund *et al*.,2021). Cutaneous bleeding in cattle always represents an exceptional situation in daily veterinary practice work. In recent years, reports on the occurrence of cases of bleeding cattle have become more abundan (Oehm *et al*., 2019a).

The only clinical sign observed in this case was focal cutaneous bleeding spots; were inconsistent with other finding (Oie, 2020). The female worm causes bleeding spots, and ovipositional bleeding was primarily seasonal it is beginning in June and peaking in September–November (Wubshet *et al*., 2019) in consistent with this finding the current case of cow in this case report came to clinic on October in agreement to the above study. On microscopic examination of the sediment sample, the distinctive parasite and its eggs were discovered, and in the stained sample relatively increased numbers of eosinophils were detected.

On the basis of, history, presence of parasite, parasite eggs and increased eosinophils and response to Ivermectin treatment the above-mentioned cases were diagnosed as subcutaneous parafilariasis. Although clinical signs are very typical, the definitive diagnosis should be based on the demonstration of the parasite or its embryonated eggs in lesions or exudate, as demonstrated in this case report. In this case Parafilariasis treated with Ivermectin 1ML/50kg injectable 3 times by 2week interval of and one time pour on in lesion in agreement .with report published Oie (2020).

In conclusion this case report illustrates the presence of Parafilaria bovicola in Bishoftu and its treatment protocol. It remains to be determined whether these case reports are isolated cases or whether parasite is becoming endemic in Bishoftu. Evolution towards an endemic situation needs to be investigated.

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4.2. Case of Sheep and Goat

4.2.1 Coccidiosis in lamb

Abstract

Coccidiosis is a parasitic infection that affects the gastrointestinal tract of animals. It is caused by the protozoan Eimeria species and can lead to serious health issues in infected animals. A 6-month-old lamb with signs of anorexia, depression, and diarrhea is presented in this case report. The lamb was intensively managed together with the whole flock. During the clinical examination, the lamb had a high fever with a rectal body temperature of 40.9 °C and was found to have passed semi-solid feces containing blood. The conjunctiva mucus membrane was slightly congested, and the lamb was depressed. Oocytes of coccidia were detected in the fecal sample, and the lamb was treated with sulfadimidine sodium at 1.5 ml/2.5 kg/day IM or three consecutive days, along with supportive care including proper nutrition and hydration. The lamb responded well to the treatment and soon showed signs of improvement. This case study recommends the early detection and appropriate treatment of coccidiosis in lambs.

Keywords: *Coccidiosis, lamb, oocysts, Sulfadimidine sodium*

Introduction

Farm animals are susceptible to gastrointestinal disease coccidiosis. The causative agent is Eimeria spp. Protozoa, or unicellular microorganisms, like eimeria are typically found in soil. Each parasitized cell undergoes hyperplasia or dies because coccidia are obligate intracellular parasites that are host-specific (Lenira, 2009) and develop inside the cytoplasm of epithelial cells. The involved Eimeria species, the amount of infective oocysts, stress, and a number of host-related factors, such as age, physical condition, genetic susceptibility, and the level of immunity that has developed from prior low levels of infection, all affect the mechanisms and severity of tissue damage. Young animals have a lower rate of epithelial turnover, making them more prone to disease (Haile, 2018).

The life cycle of an Eimeria species consists of an exogenous stage for oocyst maturation (sporogony) and an endogenous parasitic stage inside the host with an asexual and then a sexual multiplication. The unsporulated oocysts are excreted in the feces and, depending on the species of Eimeria and the humidity, oxygen content, and temperature of the surrounding environment, develop into the infectious stage after 2–7 days. The original single cell divides into four sporoblasts, each of which matures into a sporocyst, and two sporozoites develop within each sporocyst. The pathogenesis starts when a sporozoite released from a sporocyst in the gut lumen infects a cell in the intestinal mucosa (Quiroz-Castañeda & Dantán-González, 2015).

Young animals that are exposed for the first time are frequently more susceptible to a severe disease and clinical disease than other animals because specific immunity to each coccidial species develops after infection. Most lambs appear to be protected against re-infections later in the grazing season by the immunity brought on by the initial infection. (Tellez *et al*., 2014). Clinical coccidiosis is affected by a number of factors, including age, genetic susceptibility, physical condition, level of immunity, and stressors including bad weather, weaning, dietary changes and traveling (Hashemnia *et al*., 2011). The primary sign is diarrhea. According to the prepatent period of each species, the first

appearance of oocysts coincides with the change in the appearance of the feces. The color of the feces frequently varies from brown to yellow or black tarry, and they are frequently watery with clumps of mucus. The infected animals quickly exhibit severe dehydration, pale conjunctiva, listlessness, gastrointestinal pain, tenesmus, and weight loss during the period of diarrhea (Mohammed & Alobaidii, 2021).

Due to a loss of appetite the animal's general health has gotten worse. Particularly in young animals between the ages of 2 and 4 months, coccidiosis can occasionally be characterized by rapid mortality without prior digestive symptoms (Uzal *et al*., 2016). As soon as possible, treatment must be administered, taking into account the entire group of animals (age, paddock), as even animals with no outward symptoms could pollute the environment. The animals must be relocated to a cleaner environment as part of the treatment (Chartier and Paraud, 2012).

Case description

A 6-month-old lamb was brought to Feseha Gebereab Memorial Veterinary Teaching Hospital of AAU-CVMA, Bishoftu, on November 20, 2022, with a major complaint of straining in an attempt to pass feces, and the feces contained blood. The lamb's feed intake was also reduced, which started three days before admission. The lamb was managed intensively with other members of the flock. On clinical examination, the lamb was febrile with a rectal body temperature of 40.9 ^{o}C; the respiratory and heart rates were within the normal limits. The evacuated fecal matter was semi-solid and blood-tinged, as indicated in Figure B. The conjunctiva mucus membrane was slightly congested, and the lamb was depressed. Based on the history and clinical findings, lists of differential diagnoses were made, including coccidiosis, salmonellosis, and colibacillosis, from which coccidiosis was tentatively diagnosed.

Figure 23: A clinical examination of coccidiosis affected sheep (A); Blood-tinged fecal matter (B)

Laboratory findings

For further investigation of the case, fecal samples were collected directly from rectum and immediately processed before the lamb dispatched at Addis Ababa University College of Veterinary Medicine, parasitology-laboratory, Bishoftu. Fecal examination for coccidian oocytes was carried out using floatation method as described by Hansen and Perry (1994). Three grams of feces was added to 42 ml of saturated salt solution in a graduated cylinder. The contents were then mixed thoroughly using a glass rod, and were poured through a tea strainer into another beaker. The filtrate was then filled to 10ml test tube until convex meniscus formed and the cover-slip was putted on the top thoroughly and kept for 20 min in the test tube rack. Finally, the cover-slip was carefully lifted and placed on glass-slide and viewed under a compound microscope X40 objective lens. Fortunately, the floatation technique was found helpful and no further investigations were performed. Accordingly, typical coccidian/Eimeria oocytes were appreciated under microscope with X40 objective lens. Therefore, based on the history, clinical findings and laboratory results, as indicated in figure 24 a definitive diagnosis of coccidiosis was made and treatment regimen was arranged.

Figure 24: Coccidian/Eimeria oocytes

Treatment and outcome of the case

Sulfadimidine sodium at 1.5 ml/2.5 kg/day for three consecutive days along with advising of the farmer gives supportive care including proper nutrition. The lamb arrived with a rectal body temperature of 38.9°C on the next therapy. The lamb's appetite was also restored to normal, according to the owner report on phone.

Discussion

Coccidiosis is a common parasitic disease affecting sheep, caused by protozoan parasites of the genus Eimeria (Besier, 2016). Coccidiosis is a parasitic disease of the gastrointestinal tract caused by the protozoan parasite, Eimeria. This parasite is found in the intestinal lumen animals, and is typically transmitted via the fecal-oral route. Coccidiosis can cause significant clinical signs, including diarrhea, anorexia, and weight loss (McDougald, 2016). Coccidiosis is a significant cause of economic losses in the sheep industry due to decreased growth rates, reduced feed efficiency, and increased mortality rates, especially in young lambs (Rehman *et al*., 2017). In this paper, we will discuss a clinical case report on coccidiosis in a lamb which includes clinical signs, diagnosis, treatment, and the outcome of the case. The predominant symptoms of babesiosis observed in the present case report were blood contain feces and reduce feed

intake, with rectal body temperature of 40.9o C; the respiratory, and heart rates were within the normal limits with agreement with published reports of McDougald (2016).

The diagnosis of Coccidiosis is Laboratory testing revealed the presence of Eimeria ovinoidica and Eimeria crandallis in the intestinal contents of the lamb. This indicated the presence of coccidiosis in the animal, and confirmed the diagnosis(Molina, J. M. and Ruiz, 2018). the present case was diagnosis through history, clinical sign and laboratory finding with agreement with published reports of Molina, J. M. and Ruiz, (2018). Treatment of coccidiosis involves the use of oral or injectable anticoccidial drugs (Besier, 2016). In addition, the use of antibiotics and other antimicrobials can lead to the emergence of resistant strains of bacteria, which can further complicate the management of coccidiosis (Odden, 2018). The present case treated with ivermectine and sulfadimethoxine. In agreement with published reports of Besier& Odden, (Besier, 2016. & Odden, 2018).

In conclusion, coccidiosis is a significant parasitic disease affecting sheep that can cause significant economic losses. Prevention through good management practices and prophylactic treatments is crucial (Besier, 2016), and treatment should only be used when necessary to prevent the emergence of drug-resistant strains (McDougald, 2016). Further research is needed to develop more effective control strategies, including vaccines, to reduce the impact of coccidiosis in sheep production (Rehman *et al*., 2017).

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4.2.2 *Pneumonic pasteurollosis in ewe*

Abstract

Different Pasteurella specious causes pneumonic pasteurellosis, an economically significant and extensively spread livestock respiratory bacterial infection. This report presents the clinical case of a three-year-old ewe with clinical signs of coughing, profuse, muco-purulent bilateral nasal discharges and depression. On the history the owner also told me that the animal had a recent transportation. Up on physical examination the ewe was febrile, with a rectal body temperature of 40.9 ^{0}C, and had 38 breaths/mean and 92 beats/mean respiratory and heart rates, respectively. The case was diagnosed as pneumonic pasteurellosis based on history, clinical pictures and laboratory results. The ewe successfully responded to antibiotic therapy. Timely detection and proper treatment of pneumonic pasteurellosis can be crucial for a positive outcome. It is therefore imperative to implement preventive measures to minimize the likelihood of this disease's occurrence.

Keywords: *Antibiotic, Ewe and Pasteurella multocida*

Introduction

Pneumonic pasteurellosis of sheep, also known as "shipping fever," is a highly contagious respiratory disease caused by *Pasteurella* specious including *Pasteurella multocida, Mannheimia heamolytica* and *Pasteurella trehalosi* (Blackall & Bojesen 2019). The disease primarily affects sheep and can also infect other domesticated and wild animals (Dagleish *et al*., 2015).

The bacteria are transmitted through direct contact with infected animals or through contact with contaminated equipment, feed, or water sources (Griffin *et al*., 1989). The disease often occurs in stressful situations such as transport, handling, or changes in feed or environment, which can weaken the immune system of the animals and make them more susceptible to infection (West & Bateman 2018). Symptoms of pneumonic pasteurellosis include fever, depression, loss of appetite, rapid breathing, coughing, nasal discharge, and sometimes death (Caswell & Williams 2016). The disease can be acute and rapidly fatal, especially in lambs and young animals, or chronic with a prolonged recovery period (Jackwood & Saif 2018).

Diagnosis of the disease can be made through clinical signs, postmortem examination, and laboratory tests such as bacterial culture and serological assays (Snowder *et al*., 2006). Treatment usually involves the use of antibiotics, supportive care, and management practices to reduce stress on the animals (West & Bateman 2018). Prevention of pneumonic pasteurellosis involves proper management practices such as maintaining good hygiene, providing adequate ventilation and space, and reducing stress factors. Vaccines are also available and can be used to prevent the disease in susceptible animals (Jackwood & Saif 2018).

Case description

A female, adult three years old sheep was presented to Feseha Gebereab Memorial Veterinary Teaching Hospital of AAU-CVMA on November 05, 2023 with complains of inappetence, coughing, nasal discharges and weakness. As the owner complained, the sheep was bought from the rural market recently. Up on clinical examinations the ewe was febrile with body temperature of 40.9 ^{0}C, 38 breaths/mean and 92 beats/mean respiratory and heart rates respectively. The ewe had profuse, muco-purulent bilateral nasal discharges. Generally, the ewe was dull and lethargic as indicated (Figure 25). Based on medical history and clinical signs, pneumonic pasteurollosis was tentatively diagnosed, while PPR and other respiratory diseases were listed as differential diagnoses.

Figure 25: Pneumonic pasteurollosis suspected ewe during its first visit of VTH

Laboratory diagnosis and findings

The nasal swab was collected after external part of the nose was disinfected. A sterile cotton-tipped swab inserted into the nostril and rotated against the wall of the nasal cavity. The swab placed in the labeled sterile test tube that contains 9 ml of peptone water and immediately submitted to Addis Ababa University College of Veterinary Medicine Microbiology laboratory and incubated at 37^{0}C for 24hrs. The next day a loop full of the broth cultures were taken and streaked over macConkey agar and blood agar medium with 7% of sheep blood. After 24hrs of incubation at 37 ^{0}C, there was growth on the

blood agar but no growth on macConkey agar medium. Besides, biochemical tests including catalase, and indole tests were also performed. All tests were positive. Therefore, it was concluded that bacterium was *P. multisoda* based on growth pattern on the cultured medias (growth blood agar with no zone of hemolysis) as indicated in the figure 26A and biochemical test results (indole production). Finally, gram staining was preformed gram-negative short road bacteria was observed under microscope as indicated in the figure 26B. Therefore, based on the integrated results of laboratory findings, clinical findings and history, the case was confirmed as pneumonic pasteurollosis caused *P. multisoda*.

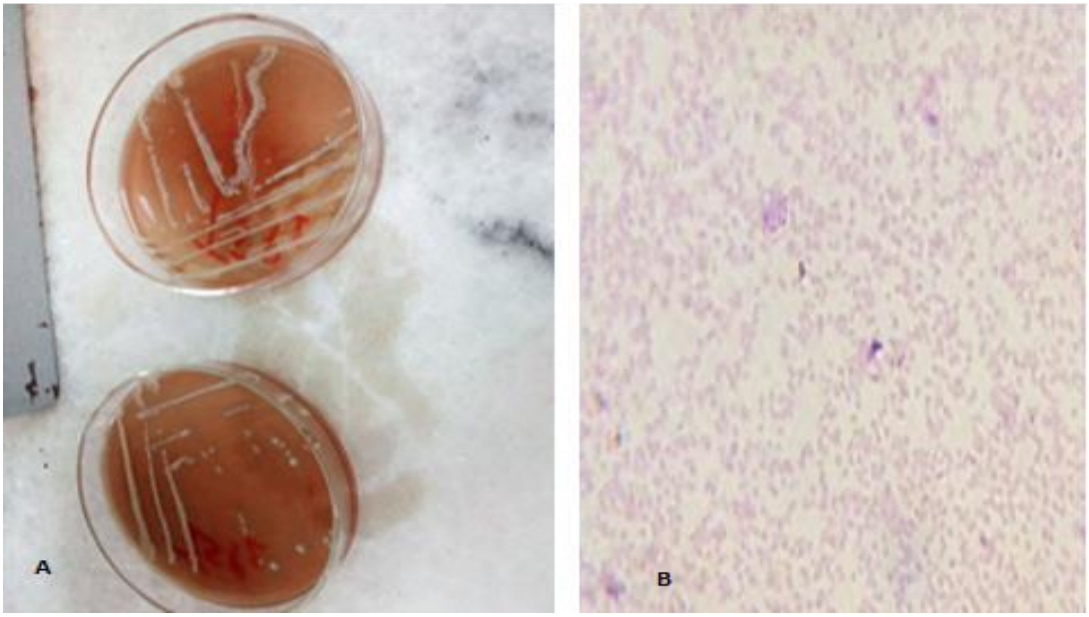

Figure 26: (A) Growth *P. multocida* on blood agar (B) Gram negative short rod bacteria

Case management and treatment outcome

For the treatment of the case a broad-spectrum antibiotic, pen-strip (combination of procaine penicillin and Dihydrostreptomycin) (Chongqing Fantong Animal Pharmaceutical Co.Ltd, China) 1ml/20kg/day intramuscularly for 5 consecutive days was arranged. The ewe was presented 24 hours post therapy with body temperature reduced to 39.2 ^{0}C. After 5 days therapy the ewe was fully recovered.

Figure 27: Ewe after 5 days treatment to Pneumonic pasteurellosis

Discussion

Pneumonic pasteurellosis is a significant concern in sheep production due to its high mortality rate and the potential for economic losses (Moustafa and Seida, 2019; Awan *et al*., 2021). Therefore, proper management practices, including good hygiene, adequate ventilation and space, and reducing stress factors, are essential in preventing the disease (Awan *et al*., 2021; Saeed *et al*., 2021). In addition to management practices, vaccines can also play a crucial role in preventing pneumonic pasteurellosis in susceptible animals (Saeed *et al*., 2021). Several types of vaccines are available, including killed whole-cell and subunit vaccines, and they have been shown to be effective in reducing the severity and incidence of the disease. Clinical signs of the disease include coughing, difficulty breathing, (Saeed *et al*., 2021; Moustafa and Seida, 2019). In this paper, we will discuss a

clinical case report on pasteurellosis in ewe which includes clinical signs, diagnosis, treatment, and the outcome of the case. The predominant symptoms of pasteurellosis observed in the present case report were febrile with body temperature of 40.9o C, and had 38 breaths/mean and 92 beats/mean respiratory and heart rates, respectively. The ewe had profuse, muco-purulent bilateral nasal discharges, coughing and depression which is in agreement with report on published paper (Saeed *et al*., 2021; Moustafa and Seida, 2019).

Diagnosis of pneumonic pasteurellosis is based on clinical signs, post-mortem examination, and laboratory tests such as bacterial culture and serological assays (Awan *et al*., 2021). Timely diagnosis is crucial for the effective treatment of the disease, which typically involves the use of antibiotics, supportive care, and management practices to reduce stress on the animals. The Diagnosis the current case was based on history, clinical signs and laboratory result. The sheep was treated with antibiotic which is in agreement with report on published paper (Awan *et al*., 2021; Saeed *et al*., 2021).

In conclusion, pneumonic pasteurellosis is a serious disease that requires careful management and timely intervention to prevent its spread and minimize its impact. A multi-faceted approach that includes good management practices, vaccination, and appropriate treatment can help to ensure the health and welfare of sheep and the sustainability of sheep production systems (West & Bateman 2018).

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4.2.3 Infectious keratoconjunctivitis /Pink eye in goat

Abstract

Infectious keratoconjunctivitis, commonly known as pink eye, is a contagious bacterial disease of the eye. This case report summarizes the case of infectious keratoconjunctivitis in a newly purchased goat that was presented to the Fisseha Gebreab Memorial Veterinary Teaching Hospital at AAU CVMA with the main complaints of eye problems and lack of appetite. On physical examination, the goat was febrile (40 °C), and the heart and respiratory rates were in the normal range. The most prominent abnormality observed was redness in both eyes, with excessive lacrimation and conjunctivitis in the left eye. Ocular swabs were collected by sterile tube with peptone water and cultured on blood agar and nutrient agar. The smear was prepared from colonies on nutrient agar, stained with Gram's staining, and observed under a microscope with a 100X objective lens. Short rod-shaped gram-negative bacteria were observed. The goat was treated with 10% oxytetracyline and tetracycline eye ointment. After seven days of treatment, the goat was fully recovered. The chances for recovery are high if cases of pink eye are reported in time and immediate treatment is instituted, as in the present case. Quarantine of newly purchased animals, proper feeding, and fly control are recommended.

Keywords*:* *Goat, Gram-negative bacteria, Infectious keratoconjunctivitis, tetracycline eye ointment* and *10% oxytetracyline*

Introduction

Pink eye, sometimes referred to as infectious keratoconjunctivitis, is an infectious bacterial condition of the eyes. This infection will lead to inflammation of the conjunctiva, cornea, and eyelid lining tissue, which may develop to ulceration, induce pain, and worsen, which may result in temporary or permanent blindness. While *Moraxella bovis*, a less common causative agent in caprine, is a significant cause of pink eye in cattle, *Moraxella ovis* is the causative agent for pink eye in goats (Jesse Abdullah *et al*., 2014). Pinkeye is a highly contagious infection that spreads through contact. When new goats are introduced to the herd, when they are moved or transported, and when goats are under extreme stress from extremely dry or cold weather, outbreaks frequently happen. From one eye to the other and from animal to animal, infection can spread quickly (Browining, 2007).

A widespread eye condition known as infectious keratoconjunctivitis typically manifests itself as seasonal and localized outbreaks. Numerous instances of large incidence variations some of which reached 90% have been made. The summer is when disease are most common. The symptoms of IBK are usually described as including photophobia, intense lacrimation, eyelid and conjunctival edema, and corneal ulcers (Rodríguez & Uk, 2006). However, in moderate cases of IBK, acute infections can result in permanent eye damage and possibly blindness. Just two of the risk factors for IBK include exposure to dust and tall vegetation (Glover *et al*., 2022). Although Moraxella bovis (M. bovis) invading the surface of the eye is the most common cause of pinkeye, other viruses and bacteria have also been related to the condition (Strickland, 2008).

A diagnosis is the process of obtaining data to categorize disease. Pathognomonic clinical signs are absent in most eye diseases. IBK's often reported clinical symptoms could be another one of several ovine eye conditions in disguise. A common error in disease diagnosis is the use of non-discriminatory information to establish a diagnosis. Clinical symptoms alone cannot differentiate IBK from other keratoconjunctivitis causes. As with

other diseases, IBK must be diagnosed utilizing a combination of information from signalment, history, clinical symptoms, and/or laboratory studies (Kneipp, 2021).

The most crucial factor in containing a disease epidemic is treating infected animals to prevent further transmission. Early identification of animals exhibiting the initial clinical symptoms (tearing, squinting, and blinking), followed by fast, efficient treatment, is crucial for preventing spread and limiting ocular damage. Affected animals should be isolated from the herd, if at all possible, to stop the spread of the disease. The best course of action to stop the spread of pinkeye in the herd may be to administer an injectable antibiotic to all susceptible animals together with a topical fly repellent in situations when a producer is unable to treat new cases as soon as they arise. The duration of the carrier condition, when spread typically happens, is reduced by prompt and successful treatment with the appropriate antibiotic to unaffected herd mates(Arnold *et al*., 2012).Thus the present case report described the Infectious keratoconjunctivitis/ Pink eye in goat.

Case description

On March 12, 2023, a 1-year-old goat was brought to the Feseha Gebereab Memorial Veterinary Teaching Hospital of AAU-CVMA with the main complaints of eye problems and a lack of appetite. The goat was purchased within two weeks and managed extensively with its current deworming history. Up on clinical examination, the goat was dull and depressed, as indicated in Figure 28A. The goat was pyrexic (40 °C), but the heart rate and respiratory rate were in the normal range. The most prominent abnormality observed was redness in both eyes with the presence of chemosis in the left conjunctiva with excessive lacrimation (Figure 28B). The differential diagnoses at this point in time were pinkeye, trauma, and malnutrition.

Figure 28: Pink eye first day clinical examination in goat

Sample collection and Laboratory finding

Ocular swabs were collected from both eyes by sterile tube with peptone water and cultured on blood agar then incubated at 37 ^{0}c for 24hrs and after that the pure colony subculture on nutrient agar and incubated at 37 ^{0}c for 24hrs and finally smear was prepared. The prepared smear then stained with Gram staining procedure (Annex 3) and a drop of oil immersion was added on the slide and observed under 100X binocular microscope. Gram-negative and short rod-shaped bacteria were observed as indicted on figure below. Therefore, based on the history, clinical findings and laboratory result the goat was finally diagnosed with pinkeye caused by Moraxella.

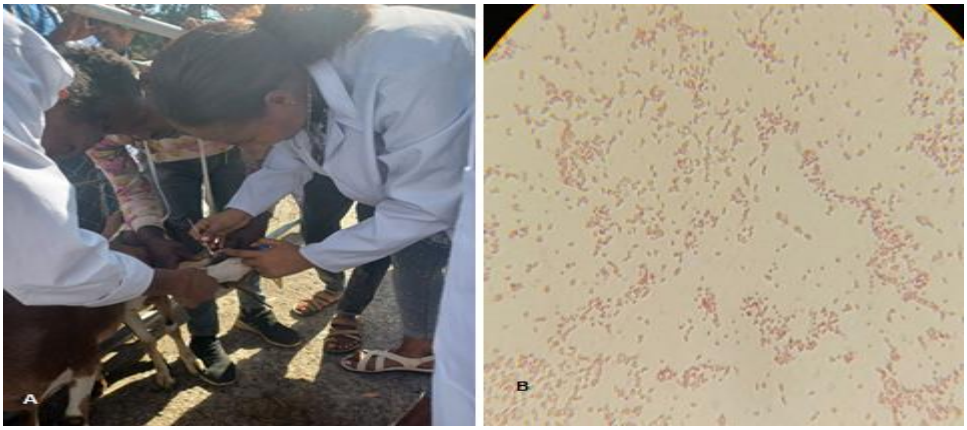

Figure 29: Infectious keratoconjunctivitis affected goat (A) Showing sample collection and (B) Gram stain result

Treatment and outcome the case

The goat was administered 10%Oxytetracyline (20mg/kg) injections intramuscularly for 5 days. Tetracycline eye ointment was prescribed to be applied topically three times daily (TID) for seven days. The signs of conjunctivitis have decreased on the fifth day of post treatment. Thus, the prognosis was good since the goat had showed positive response to the treatments given .After seven day treatment the goat was fully recovered as indicated on figure below

Figure 30: Goat after recovery from Pink eye

Discussion

Pink eye disease has been reported to progress through four stages according to severity. At stage 4, there is complete ulceration of the cornea, protrusion and sticking of the iris to the cornea with partial or complete blindness(Jesse Abdullah *et al*., 2014). The present case was diagnosed at the first stage of the disease since the abnormality was redness in both eyes with presence chemosis of the left eye conjunctiva with excessive lacrimation. Stress has been reported as one of the predisposing factors to pink eye disease (Browining, 2007).In the present case report, the goat was said to have been transported from Adulala local market and developed the condition two weeks on arrival into the farm. Transportation being one of the causes of stress could have precipitated the condition in the goat. Other predisposing factors include bright sunlight(Clifford-Rather, 2011). Poor hygiene condition in farms have been reported to be another predisposing factor to the disease, as this will attract flies which are mechanical vectors for the transmission of the causative agent (Browining, 2007). The thorough management of the current case may have made the goat's condition more susceptible to the predisposing factors mentioned above literatures.

Both systemic and topical antibiotic treatments have been administered in the present case. Long acting oxytetracycline (20mg/kg) was administered intramuscularly as recommended (Jesse Abdullah *et al*., 2014). Topical treatment has been reported to speed up the recovery process of the affected animal (Jesse *et al*., 2017) .The chances for recovery are high if cases of pink eye are reported in time and immediate treatment instituted as reported in the present case. Quarantine of newly purchased animals, adequate quality feeding and fly control is recommended. In the present case the owner was advised to isolate the goat from the rest of the flock until full recovery is achieved.

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4.3. Cases Reports of Horse

4.3.1. Epizootic lymphangitis

Abstract

Epizootic lymphangitis is a debilitating fungal disease caused by some isolates of Histoplasma capsulatum, which mainly occurs in equids. This case study summarizes the case management of a horse that presented to SPANA Hospital with a history of one small swelling a month ago. A few days ‘later, a swelling line appeared, starting from the pastern joint to the shoulder area. Small swellings had appeared along the swollen line, and the swellings started to produce creamy-like pus. On clinical examination, there was much pus discharging from nodules along the lymphatic vessel of the left forelimb. No other nodules were seen in other parts of the horse‘s body. Body parameters were: temperature 37.8 ℃, respiratory rate 24 breaths per minute, and pulse rate 44 beats per minute. All were in the normal range. A pus sample was collected, and a smear was prepared, stained with Giemsa staining, and observed under a 100X magnification lens. The result showed a halo, unstained capsule-like structure, which confirmed the yeast form of Histoplasma capsulatum. The horse was treated with local flushing of the nodules with 4% tinctured iodine for three weeks. After the end of treatment, all the nodules turned into scars. Epizootic lymphangitis is an economically important disease in some areas of the world, particularly where large numbers of horses are assembled, like in Bishoftu. Different treatment modalities are available, some of which are successful in treating early cases of the disease, but the cost of treatment is not affordable for farmers. Effective prevention and control of epizootic lymphangitis are based on euthanizing infected equids and the application of strict biosecurity measures.

Keywords*: Epizootic lymphangitis, Horse, Gimesa stain and Histoplasma capsulatum*

Introduction

Epizootic lymphangitis is a debilitating fungal disease, caused by some isolates of Histoplasma capsulatum, which mainly occurs in equids. Epizootic lymphangitis results from infection by a dimorphic fungus, Histoplasma capsulatum, which exists as yeast in animal tissues and a saprophytic mycelium (mold) in the environment. This organism is a member of the family Ajellomycetaceae and order Onygenales (Oie., 2019). The organism can survive in dust and soil for extended periods of time, especially under warm moist conditions.It is highly resistant to the actions of physical and chemical agents. The disease is common in various parts of Africa, the Middle East, Russia, and Asia (Mahajan, 2021). Most outbreaks occur in humid and hot climates; when large numbers of equids are stabled together for transportation needs, in military situations or racing The disease is currently prevalent in Ethiopia; with prevalence in horses ranging from 0 - 39% in various regions of the country (Molla *et al*., 2022).

Although epizootic lymphangitis is endemic in several countries causing significant economic and welfare issues, it is an OIE non-listed disease. In Ethiopia, studies showed that it is a widespread disease affecting 18.8–26.2% of carthorses and 21% of cart mules. Based on the route of infection, epizootic lymphangitis can occur as cutaneous, respiratory, ocular, and asymptomatic forms (Abdela *et al*., 2021). The disease can be diagnosed based on the clinical examination of the lesions, microscopic examination of the yeast form of the fungus in pus, serological tests or skin hypersensitivity testing (Molla *et al*., 2022).

Treatment and euthanasia were critical elements of the intervention to contain spread of EZL. Treatable cases were treated by incision of nodules when present, application of tincture of iodine, and administration of parenteral iodides (potassium iodide, Ubiche) in drinking water. Length of treatment increased the more severe the case classification (Duguma *et al*., 2021).

Case description

An owner presented a horse to the SPANA open-air clinic. The owner mentioned that his horse had one small swelling before a month. A few days later, a swelling line had appeared from the pastern joint to the shoulder area. Small swellings had appeared along the swollen line, and the swellings started to look creamy like pus during that time. On clinical examination, there were many pus discharging nodules along the lymphatic vessel of the left forelimb. No other nodules were seen in other parts of the horse's body. Body parameters were: temperature 37.8 °C, respiratory rate 24 breaths per minute, and pulse rate 44 beats per minute. All were in the normal range.

Figure 31: Nodules along the lymphatic vessels in epizootic lymphangitis

Sample Collection and Laboratory Diagnosis and Result

Sample was collected using syringe and needle pus was aspirated from a nodule that was not ruptured and immediately smear was prepared from the pus sample and allowed to air day. The smear was stained with Gimesa stain and observed under 100X magnification lens of a binocular microscope after a drop of oil immersion added on top of the slide. The result showed a halo, unstained capsule-like structure which confirmed the yeast form of Histoplasma capsulatum.

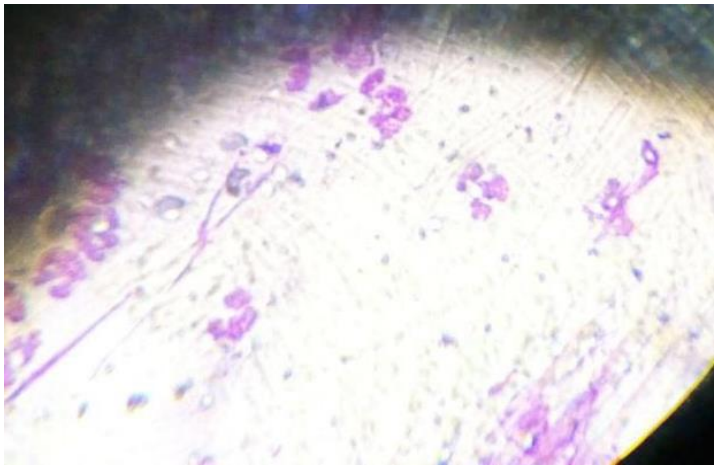

Figure 32: Halo structure; yeast form of HCF

Treatment and Outcome of the case

The area of infection was shaved and any nodule that was not ruptured was incised with Scalpel blade continued with removal of the pus and flushing of the nodule with 4% tincture iodine for three week (Oie., 2019). The owner was strongly advised to clean the infected area with soap and water then with 4% tincture iodine every day. There were no new nodules emerged after the three weeks of treatment. All the nodules were changed in to scar 5 weeks as indicated on figure.

Figure 33: EL affected horse on treatment and its outcome (A); Treatment with 4% tincture iodine (B); EL affected horse after recovery

Discussion

Epizootic lymphangitis (EL), caused by Histoplasma capsulatum variety farciminosum (HCF) is a contagious, chronic disease of equines, characterized by development of nodular lesions in the lymph nodes, lymphatic vessels and skin. It is one of the most important diseases of equines in Ethiop. EL has been reported in many countries (Hadush *et al*., 2020)

Epizootic lymphangitis is a chronic disease of horses that is caused by fungus. The clinical signs of the disease seen in this case; nodules, discharging creamy pus, found along the lymphatic vessel were similar with the clinical signs of the disease mentioned on literatures (Hadush *et al*., 2020; Molla *et al*., 2022).

As in literatures the disease does not have effective treatment so far (Ameni, 2006). The trial of Potassium Iodide treatment by SPANA is showing better result in the treatment of early cases of the EZL but the cost of the treatment was not affordable by the owner. Effective prevention and control of epizootic lymphangitis is based on euthanizing infected equids and the application of strict biosecurity measures.

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4.3.2 Case report on Tetanus in horse

Abstract

Tetanus is an acute and fatal nervous system disorder caused by the neurotoxin Clostridium tetani, and it affects humans, domestic animals, and wildlife. The two-year-old, 240 kg horse was presented on February 6, 2023, to SPANA Hospital, Bishoftu. In the anamnesis, the owner reported that a few days later, there was swelling and cloudiness of the eye and stiffness in the limbs. During physical examination, the animal showed a heart rate of 60 beats per minute, a respiratory rate of 30 per minute, and a rectal temperature of 37.5 °C. The horse's clinical signs were spasticity, rigid gait, distant hind limbs, prolapse of the third eyelid, hyperesthesia, an extended neck, a tail away from the body, distended nostrils, and erect and immobile ears. The horse was an inpatient in a dark room at the SPANA clinic with its ears stuck down with cotton for fourteen days and treated following tetanus management protocols. ACP (Acepromazine, Elanco) 2 mg/ml at a dose of 0.03-0.125 mg/kg body weight, procaine penicillin G at a dose of 20,000 IU/kg/day for seven successive days, and tetanus antitoxin, or TAT, with a 100 IU/kg therapeutic dose were administered IM. To control muscle spasm, halt further propagation of the agent, and neutralize the circulating toxins, respectively. Therefore, after fourteen days of implementation of the treatment, the animal had successfully recovered. Tetanus is a serious disease with a high case fatality rate. Priority should be given to educating communities about the use of appropriate packsaddles and harnesses made of natural materials for wound avoidance.

Keywords*:* *Tetanus, Horse, Treatment outcome*

Introduction

Tetanus, a severe and often fatal disease of the neurological system brought on by the neurotoxin Clostridium tetani, can harm both domesticated animals and wild animals. Although immunization can prevent it, the issue nonetheless poses a threat to the lives of animals. At least 209 species of anaerobic organisms belong to the genus Clostridia, and C. tetani is one of the most well-known pathogens in this group that produces exotoxins. The spores of C. tetani may be isolated from soil, human and animal feces, and/or can resist anaerobic and extremely high temperatures in both indoor and outdoor habitats (Melo & Ferreira, 2022).

The deep puncture wound serves as the major point of entry. In horses, puncture wounds to the feet are typical entry points, and in newborn equids, the umbilicus still serves as the primary entry point. According to reports, horses are the domestic species most vulnerable to the effects of tetanus toxin (Sharma *et al*., 2021).

Tetanus clinical symptoms are caused by a complex of exotoxins that the organism produces when it is anaerobically growing. The main neurotoxic produced by the bacterium, tetetanospasmin, is what causes the majority of clinical symptoms (Ribeiro *et al*., 2018). Tetanus manifests clinically as localized and generalized forms in horses, as well as acute and subacute forms. Spastic paralysis rapidly progresses from the head (masticatory, auditory, and third eyelid muscles) to the respiratory muscles, then to the limbs in the acute type. Sweating occurs together with generalized convulsions. Respiratory failure can bring death within a day or two. The subacute types take 1-3 weeks for the symptoms to appear. Some animals might regain health. Early warning indications include hyperesthesia and third eyelid prolapse. As a result of the mastication muscles' paralysis, swallowing and eating are challenging (Sharma *et al*., 2021). The diagnosis of tetanus is based on immunization history and historical and clinical findings, whereas less emphasis is placed on laboratory testing (Melo & Ferreira, 2022).

Eliminating the contaminating bacteria, neutralizing the remaining toxin, controlling muscle spasms, applying wound dressings if necessary, maintaining nutrition and hydration, and also providing supportive treatment, such as stalls with a high bed, quiet surroundings, and darkness are all necessary for the treatment to be effective (Oliveira & Pereira, 2020).

Case description

A two-year-old, 240 kg horse presented in the February 6, 2023, SPNA clinic in Bishoftu. In the anamnesis, the owner reported that a few days before the day of examination, he observed swelling and cloudiness on the eye and stiffness on the limb. The horse did not receive any type of vaccine or undergo surgical procedures. The animal has always fed, defecated, and urinated normally. During physical examination, the animal showed a heart rate (HR) of 60 beats per minute (bpm), a respiratory rate (RR) of 30 per minute (mpm), and a rectal temperature (RT) of 37.5 °C. On the horse, clinical signs such as spasticity, rigid gait, distant hind limbs, prolapse of the third eyelid, hyperesthesia, neck extended, tail away from the body, distended nostrils, and erect and immobile ears were observed Figure 34. Additionally, in the physical examination, small wounds in both hooves were found. The horse was inspected, as was the entire bodies of the animal and small injuries were found. Based on the physical examination of the animal, as well as the clinical signs presented, the diagnosis of tetanus was established.

Figure 34: Tetanus affected horse: (A): Showing prolapse of the third eyelid, (B): limb and tail stiffness.

Treatment protocol and outcome

The horse were admitted in dark room and ear stuck down with cotton in SPANA clinic for fourteen days and treated following tetanus management protocols indicated in (Feyisa, 2021) i.e. to halt further propagation of the agent, neutralize the circulating toxins, control of muscle spasm. Accordingly, ACP (Acepromazine, Elanco) 2 mg/ml at a dose of 0.03-0.125/ kg body weight, SID for two days, as muscle relaxant was administered IM. Procaine penicillin G at a dose of 20,000 IU/ kg/day BID for seven successive days was given in IM. Besides, Tetanus antitoxin or TAT with 100 IU/kg STAT therapeutic dose was given IM. In addition to that in the third day three bags of IV fluid were administered though IV because of horse's gradual appetite loss. Then after five days of treatment, a return in the third eyelid prolapse, stiffness and hyperesthesia were observed. Therefore, after fifteen days implementation of the treatment the animal had already recovered as indicated on figure below.

Figure 35: Tetanus affected horse after recovery

Discussion

Considering the clinical signs, data from the anamnesis and physical examination of the horse the diagnosis of tetanus was established in agreement with Avante *et al*.,. (2016), hyperesthesia, spasticity, ear position, flag tail, extended neck, third eyelid prolapse, muscular stiffness of the head and neck, mandibular trismus are signs of tetanus and some of them could be observed in the mentioned horse. The clinical signs occur due to the *C. tetani* toxins which are generated by the creation of an anaerobic environment in wounds, abscesses caused by injection, punctate wounds in hooves or soft tissues. The horse in this report had the hooves and the entire body inspected, however small wounds were found in agreement with the study (Dammessa, 2017). Tetanus is a serious illness with a high case fatality rate. Priority should be given to educating communities about the use of appropriate packsaddles and harnesses made of natural materials for wound avoidance.

The current case was treated with administration of Procaine penicillin G, Tetanus antitoxin (TAT), Acepromazine, and intravenous fluid therapy in agreement with the published case report on Feyisa, (2021).

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4.4 Case Report of Poultry

4.4.1 Infectious Bursal Disease (IBD)

Abstract

Infectious bursal disease (IBD) is an infectious disease caused by a virus belonging to the Birnaviridae family. It is one of the most common diseases in chickens and is a major cause of mortality in broilers. This clinical case report summarizes a case of IBD in a 29-day-old Cobb 500 broiler breed from a smallholder poultry farm in Bishoftu. The birds showed signs of depression, decreased feed intake, ruffled feathers, and a mortality rate of 12%. On the postmortem examination, there were changes in different body parts of the cadaver, including ecchymotic hemorrhages on the leg muscles, an edematous bursa of Fabricius, and enlargement and color change on the liver. Two pools of tracheal and cloacal swabs and tissue samples were sent to the National Veterinary Institute (NVI) for confirmatory diagnosis. A conventional polymerase chain reaction (PCR) test confirmed that the cause of the disease is the IBD virus. Based on history, clinical signs, post-mortem examination, and laboratory results, the case was finally diagnosed as IBD. The case was treated with a combination of vitamins and antibiotics. After treatment, the chickens showed improved appetite and overall health. In conclusion, IBD is a serious issue for the poultry industry, and it is important to take steps to prevent and control it. It is also noted that prompt diagnosis and proper management of IBD are essential in order to prevent high mortality rates. Moreover, it is important to monitor birds for signs of IBD, isolate sick birds immediately, and treat them with a combination of antibiotics and multivitamins. This is also good for secondary bacterial complications and to stimulate the bird's immunity, as the IBD virus is an immunosuppressive disease.

Keywords: *Antibiotic, Broiler, Infectious bursal disease, Postmortem examination and Vitamins*

Introduction

Infectious bursal disease (IBD), is a viral infection that mostly affects the lymphoid tissues of young poultry and is acute and extremely contagious. The disease known as "Gumboro disease" is brought on by the infectious bursal disease virus (IBDV), a member of the genus AviBirnavirus and family Birnaviridae. It is an icosahedral, non-enveloped double-stranded RNA virus (dsRNA) with a bisegmented genome. Segment B is shorter and only encodes one viral protein, while segment A is longer, 3261 nucleotides long, has two open reading frames (ORF), and encodes the four viral proteins VP2, VP3, VP4, and VP5. There are two serotypes of IBDV (serotypes 1 and 2) (Barman *et al*., 2014).

IBDV is now widespread throughout the world and is found in all significant poultry-producing regions (Hayet *et al*., 2021). IBDV serotypes I and II can infect turkeys, ducks, and ostriches both spontaneously and artificially, as evidenced by their isolation and serological response. However, only hens exhibit clinical illness. Serotype I viruses can infect any breed of chicken, but white leghorns have the worst clinical signs, lesions, and mortality rates. The mortality rate from infections brought on by the classic strain can range from 1-60% in flocks that are completely susceptible, with a severe morbidity of up to 100% (Wagari, 2021).

The most common route of infection is through the mouth, but conjunctiva and the respiratory system may also be important (Barman, *et al*., 2014). The virus affects lymphoid tissue, killing B lymphocyte cells in BF, the spleen and cecal tonsil-lymphocyte relatively unharmed. Four to five hours after an oral infection, the virus can be identified in macrophases and lymphoid cells of the cecum, duodenum, jejunum, and kuppfer cell of the liver. Many of the bursa's cells have antigen by 11 hours following the infection through the blood stream. Up to 14 days after infection, viral antigen can be discovered in the bursa when the virus affects other organs such the spleen, harderian gland, thymus lymphocyte, and their precursor (Teshome *et al*., 2015),

Infectious bursal disease virus has short incubation period of 2-3 days and the infection generally last 5-7 days. The tendency of birds to engage in venting is one of the initial symptoms of IBDV infection. According to Hayet *et al*.,. (2002), the various clinical symptoms of IBD include despondency, reluctance to move, white and watery diarrhea, dehydration, ruffled feathers, trembling, and gasping. The flock history, clinical symptoms, and postmortem lesions are taken into account for the diagnosis. IBDV infection is confirmed by the pathological change seen at the bursa of Fabricius, histophatological investigations, and the detection of viral antigen by immunohistochemistry Barman, *et al*., 2014). Agar gel precipitin assay or antigen capture enzyme linked immunosorbent assay (AC-ELISA) can be used to demonstrate viral antigen. IBDV diagnosis commonly uses the molecular instrument polymerase chain reaction (PCR) (Teshome *et al*., 2015).

To control secondary agents like antibiotics and vitamins that impact immunosuppression, birds may benefit from drugs to treat symptoms. There is no evidence that treatment intervention can change how the viral infection develops (Wagari, 2021). Thus, the case of infectious bursal disease on broiler is presented in the current case report.

Case description

Two 29-day-old Cobb 500 breed broilers were brought to Feseha Gebereab Memorial Veterinary Teaching Hospital at AAU-CVMA on February 2, 2023, from a smallholder poultry farm with the complaints of, in appetence, severe depression and the sudden death of chickens. The farm owner mentioned that he had more than 1000 chickens, but around 120 died within 3 days after a 15-day IBD vaccination. Further investigation revealed that the owner brought the current flock just after the last flock. On physical examination, there were depression, weakness, ruffled feathers as indicated (Figure 36A), and whitish diarrhea as indicated (Figure 36B). During postmortem examination, there were ecchymotic hemorrhages on the leg muscles (Figure 37A), an edematous bursa of Fabricius as indicated (Figure 37B), and an enlargement and cooler change on the liver,

which is pale (Figure 37C). Based on history, clinical signs, and post-mortem examination, the case was tentatively diagnosed as infectious bursal disease.

Figure 36: IBD affected broiler: (A); Shows depression, weakness, ruffled feathers (B); Whitish diarrhea

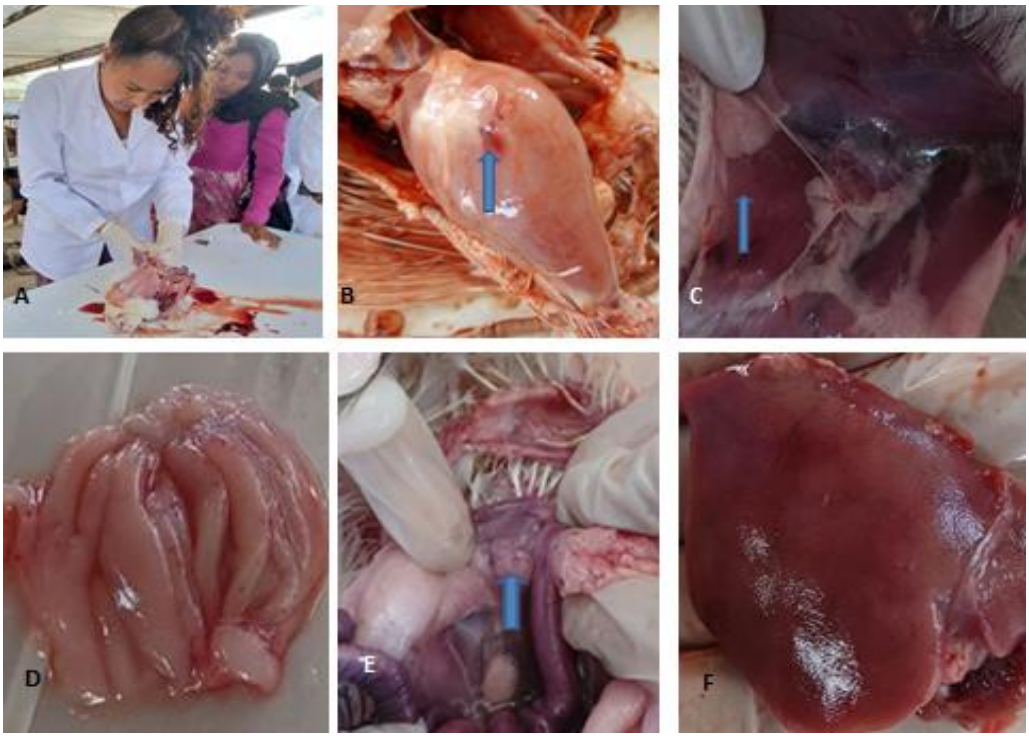

Figure 37: Post mortem examination of IBD affected birds: (B& C); Ecchymotic haemorrhages on the leg muscles (D&E); Edematous bursa of Fabricius; (F); Enlarged and pale color liver**.**

Laboratory diagnosis and result

Pooled sample from two chicken's bursa of Fabricius tissue were placed in cryovial sterile tubes which contain virus transport media (VTN) and submitted with a cold chain to the NVI (National Veterinary Institute) for confirmatory diagnosis. A polymerase chain reaction (PCR) test confirmed that the cause of the disease is the IBD virus (Annex 5). Based on history, clinical signs, post-mortem examination, and laboratory results, the case was finally diagnosed as infectious bursal disease.

Treatment and outcome of the case

The treatment of IBD in chickens is usually supportive and includes vitamins and antibiotics to prevent secondary infections. The present case was treated with a combination of vitamins and an antibiotic, which is Enrofloxacin oral suspension 1 ml per one liter of drinking water and Resergen oral immune stimulant suspension at a dose of 1 g/5 liters was administered for 5 days. After three days of therapy, the owner reported that all the chickens showed improved appetites. After five days of treatment, the overall health of the chickens was improved, as the owner's reports. Finally, it was suggested to the owner to fix the house downtime between the batches.

Discussion

Infectious bursal disease (IBD) is a contagious viral infection that affects chickens, particularly broilers, with potentially devastating economic losses for the poultry industry. The disease is caused by a virus belonging to the family Birnaviridae and is characterized by immunosuppression, which can lead to increased susceptibility to secondary bacterial and fungal infections (Ahmed, A & Saif, Y., 2020). The clinical signs of IBD vary according to the age of the birds. In young birds, it is characterized by anorexia, lethargy, weight loss, and increased mortality. In some cases, the birds may also have an enlarged bursa of Fabricius and anemia. The clinical signs obtained in the current

case report were in agreement with the review published by Nguyen, D. T., & Swayne, D. E (Nguyen, D. T., & Swayne, D. E. 2016).

The diagnosis of IBD in broilers is usually made based on clinical signs and a post-mortem examination. A post-mortem examination is important for the diagnosis of IBD because it can help identify the presence of lesions. In addition to post-mortem examination, laboratory testing is also important for the diagnosis of IBD including molecular technique. This test detects the presence of the virus in the bird (Santos, R., & Bicalho, R. 2018). The diagnosis of the current case was based on clinical signs and post-mortem examination, like the ecchymotic hemorrhages on the leg muscles, the edematous bursa of Fabricius and the enlargement and cooler change on the liver, which were in agreement with what was published in Santos, R., & Bicalho, R (2018).

In conclusion, infectious bursal disease is a serious issue for the poultry industry, and it is important to take steps to prevent and control it. Biosecurity measures, such as avoiding contact between healthy and infected birds, as well as vaccination programs, are the most effective ways to prevent IBD. Moreover, it is important to monitor birds for signs of IBD, and to isolate sick birds immediately. But above all, as it is recommended in this case report, the owner should have to keep the house down. Because it might be the one that exposes the flock to this disease. Treatment with a combination of antibiotics and multivitamins is also good for secondary bacterial complications and to stimulate the immune system of the bird, as the IBD virus is an immunosuppressive disease.

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4.4.2 Newcastle disease

Abstract

Newcastle disease (ND) is one of the most important viral diseases. It is an acute infectious viral disease of domestic poultry and other species of birds, regardless of variation in sex and age. This case report provides Newcastle Disease case management at a smallholder poultry farm in Bishoftu, Ethiopia, where the owner kept more than 1000 hens. A total of 8 to 10 chickens died suddenly per day over the course of the disease. There were 168 chicken deaths in total. On the day of the visit, 15 chickens had greenish, watery diarrhea, depression, and a twisted neck. Hemorrhage in the trachea, gut, and cecal tonsil was discovered during post-mortem examinations for gross pathological evaluation on one of the sick chickens. For further confirmation of the case, pulled tracheal and cloacal samples were taken using sterile swabs from affected chickens, and virus transport medium (VTM) was used to transport and store the sample until processing. Then a real-time reverse transcriptase polymerase chain reaction (RT-PCR) confirmatory diagnosis was done at the National Animal Health Diagnostic Center (NAHDIC), Sebeta. Enrofloxacin oral suspension (1 ml per one liter of drinking water) was administered to 30 chickens with evident clinical signs for 5 days. All 817 chickens were administered Resergen oral immune stimulant suspension at a dose of 1 g/5 lit until good progress was being made. After the first mortality, two or three deaths per day were documented. Even though the mortality rate reached 18.3%, the deaths stopped a week later, and the surviving flock returned to normal status. In conclusion, Newcastle disease is one of the most significant animal diseases in the world, both in terms of the number of animals affected annually and the significant financial losses it causes the poultry sector. To prevent financial losses and treat the condition as soon as possible, rapid and accurate ND detection and confirmation are crucial. Hygiene and immunization are the two main methods for preventing Newcastle disease.

Keywords: *Enrofloxacin, Immune stimulant and Newcastle disease*

Introduction

One of the most significant viral infections is Newcastle disease (ND). Regardless of variations in sex and age, it is an acute infectious viral disease that affects domestic poultry and other species of birds (Ashraf & Shah, 2014). Newcastle disease (ND), also referred to as avian paramyxovirus (APMV) or Newcastle disease virus (NDV), is an illness brought on by infections with virulent viruses from the genus Avulavirus and species avian avulavirus 1 (Bello *et al*., 2018a). All nations that raise poultry record cases of NCD. Although all bird species are likely vulnerable to infection, the disease typically affects chicken, albeit it may differ significantly from one avian species to another with any given viral strain. Every age group is at risk. Different species have different rates of morbidity and mortality, and with the strain of virus (Alemneh, 2019).

The NCD virus is transmitted and disseminated through the excretions of infected birds that contain the virus. The primary method of NCD infection is through aerosol exposure. Additionally, tainted feed, water, footwear, clothes, equipment, and the actual habitat of the fowl are all implicated. Therefore, the primary means through which viruses transmit between flocks are the movement of live birds, the movement of poultry products, the consumption of tainted poultry food or water, and non-avian hosts (Mesfin & Bihonegn, 2018). Greenish diarrhea, nervous system disorders, and respiratory issues are some symptoms of this disease. The strain of the virus and the bird's immunity will determine how severe it is.Incubation times range from 2 to 15 days. As lentogenic is less virulent, velogens and mesogens are also known as virulent NDV. In places where live vaccine inoculation was conducted, fewer cases were observed (Samad *et al*., 2022).

The history, clinical symptoms, and post-mortem findings are used to make a clinical diagnosis, which is a strong preliminary diagnosis. However, because many other diseases, such as infectious bronchitis, infectious laryngotracheitis, and avian influenza, can have similar symptoms, laboratory confirmation is required. The ND virus can be identified by the hemagglutination and hemagglutination inhibition tests, virus neutralization tests, enzyme linked immunosorbent assays, plaque neutralization tests,

and reverse transcriptase polymerase chain reactions (RT-PCR) (Ashraf & Shah, 2014). Sanitation (cleaning, disinfection, limiting contact to wild birds, and personal hygiene) and vaccination, which is the most effective method, are the general ways to controlling Newcastle disease. Newcastle disease has no known cure, however antibiotics can be used for 3-5 days to stop subsequent bacterial infections such *E. coil* (Butcher *et al*., 2022).

Case description

On January 22, 2023, the owner was present at the veterinary teaching hospital in Bishoftu, Ethiopia, with their one chicken, which had been severely affected by an unknown disease from one of the poultry farm in Bishoftu. The farm owner mentioned that he had more than 1,000 fourteen-week-old chickens. The owner mentioned that there was a sudden death of chickens (a total of 8 to 10 chickens died in one day's ‘time) after the ND vaccination at 12 weeks. The total mortality was 168. During the physical examination, there was depression, as indicated in Figure 38A. On the day of the visit to the farm, there were around 30 chickens with sneezing, greenish-watery diarrhea, depression, and a twisted neck, as indicated in figure 38B, and dead birds as indicated in figure 38C. Based on history, clinical findings, and a postmortem picture, the case was tentatively diagnosed as New Castle disease.

Figure 38: ND affected birds: (A) Infected chickens in VTH during the first visit, (B) Greenish watery diarrhea during farm visit (C) Diseased chickens due to NCD

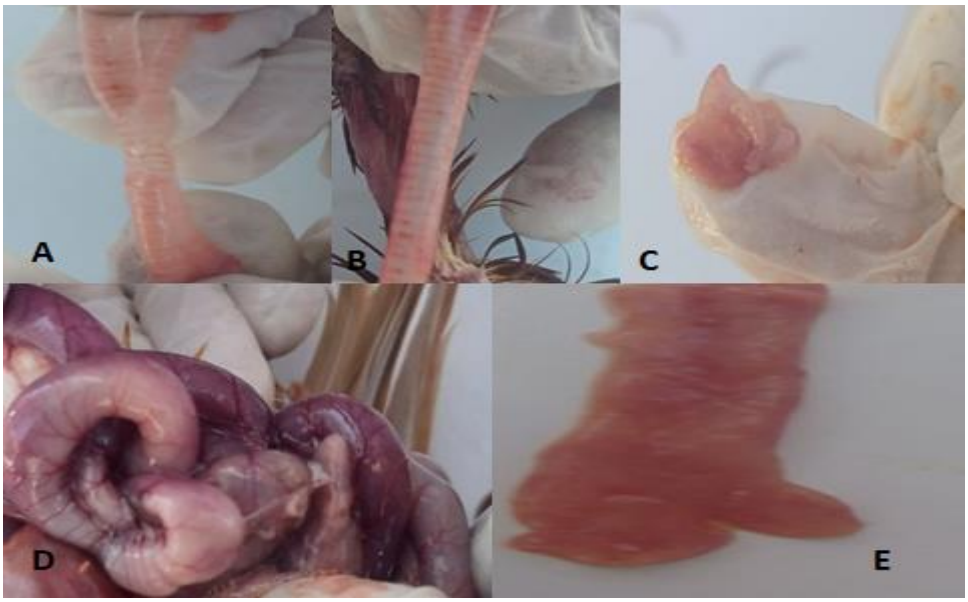

Figure 39: ND affected bird Post-mortem findings: (A and B): Tracheal hemorrhage, (C) Petechial hemorrhage in cecal tonsil, (D and E) Hemorrhage in the intestine.

Laboratory finding

For further confirmation of the case, pulled tracheal and cloacal samples were taken by using sterile swab from affected chickens and virus transporting media (VTM) was used to collect and store the sample until processes. Then Real-time reverse transcriptase polymerase chain reaction (RT-PCR) confirmatory diagnosis was done at National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta. Therefore, the laboratory finding complemented the tentative diagnosis and confirmed the chickens were affected by NCD (Figure 40).

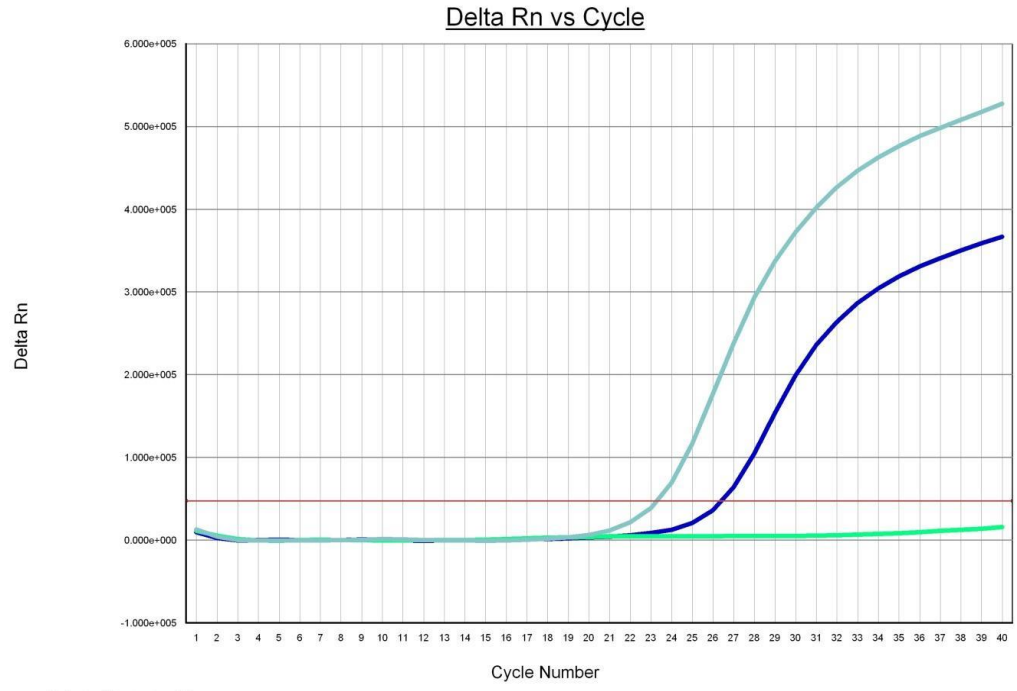

Figure 40: Real-time reverse transcriptasepolymerase chain reactions (RT-PCR) result.

Case management and out come

The case was treated with antibiotic and vitamin to reduce secondary bacterial complications, enrofloxacin (ashienro 10%) oral suspension 1 ml per liter of drinking water was given to 30 diseased chickens for 5 consecutive days, and an immune stimulant called Resurgen (Quadragen Vet Health Pvt. Ltd) oral suspension containing essential oils, vitamin E, and selenium was given to all 802 chickens in a dose of 1 g/5 lit until good progress was being made. After the first mortality, two or three deaths were recorded each day. Even though the mortality rate reached 18.3 %, the deaths stopped a week later and the surviving flock returned to normal status as indicated in Figure 41.

Figure 41: NCD affected layers after recovery

Discussion

Newcastle disease in these clinically affected chickens involves different systems and organs. Signs associated with GIT include anorexia and diarrhea, signs and signs associated with nervous symptoms like twisting of neck were highly visible signs. These clinical signs are in line with the clinical signs listed by (Bello *et al*., 2018b). The post mortem findings indicate the presence of haemorrhage in gastrointestinal tract and respiratory tract that include tracheal. This is in agreement with the review of (Alemneh, 2019). Since the flock was brought from a farm that produced pullets, the vaccination

history of the flock is unknown from a prevention and control standpoint. However, the owner vaccinated the flock at 12 weeks of age after the flock was brought, and mortality was 18.3%. This could be as a result of the vaccine's challenge in protecting them from more complex cases and mortality because as (Dimitrov *et al*., 2017) says that ND vaccination usually protects the bird from the more serious consequences of disease in the presence of the virus Regarding vaccination the challenge in this small scale farm is about the vaccine itself. As the remained flock has to be vaccinated protecting the proper vaccination procedure is very important for its effectiveness. Enrofloxacine was used in this case to combat secondary bacterial complicetions. The reason behind was that the flock was this group of drugs has a very good action in different body systems including GIT and respiratory systems and immunohistopathological response in Newcastle diseas; this is in agreement with (Sureshkumar *et al*., 2013).

In conclusion, Newcastle disease is one of the most significant animal diseases in the world, both in terms of number of animals affects annually and the significant financial losses it takes on the poultry sector. To prevent financial losses and treat the condition as soon as possible, rapid and accurate ND detection and confirmation are crucial. Hygiene and immunization are the two main methods for preventing Newcastle disease.

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4.4.3 *Salmonellosis in Broilers*

Abstract

Salmonellosis is a bacterial infection caused by the Salmonella bacteria which most commonly affects poultry, but can also affect humans, mammals. This case study was summarizing at a flock of broiler which were suffering from Salmonellosis. Clinical signs of the infection was included ruffled feathers, diarrhea (yellowish in color), and weight loss. Diagnosis of the infection was made through history taking, clinical signs observed and laboratory testing of fecal samples from the affected birds. Treatment of the flock consisted of the administration of antibiotics, supportive care, and strict biosecurity measures. The outcome of the case was good, with all birds recovering and returning to normal within one week. This study demonstrates the importance of early detection and intervention when dealing with Salmonellosis in poultry. It also highlights the effectiveness of antibiotics in treating the infection and improved sanitation, proper nutrition, and vaccination are also important for preventing and controlling salmonellosis in broilers to prevent the spread of the disease.

Keywords: *Diarrhea, Poultry, Salmonella, Treatment,*

Introduction

Salmonellosis is a serious zoonotic disease caused by the bacteria Salmonella spp. It is a significant factor in the morbidity and mortality of both human and animals, particularly poultry. As one of the primary causes of human salmonellosis, broilers are particularly sensitive to the disease (Castro-Vargas *et al*., 2020). One of the most prevalent bacterial infections in poultry, Salmonella infection is brought on by a range of Salmonella species and results in significant economic losses due to mortality and decreased production. (Rajagopal & Mini, 2013). Salmonella spp. are Gram-negative, short, plump-shaped rods that can be found in the environment, in the gastrointestinal tracts of animals, and in food products. They are also non-spore-forming, non-capsulated, aerobic, and facultatively anaerobic organisms (Fàbrega, 2013).

Avian salmonella infection may occur in poultry either acute or chronic form by one or more member of genus Salmonella(Hossain *et al*., 2006). Around the world, it is a serious chicken disease. Salmonella gallinarum (S. gallinarum) and Salmonella pullorum (S. pullorum) are salmonellae that are specific to avian hosts. Salmonella enterica biovars Gallinarum causes poultry typhoid, whereas Salmonella pullorum causes Pillorum sickness.(Kumari *et al*., 2013). Although they may not have the same zoonotic potential as Salmonella typhimurium or Salmonella enteritidis, they can nevertheless significantly reduce chicken survival rates, which would result in a significant economic loss (Sanni *et al*., 2022).

Consuming infected food or water triggers the start of Salmonella's pathogenesis. Salmonella can survive a wide range of temperatures, including those in refrigerators, and can persist in dry environments for months or even years. Once swallowed, the organism is absorbed by the intestine where it can attach to the mucosa and infiltrate the epithelial cells of the intestine. Adhesins, a class of proteins found on the surface of the organism, are responsible for this attachment. The creature can generate poisons that harm the epithelial cells once it has adhered to the intestinal mucosa, which enables the organism to penetrate deeper into the tissues.(Santos *et al*., 2003). Another significant

route of transmission is thought to be the cloacal and nasal routes of infection in newly born chickens. Early infection of chicks may happen by vertical transmission from an infected ovary, oviduct, or from the infected eggs as they pass through the cloacal feces of infected or carrier chickens (Kabir, 2010).

In grown-up chickens either an acute enteritis with greenish diarrhea or a chronic genital disease with decreased egg production are brought on by salmonellosis. Ruffled feathers, insatiability, thirst, yellow diarrhea, dejection, and reluctance to move are other persistent symptoms.(DALAI *et al*., 2015). Salmonella is a common pathogen found in poultry and can cause a variety of pathological changes in a variety of organs. The gastrointestinal tract, respiratory system, reproductive organs, liver, kidneys, and heart can all be affected by Salmonella, leading to decreased functioning, increased risk of infection, and even death (Zulqarnain *et al*., 2021). Salmonellosis in poultry can be diagnosed using a variety of techniques, including history, clinical findings, laboratory cultures, serological testing, molecular assays, post-mortem exams, and fecal or oropharyngeal swabs (Oie., 2019).

One of the most commonly used treatments for salmonellosis in poultry is antibiotics. Such treatments are typically administered either orally or through injection. However, antibiotics are not always the most effective approach in treating salmonellosis. In some cases, the bacteria may become resistant to the antibiotics, or the bacteria may remain in the bird's intestines after treatment, meaning the infection can return. Additionally, long-term antibiotic use can cause other health issues in poultry, such as antibiotic-resistant infections and the development of antibiotic residues in eggs (Randall *et al*., 2006).

As an alternative to antibiotics, vaccination is also used to control salmonellosis in poultry. Vaccines are available for many of the most common types of Salmonella, including Salmonella Enteritidis and Salmonella Typhimurium. Vaccination can be effective in preventing and controlling the disease, as it stimulates the bird's immune system to produce antibodies against the bacteria. Vaccination alone is not sufficient for complete control of the disease, however, as it may not be effective in preventing the spread of the disease from bird to bird (Hu *et al*., 2021).

Case description

A phone call was received from one poultry farm in Bishoftu. The owner of the farm mentioned that he had 1500 chickens. But they reduced their feed intake and showed decreased body, ruffled feathers, diarrhea (yellowish in color) of some of the chicken. The farm owner managed to isolate thus diseased chicken in to isolation pen. Chickens in the main shed of the farm also show clinical sign but; depression, yellow diarrhea and refused to move as indicated on figure as below. The body temperatures of selected 3 chickens were measured and it ranges between 39.1^0c to $41.^0$c, which was in a normal range. The feed in the store was in the isolation room without keeping the hygienic condition of the house.

Figure 42: Salmonella affected bird during clinical examination

Laboratory finding

Pre-enrichment was done by using buffered peptone water at sample collection step. Swab was putted in a test tube with 10ml sterile buffered peptone water and incubated at 37°C for 24 hrs. And Rappaport-Vassiliadis medium with soya (RVS broth) was used for secondary enrichment. 0.1 ml of the pre-enriched culture was transferred to a tube containing 10 ml of the RVS broth and incubated the inoculated RVS broth at 41.5 °C for

24 hrs. A sample suspected to be positive as indicated figure A at enrichment step was cultured on selective media (XLD). On XLD, salmonella have a slightly transparent red colony with black center as indicated on figure B. Based on the clinical signs, history and laboratory result, the current case was confirmed as Salmonellosis.

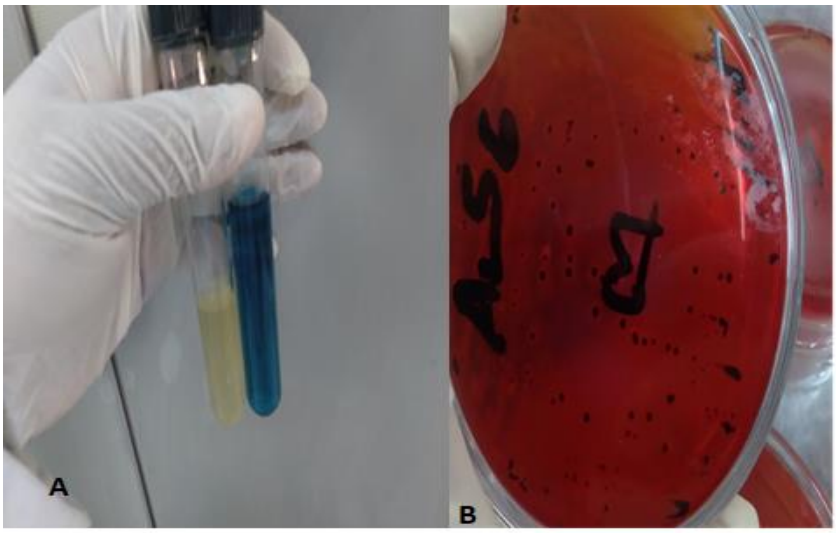

Figure 43: Showing the positive result on RVS (A) and XLD (B)

Treatment and Outcome of the case

Sulphadimidine sodium 1 kg per 2000 litres of drinking water for 5 day was given to all chicken in the farm. To compensate the fluid loss due to diarrhea, for the chickens in isolation pen, one table spoon of salt was added per every one liter of water. This oral hydration therapy continued for 5 days. After the end of the treatment the diarrhea stopped. After a week, the chickens in the isolation pen were bright with good feed intake as the owner report through phone.

Discussion

Clinical cases of salmonellosis in poultry can cause significant health concerns for humans and animals. This paper will discuss the clinical case of salmonellosis in poultry, with a focus on the clinical signs and symptoms, diagnosis, and treatment of the infection. In broilers, the clinical symptoms of salmonellosis include lethargy, decreased feed intake, and watery droppings. The clinical signs observed in the present case were depression, decreased feed intake and yellow diarrhea and refused to move which is in agreement with (Oliveira *et al*., 2019). Diagnosis of salmonellosis in broilers is based on clinical signs and laboratory testing. In the laboratory, the bacterium is identified by culturing the organism from the affected bird's feces. The diagnosis of the current case was in line with literature Pervaiz, M., & Sultana, S. (Pervaiz, M., & Sultana, S. 2015).

Treatment of salmonellosis in broilers is primarily accomplished through the use of antibiotics. The most commonly used antibiotics for salmonellosis in broilers are sulfonamides, tetracyclines and fluoroquinolones. The current case is also treated by sulfonamides which is in line with the work of Tan *et al*., (Tan *et al*., 2022). In general, salmonellosis is a serious disease that can cause severe economic losses in the poultry industry. Early diagnosis and prompt treatment with antibiotics are important for controlling the spread of the disease and minimizing economic losses. Improved sanitation, proper nutrition, and vaccination are also important for preventing and controlling salmonellosis in broilers

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4.5. Cases Report on Dogs

4.5.1 Canine Babesiosis

Abstract

Babesiosis is a disease of the red blood cells caused by the protozoal parasite Babesia *canis,* and *gibsoni* and it is a major cause of canine morbidity and mortality. This case report describes the clinical signs, diagnosis, treatment, and outcome of a case of babesiosis in a two-year-old male German Shepherd. The dog presented with clinical signs of pale mucous membranes, lethargy, a body temperature of 38.5 oC, an increased heart rate (132/min) and respiratory rate (56/min), along with bilateral enlarged lymph nodes. The diagnosis was based on a complete history, clinical signs; a blood smear was also performed and revealed small *Babesia(B.* gibsoni). Then the dog was treated with Diminazene aceturate 3.5 mg/kg IM is prescribed for 5days, Ivermectin at 0.2 mg/kg SC and supported with intravenous fluid therapy. The Dog was also administered with multivitamin 1ml/10kg IM. After four days of post-treatment, the owner reported that the dog had died. This case report highlights the importance of diagnosing and treating babesiosis in dogs, as it can be a life-threatening condition. Treatment should be based on the individual case and should include supportive care as well as specific anti-parasitic therapy. Early diagnosis and prompt treatment are essential for a positive outcome.

Keywords*: Babesiosis, Dog, Diminazene aceturate, Ivermectin, blood smear*

Introduction

Babesiosis is a blood protozoan infection that can affect both domestic and wild animals and is spread by ticks. Babesia species are apicomplexan parasites spread by ticks that affect a variety of vertebrate hosts. Historically, the host specificity and appearance of the intra-erythrocytic forms (piroplasms) have been used to identify distinct species (Hordofa & Adugna, 2017). A variety of Babesia species can cause canine babesiosis, one of the most prevalent haemoprotozoan diseases in existence today. Babesia canis, a large , and Babesia gibsoni, a tiny, are two commonly recognized canine pathogens..(Sivajothi *et al*., 2014).

The protozoan parasites of the genus Babesia are the source of the dangerous and potentially fatal disease known as canine babesiosis. The condition is a widespread occurrence in canines everywhere and is regarded as one of the most significant canine tick-borne diseases. (Baneth, 2018). Babesiosis in dogs can be caused by several different species of Babesia, but B. canis and B. gibsoni, is the most reported species globally (Solano-Gallego *et al*., 2016). Other species such as B. vogeli, and B. conradae have also been reported in different parts of the world (Baneth, 2018).

Babesia parasites are primarily transmitted to dogs by the bite of infected ticks, particularly those from the genus Rhipicephalus, which are frequently found in warm, humid. climates (Dantas-Torres, 2010). The parasites then enter the dog's red blood cells, grow there, and decimate the cells, causing anemia and other clinical symptoms. (Baneth, 2018).

Fever, anorexia, lethargy, weakness, pale gums, jaundice, hemoglobinuria, and other systemic indications are some of the mild to severe clinical manifestations of canine babesiosis. (Solano-Gallego *et al*., 2016). The kind and subtype of the Babesia parasite, the age and immune status of the dog, the occurrence of co-infections with other tick-borne diseases, and other variables may all affect how severe the clinical indications are. (Baneth, 2018).

A combination of clinical indicators and laboratory testing are necessary for the diagnosis of canine babesiosis. Since clinical symptoms might be non-specific and can resemble those of other diseases, a thorough history and physical examination are crucial to take into account when reaching a diagnosis (Irwin *et al*., 2009). Babesiosis is most frequently diagnosed using blood testing, and a combination of tests is typically necessary for a conclusive diagnosis. (Solano-Gallego *et al*., 2016). While PCR is a very sensitive molecular diagnostic test that detects Babesia DNA in the blood, blood smears are frequently used to find Babesia parasites inside the red blood cells (Baneth, 2018). Serology tests are also helpful for identifying parasite exposure; however, they might not be accurate in identifying acute infections (Irwin *et al*., 2009). In order to determine the severity of the condition and offer supportive care, haematological and biochemical tests may also be carried out (Solano-Gallego *et al*., 2016).

Canine babesiosis can be treated using a variety of treatment modalities. Effective therapies include clindamycin, diminazene aceturate, imidocarb dipropionate, and doxycycline. Blood transfusions improve a patient's clinical outcomes. In situations of infection with microscopic Babesia species, relapses are frequently reported and clinical and parasitological cure is frequently not achieved. Anti-inflammatory medications, fluid treatment, and other supportive therapies are frequently used..(Nalubamba *et al*., 2015) . Thus, the present case report describes the case of babesiosis in dog.

Case description

A two-year-old male German Shepherd dog was presented to the Veterinary Teaching Hospital, Bishoftu, Ethiopia, in November 2022 with a history of weakness, inappetence, and reddish-colored urine for the past two days. Clinical examination of the dog revealed a body temperature of 38.5 ^{o}C, a light yellowish pale mucus membrane, an increased heart rate (132/min) and respiratory rate (56/min), weakness as indicated in the figure, and bilateral enlarged lymph nodes.

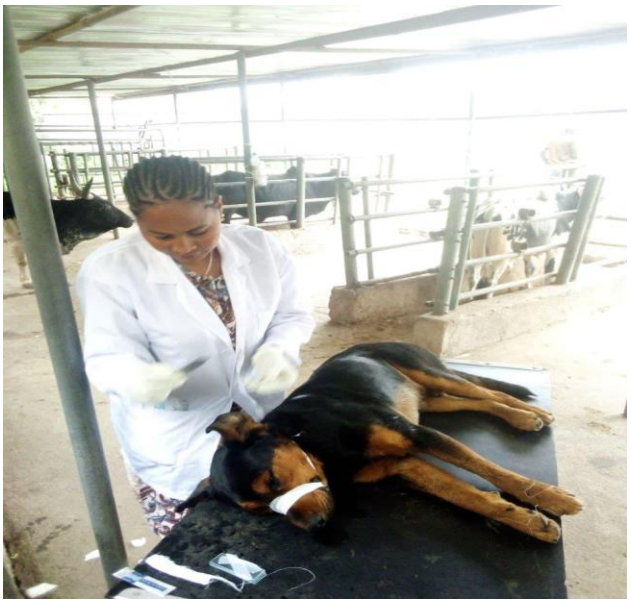

Figure 44: Canine babesiosis clinical examination on the first day at VTH

Laboratory examination

Blood was collected from ear vein and a thin smear was made. It was then heat fixed and Giemsa staining was done following the standard protocol. The result was pear-shaped piroplasms were appeared inside the erythrocytes as indicated in the figure below .

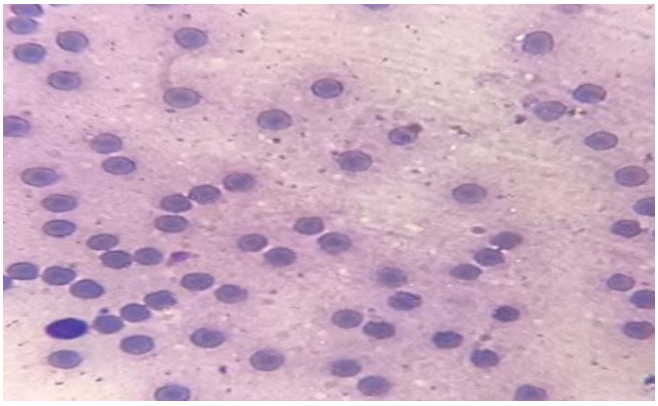

Figure 45: Laboratory result from Babesia affected Dog: small seen with in RBD in a Giemsa-stained blood smear

Case management and treatment outcome

Diminazene aceturate 3.5 mg/kg body weight is a prescribed intra-muscularly for 5days and Ivermectin at 0.2 mg/kg SC with fluid therapy. The Dog was also administered with multivitamin 1ml/10kg IM, as a supplement for iron and vitamin to increase RBC formation. administered in the first day and on the second day, the dog was in a good health as indicated in the figure as below but the owner of the dog was interrupted the treatment for Two days. After five days of post treatment, the owner reported that the dog has died.

Figure 46: The outcome of Canine babesiosis case treatment after 24 hours

Discussion

Babesiosis is a highly prevalent tick-borne disease affecting a wide range of host species worldwide, especially in tropical and subtropical countries where abundant tick population is present. Based on the history, clinical signs and laboratory result the case was finally diagnosed as babesiosis. The predominant symptoms of babesiosis observed in the present case report were pale mucus membranes, anemia, hemoglobinuria and depression were in agreement with published reports of Peter J Irwin (Irwin, 2009). Detection and treatment of babesiosis are important tools to control babesiosis. Microscopy detection methods are still the cheapest and fastest methods used to identify Babesia parasites although their sensitivity and specificity are limited and the current case

report was performed by microscopic detection of babeseia parasite (Sivajothi *et al*., 2014).

In conclusion, canine babesiosis is a serious disease that can cause significant morbidity and mortality in dogs. Early diagnosis and treatment are crucial for successful outcomes in dogs with babesiosis, and veterinarians should remain vigilant for this disease, especially in areas with high tick populations. Further research is needed to develop more effective diagnostic and treatment strategies for canine babesiosis.

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4.5.2 Flea Allergy Dermatitis in Dog

Abstract

Flea bite hypersensitivity, also called flea allergy dermatitis (FAD) is the most common skin allergy encountered in small animal veterinary medicine. In some regions of the world, it is the most commonly seen canine disease. This case study summarizes the case management of Flea allergy dermatitis in a dog that was a phone call, from one of the exotic breed dog owner for veterinary service on December 20, 2023, with history of pruritis before a week, worsening during day time even while eating the feed. According to the owner`s complaints, the dog bite his back and scratching it to inanimate objects and the dog was kept in the house and the house was infested with a flea. Upon physical examinations, close examination of the skin and hair coat of the dog revealed the presence of adult fleas around, tail and the ventral part of the abdomen. Alopecia on the tail area was observed on the dog and the body parameters (temperature, respiratory rate and pulse rate) were in normal range but the dog was itching and biting itself. The case was treated by Ivermectin, Dexamethasone eye drop topically and Penstrip were managed; the owner also informed to spray their dog and the housing of dogs. After a tow week the Dog was completely recovered. In conclusion, Flea allergy dermatitis is a skin condition that arises from a hypersensitivity to saliva from the flea. Since some dogs are hypersensitive to the saliva of fleas, dogs that had a sudden onset of severe itching and hypersensitive reactions should be checked for the presence of fleas. Avoidance of flea bites remains the best long-term solution to flea allergy dermatitis, which is accomplished by implementing an aggressive flea control program that may also include environmental controls.

Keywords*: Dexamethasone Flea Allergy Dermatitis, itching, ivermectin,*

Introduction

In small animal veterinary medicine, flea bite hypersensitivity, often known as flea allergy dermatitis (FAD), is the most frequent skin allergy. It is the most prevalent dog sickness in several parts of the world. Areas where fleas cannot live, such as those above 1500 feet in elevation or with minimal humidity, are devoid of this disease. The cat flea is one of more than 2000 known species and subspecies of fleas (*Ctenocephalides felis felis*) is the species most frequently found infesting dogs, cats, and all caged pets(Lam & Yu, 2009).

When animals become sensitized to the antigenic substance in flea saliva, flea bite hypersensitivity shows as pruritic dermatitis. Fleas squirt saliva during feeding that is made up of several histamine-like substances, enzymes, polypeptides, and amino acids that range in size from 40 to 60 kD and cause Type I, Type IV, and basophil hypersensitivity. The majority of dogs with flea allergies experience rapid skin discomfort (Wilkerson *et al*., 2004). Dogs who are regularly exposed to flea bites have low amounts of these circulating antibodies, which may explain why they either do not experience skin reactions at all or experience them much later and to a much lesser extent. This may suggest that dogs who are repeatedly exposed to flea bites build an immune tolerance on their own (Wuersch *et al*., 2006).

The flea found most commonly on both dogs and cats with a flea infestation is the cat flea, *Ctenocephalides felis*. Pets that develop FAD have an allergic response to flea saliva injected during flea feeding. The itch associated with just one flea bite persists long after that flea is gone and leads to significant self-trauma (Road & Parker, 2017). Based on the physical examination and the history, flea allergy dermatitis can be accurately diagnosed. Animals of any age or breed may get flea allergic dermatitis, which has no preference for breed or sex. Patients may experience seasonal or year-round pruritus depending on where they reside. Animals' caudal sides frequently display clinical signs, particularly in dogs(Lam & Yu, 2009). The frequency of flea contact, the length of the condition, the presence of secondary or concurrent skin illnesses, the level of hypersensitivity, and the

effects of prior or ongoing treatment are only a few of the factors that might affect the clinical signs of FAD. Other than the odd scratching brought on by irritation from flea bites, non-allergic animals may not exhibit many clinical symptoms (Bensignor, 2013).

Fleas are an allergen that must be removed in order to effectively treat FAD symptoms. The veterinary practitioner must work toward this objective while still controlling the illness in order to decrease and ultimately relieve the patient's suffering. Over time, there have been significant changes in flea management methods. With simple dosage formulations and extended residual activity, insecticides and insect growth regulators have significantly increased owner compliance and helped eradicate recurrent infestations(Carlotti & Jacobs, 2000). Thus, the present case report described the Flea allergy dermatitis in dog.

Case description

There was a phone call, from one of the exotic breed dog owner for veterinary service on December 20, 2023, with history of pruritis before a week, deteriorating during day time even while eating the feed. According to the owner`s complaints, the dog bite his back and scratching it to inanimate objects and the dog was kept in the house and the house was infested with a flea. Upon physical examinations, close examination of the skin and hair coat of the dog revealed the presence of adult fleas around, tail and the ventral part of the abdomen. Alopecia on the tail area was observed on the dog as indicated in Figure below and the body parameters (temperature, respiratory rate and pulse rate) were in normal range but the dog was itching and biting itself. Based on history taken, clinical signs observed and presence of fleas on the body of the dog, it is finally diagnosed as Flea Allergy Dermatitis.

Figure 47: Flea Allergy Dermatitis affected dog during clinical examination

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Case management and treatment outcome

In this case report the dog was treated by Ivermectin 1ml/50kg, Dexamethason eye drop topically to reduce pain during irritation and Penstrip 1ml/20kg, for secondary bacterial complication the for three-day case were managed; the owner also informed to spray their dog and the housing of dogs. After three days of end of treatment, when visited at the house the dog for check-up, the dog was bright and the owner confirmed that the dog stopped itching after the treatment. After a tow week the Dog was completely recover as indicated figure.

Figure 48: Flea Allergy Dermatitis affected dog after recovery

Discussion

Flea allergy is a very common pruritic dermatological condition in the dog. This dermatitis occurs in young adult dogs of any sex. Clinical signs are usually more severe during the warm season. They are characterized by a pruritic erythematous papular eruption affecting the caudal aspect of the dog like dorsolumbar pruritus and lesions are characteristic diagnostic criteria. Diagnosis of canine flea allergy dermatitis relies on history of housing, physical examination and lesions and presence of flea is pathognomonic sign for flea allergy dermatitis. Clinical signs and lesion distribution are strongly suggestive of the diagnosis which is agreed with the current clinical finding observed in the reported case (Laffort-Dassot, 2009b). In this case report the dog was treated by Ivermectin, Dexamethason eye (drop topically to reduce pain during irritation) and Penstrip 1ml/20kg for secondary bacterial complication for three day were managed; dog was responded to the treatment.

In conclusion, Flea allergy dermatitis is a skin condition that arises from a hypersensitivity to saliva from the flea. Since some dogs are hypersensitive to the saliva of fleas, dogs that had a sudden onset of severe itching and hypersensitive reactions should be checked for the presence of fleas. Avoidance of flea bites remains the best long-term solution to flea allergy dermatitis, which is accomplished by implementing an aggressive flea control program that may also include environmental controls.

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4.5.3 Parvovirus in dog

Abstract

Canine parvovirus infection (CPV) is an acute disease of dogs caused by canine parvovirus. Canine parvovirus can affect dogs at any age. Severe infections are most common in puppies between six weeks and six months old. The present case was presented at the veterinary teaching hospital at the College of Veterinary Medicine, Bishoftu, Ethiopia, on December 20, 2023. The three-month-old puppy, about 8kg in weight, was suffering from bloody diarrhea, loss of appetite, vomiting, and dehydration. Up on clinical examination, the puppy had a body temperature of 41.2 oC, a respiratory rate of 27 breaths per minute, and a pulse rate of 51 beats per minute. Based on physical examination, the estimated percentage of dehydration was 10% (moderate degree of skin turgor, dry oral mucous membrane, and decreased pulse pressure). On the basis of clinical signs and symptoms, it was symptomatically diagnosed as a parvovirus infection. Symptomatic and supportive treatment was performed by using a 5% dextrose infusion. To prevent the secondary bacterial complications, sulfadimidine (20 mg/kg IM/day) was given for 5 days. To stop the vomiting, an antiemetic was given orally for five days. In conclusion, canine parvovirus is a typical illness in puppies marked by severe dehydration, vomiting, and bloody diarrhea. Therefore, the goal of treating puppies with this condition should be to stop additional effacing of the intestine and reduce ongoing loss.

Keywords*: Canine parvovirus; Puppy; sulphadimidine*

Introduction

Acute canine parvovirus infection (CPV) is a condition that affects dogs. Any age of dog can contract the canine parvovirus. Puppies between the ages of 6 weeks and 4 months are particularly susceptible to severe infection. All dog breeds are susceptible. (Nandi & Kumar, 2010). One of the leading causes of morbidity and mortality in young dogs around the world is canine parvoviral enteritis. A single-stranded DNA virus that infects rapidly dividing cells in the gastrointestinal system, bone marrow, lymphoid tissue, and cardiac myocytes, canine parvovirus is a member of the genus Protoparvovirus and family Parvoviridae (Mazzaferro, 2020).

The Parvoviridae family of parvoviruses consists of tiny, single-stranded, non-enveloped DNA viruses. The feline panleukopenia virus (FPV) or a parvovirus that infected wildlife and caused hemorrhagic diarrhea are likely to have been the ancestor of the current type of CPV.CPV comes in two varieties: type 1 (CPV-1) and type 2 (CPV-2). CPV-1 was more prevalent in the 1970s; today, CPV-2 is the dominant strain in the. CPV-2 due to its greater prevalence and pathogenicity. Puppies from 6 weeks to 6 months old are most commonly affected by CPV-2.Older dogs have a lower risk of contracting parvovirus due to their protection from prior infections or vaccinations (Tabor, 2011). A very dangerous disease called CPV can spread through feces. The canine parvovirus (CPV) infection spreads quickly. among canine populations throughout and in underdeveloped nations.(Mia & Hasan, 2021).

Infection with the canine parvovirus affects household dogs and other canine family members everywhere..(Nandi & Kumar, 2010). Lethargy, inappetence, vomiting, and diarrhea are the most typical clinical symptoms of parvoviral enteritis. The diarrhea may be soft, mucoid, liquid, or hemorrhagic in appearance. Sloughing of the intestinal mucosal lining can cause the feces to appear reddish and viscous.(Mazzaferro, 2020). Testing should be done to confirm the infection even though clinical indications including fever, nausea, abdominal pain, vomiting, and bloody diarrhea in a young, unvaccinated dog are frequently very suggestive of CPV-2. A fecal antigen ELISA,

1,2,4,5 that is specific and moderately sensitive for CPV-2 (Tabor, 2011). Symptoms of a parvovirus infection typically include vomiting, severe dehydration, and bloody diarrhea. Therapeutic use of antibiotics and fluids can treat hemorrhagic gastro enteritis caused by parvovirus infection.(Munibullah *et al*., 2017). Therefore, this report describes canine parvovirus in puppy and its treatment outcome based on tentative diagnosis.

Case description

A young boy presented a 3-month-old puppy, about 8kg in weight, to a veterinary teaching hospital at the College of Veterinary Medicine, Bishoftu, Ethiopia, on December 20, 2023. The owner mentioned that he lost one puppy with signs of vomit and diarrhea two months ago. And he adopted this new puppy five days ago. Two days before he brought the puppy to the clinic, he saw his new puppy having watery diarrhea, sometimes mixed with blood, and vomiting. Then the puppy became very weak and reluctant to eat and drink. Up on clinical examination, the puppy was very weak, as indicated in Figure 49, with a body temperature of 41.2 ^{o}C a respiratory rate of 27 breaths per minute, and a pulse rate of 51 beats per minute. Based on physical examination, the estimated percentage of dehydration was 10% (moderate degree of skin turgor, dry oral mucous membrane, and decreased pulse pressure). On the basis of clinical signs and symptoms, it was symptomatically diagnosed as a parvovirus infection.

Figure 49: Parvovirus affected puppy at the first day clinical examination

Treatment and Outcome of the case

Symptomatic and supportive treatment was performed, by using by using 5% Ringer lactate infusion as indicated figureb51A. To prevent the secondary bacterial complications sulphadimidine 20mg/kg IM/SID was given for 5 days. To stop the vomit, Metoclopramide HCl 5mg BID was given orally for similar five days. The puppy was getting better each day after the commencement of treatment. After the five days of treatment the puppy started to take its food and drink by itself. After one week, it was healthy as indicated on figure (51, B&C).

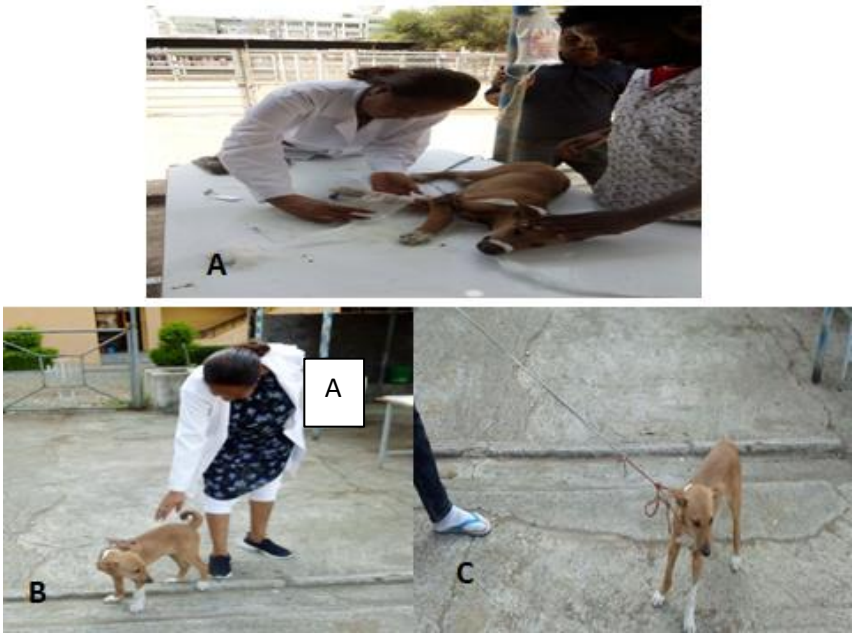

Figure 50: A supportive treatment of Canine parvovirus, by using 5 % Dextrose infusion,B and C the outcome of the Dog after one week.

Discussion

Clinical symptoms of the canine parovirus infection were anorexia, bloody diarrhea, vomiting, and dehydration in the affected canines. The affected dogs reverted to their normal pathophysiological condition between four and seven days after the administration of fluid and antibiotics along with supportive treatment in dogs(Dongre *et al*., 2015). Based on the history and clinical findings, the current case of puppy was diagnosed tentatively as canine parvovirus disease In addition to the history and clinical findings of the case the outcome of affected dogs returned to their normal condition between 5 days was in line with those reported by Dongre *et al*.,(2015). In addition, the age of the dog and the abundance of similar cases were also considered. The highest rate of occurrence of CPV below six months may be due to viral attraction for rapid multiplying intestinal crypt cells in young dogs (pups) with highest mitotic catalog due to alterations in bacterial flora as well as in the diet due to weaning may enhance the susceptibility of pups to CPV (Kalita *et al*., 2022).

The treatment protocol followed in the current case was aimed at curing the puppy via resuscitation of fluid deficit due to vomiting and diarrhea, avoiding of further effacing of the intestine by secondary bacteria and ceasing of vomiting. Hence, an intravenous lactated ringer, sulfadimidine sodium for secondary bacterial prevention and antiemetic for vomiting were administered. This is in line with the protocols stated in the literatures indicating rehydration and antimicrobial as integral therapy for puppy with parvovirus (shali & Jain, 2020).

In conclusion, canine parvovirus is a typical illness in puppies marked by severe dehydration, vomiting, and bloody diarrhea. Therefore, the goal of treating puppies with this condition should be to stop additional effacing of the intestine and reduce ongoing loss.

Limitations

Due to a lack of particular virus kit, additional laboratory confirmation was not carried out in this case; as a result, the puppy was treated based on clinical symptom and other indicators.

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4.5.4 Salmonellosis in dog

Abstract

Salmonella spp. are gram-negative, rod-shaped bacteria belonging to the family Enterobacteriaceae, order Enterobacterales. The genus Salmonella is divided into two broad species, *Salmonella enterica* and *Salmonella bongori*. This case report describes the clinical signs, diagnosis, treatment, and outcome of a case of salmonellosis in a one-year-old dog. The dog presented with clinical signs of diarrhea mixed with blood and mucus, abdominal pain, being reluctant to eat and drink, a body temperature of 41.2 oC, a respiratory rate of 28 breaths per minute, and a pulse rate of 52 beats per minute. The diagnosis was based on history-taking, clinical signs observed, and laboratory results. The dog was treated with Trimethoprim Sulfadiazine and diclofenac, along with advice to the owner to manage the dog alone with good management and maintain the hygienic status of dog housing and feeding. The dog responded well to the treatment and healed from the disease after five consecutive treatment days. The dog defecated normal stools. In conclusion, salmonellosis is an infectious disease of humans and animals caused by organisms of the two species of *Salmonella* (*S. enterica* and *S. bongori*). Salmonella pathogens are highly adaptive and potentially pathogenic to humans and other domestic animals; young dogs and cats are the most susceptible and are capable of producing serious infections of zoonosis importance. Basic hygiene practices and the implementation of scientifically based management strategies can efficiently mitigate the risks associated with animal contact.

Key words: *Diarrhea, Dog, Salmonella, Treatment*

Introduction

Salmonella spp. are Gram-negative, rod-shaped bacteria belonging to the family Enterobacteriaceae, order Enterobacterales. The genus Salmonella is divided into two broad species named Salmonella enterica and Salmonella bongori (Dróżdż *et al*., 2021). Salmonella spp. have been found in all species of mammals, birds, reptiles, and amphibians that have been investigated. These organisms have also been detected in fish and invertebrates. Infection can range from apparently "healthy" carrier animals to those that show acute signs of the infection. A spectrum of symptoms occurs with Salmonella infection, from in apparent or subclinical infections to obvious or clinical disease. Asymptomatic infections are particularly prevalent in poultry, swine, reptiles and amphibians. Salmonellosis occurs worldwide but seems to be most common where intensive animal husbandry is practiced. (Oie, 2013).

Salmonellosis is considered one of the most important foodborne zoonotic diseases. Dogs are one of the most important companion animals in the world. Close contact between dogs and humans has been recognized as a potential source of Salmonella spp. zoonotic infection.(Bataller *et al*., 2020). Transmission in dogs and cats usually occurs either directly or indirectly by the faecal–oral route. Transmission of Salmonella spp from dog to humans is possible due to the ability of Salmonella cells to survive in the environment.(Dróżdż et al., 2021).

The prevalence of Salmonella spp. in dogs is not fully understood since dogs can serve as asymptomatic carriers and excrete the bacteria with no clinical demonstrations. The dogs carrying Salmonella spp. can excrete the bacteria for more than 6 weeks.The clinical symptoms of salmonellosis in dogs include fever, loss of appetite, diarrhea, bloody diarrhea, abdominal pain, and abortion A part of these symptoms usually occurs 3-5 days after infection with the bacteria; the clinical symptoms may appear even 12 hours after exposure to bacteria. Nevertheless, the clinical salmonellosis infection in dogs is rare and the subclinical form of the disease is the dominant type in dogs (Rafiee, 2020).

Pathogenesis depends on the numbers of Salmonella ingested, the serovar or strain involved, and its virulence may deter- mine the clinical outcome of infection with Salmonella serovars. Non-specific host factors that affect the ability of organisms to establish and produce infection include gastric acidity, peristalsis, quality of intestinal mucus, lysozyme in secretions, lactoferrin in the intestinal tract and the normal resident intestinal flora. The greater susceptibility of young animals to salmonellosis may be associated in part with the lack of a well-established normal flora. In addition, a naïve or incompetent immune system renders such animals vulnerable to these enteropathogens (Khan, 1970).

Diagnosis and its differential diagnosis: Definitive diagnosis of salmonellosis is typically made by bacterial culture, usually of the feces and serological test (Demirbilek, 2018). However, diagnosis of salmonellosis is not easy because the growing Salmonella on a faecal culture does not prove that it is creating a problem because Salmonella is discovered in the feces of many dogs and cats who do not have diarrhea. If a faecal culture reveals Salmonella and your companion exhibits infection-related symptoms (fever, vomiting, watery or bloody diarrhea, etc.), it can be suspected that Salmonella is the cause (Hertzer *et al*., 2021). Campylobacteriosis, parasitic diarrhea, shigellosis, vibriosis, viral gastroenteritis and yersiniosis are differential diagnosis to salmonellosis(Metreveli *et al*., 2022).

Treatment and prevention of salmonellosis: Uncomplicated cases of salmonellosis do not warrant treatment with antibiotics but septicaemic salmonellosis can be treated with a number of antibiotics. Many isolates are resistant to one or more antibiotics, and the choice of drugs should, if possible, be based on susceptibility testing. A combination of ampicillin and enrofloxacin has been recommended for empiric therapy in dogs. Additional therapies may include nonsteroidal anti-inflammatory drugs (given to decrease the effects of endotoxemia) and antibodies to Salmonella lipopolysaccharide (Oie, 2013).

Prevention of salmonellosis dogs kept in animal shelters can be a good source for the distribution of different serovars of Salmonella in the environment. Animals, especially carnivores, as the asymptomatic carriers of Salmonella spp. play a significant role in this cycle. When salmonellosis has been confirmed in a dog, the following control measure should be considered; isolate obviously affected dog from human and animal shelters and determining the antibiotic susceptibility pattern of Salmonella species isolated from such animals can provide valuable information for the identification of ineffective medicines and replacing them with useful ones in order to promote and maintain public health(Rafiee, 2020). Thus, the present case report described the case of salmonellosis in dog.

Description of the case

A 1-year-old dog presented to the veterinary teaching hospital at the College of Veterinary Medicine, Bishoftu, Ethiopia, on December 20, 2022. The owner mentioned that the dog had diarrhea mixed with blood and mucus and abdominal pain for 5 days before becoming reluctant to eat and drink. The dog was also emaciated, as indicated in the figure below. The dog was kept in the house and fed over "enjera," which is left over from the family as a past history. Up on clinical exemption, the dog had a body temperature of 41.2 ^{o}C, a respiratory rate of 28 breaths per minute, and a pulse rate of 52 beats per minute. Based on the history and clinical signs observed, the case was tentatively diagnosed as salmonellosis, and its differential diagnoses for this case were colibacillosis and coccidiosis.

Figure 51: Salmonella affected dog during the first day

Laboratory findings and its investigation

The fecal sample was taken from the ox and sent to biomedical and parasitology laboratory of Addis Ababa University for further confirmation. Fecal floatation technique was performed for the detection of Emeria/Coccidian oocytes and the result was negative. The sample was enriched in Tryptone soya broth for 24 hours. The sample from the enriched media was cultured on XLD media and also checked for *E. coli* by cultured on EMB, no growth of bacteria was detected. Bacteria (salmonella) growth was observed on the XLD media.as indicated on figure as below. Based on the history and clinical sign observed and laboratory result the case was diagnosed as salmonellosis.

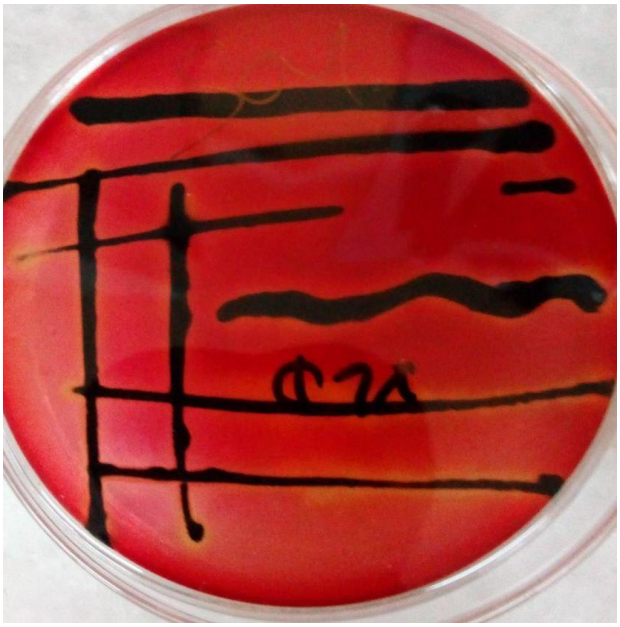

Figure 52: Laboratory result from Salmonella affected dog on XLD

Treatment and outcome

Based on history, clinical signs observed and laboratory result the case was finally diagnosed as salmonellosis. As the best medical therapy for this case Trimethoprim Sulfadiazine 30mg/kg (Hebei Yuanheng Pharmaceutical Co., Ltd, China) was administered for five days intramuscularly and 3 mg/kg/day diclofenac was given; the owner was also informed to manage the dog alone with good management and keeping the hygienic status of dog housing and their feeding. The dog was responded well to the treatment and healed from the disease after five consecutive treatment days the dog was defecated normal stool as indicated in figure as below.

Figure 53: Salmonella affected dog after therapy**.**

Discussion

Salmonella spp. infections are still a major issue around the world. These infections have the potential to result in substantial economic losses, human morbidity and mortality, and animal production losses. People, other species of livestock, and pets are all susceptible to infection and risk developing severe illnesses(Chase & Lunney, 2019). The current case was diagnosed as a Salmonellosis based on the history and clinical signs observed and also the laboratory result it was salmonellosis. The clinical changes seen in the current case report were fever, lethargy, lack of appetite and fetid diarrhea which was agreed with the clinical signs observed in (Hertzer *et al*., 2021). The treatment given for this case was Trimethoprim sulfadiazine 30mg mg/kg for five days. The treatment option used in the current case provided good response to the disease and it was in agreement with the treatment recommended for salmonellosis by Oie (Oie, 2013).

In conclusion, salmonellosis is an infectious disease of human and animal caused by organisms of the two species of Salmonella (S. enterica and S. bongori). A Salmonella pathogen are highly adaptive and potentially pathogenic to human and other domestic animals; young, dog and cat are the most susceptible and are capable of producing a serious of infections having zoonosis importance. Basic hygiene practices and the implementation of scientific based management strategies can efficiently mitigate the risks associated with animal contacts.

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4.6. Case of swine

4.6.1. Sarcoptic mange in swine

Abstract

Sarcoptic mange is a highly contagious skin disease caused by the mite *Sarcoptes scabiei*. It is a common problem in swine, with clinical signs such as severe pruritus, alopecia, and crusty skin lesions. This case report summarizes the case of sarcoptic mange in a flock of 68 swine on one of the swine farms in Bishoftu, Ethiopia. They were kept under intensive farming. The owner bought one new swine, whose history is not known, from the other farm five weeks ago and mixed them with the others on the farm. A week before, 15 swine died. There were swine with skin rubbing against the sides of the pen and skin lesions on the ear. On clinical examination, the body parameters were in the normal range, and ear shaking, severe rubbing of the skin against the sides of the pen, and skin lesions on the ear were observed. The case was diagnosed based on clinical signs. Skin scrapings of affected areas were examined under a low-power microscope. A sarcoptic mange mite was seen on the slide. The case was treated with two injections of Ivermectin, ten days apart, which usually eliminate the mite and keep the affected pigs out. Control includes spraying the house and premises. The outcome of treatment was generally good, and the swine started to show improvement within a few days of starting treatment. After two months, the case was fully recovered. In conclusion, sarcoptic mange is a common skin condition in swine that can be successfully treated with the use of topical and systemic antiparasitic agents. Early diagnosis and treatment are essential for a successful outcome, as demonstrated in this clinical case report.

Keywords*: Sarcoptic mange, Skin scrapings. Ivermectin*

Introduction

Sarcoptic mange, caused by the burrowing mite *Sarcoptes scabiei*, is a highly contagious and economically important disease in swine production worldwide (Song & Moon, 2020, Jerome Goddard, 2017). The mites are transmitted through direct contact between infected and susceptible pigs and can survive off-host for up to two weeks (OIE, 2019). Sarcoptic mange is a common skin disease in swine caused by the *Sarcoptes scabiei* mite. It is characterized by intense itching and a variety of clinical signs including alopecia (hair loss) and crusting. Clinical case reports of Sarcoptic mange in swine have been documented in many countries The clinical signs of Sarcoptic mange in swine include intense itching, hair loss, and crusting of the skin). In addition, pruritus (severe itching) may be present (Wittmann, *et al*., 2018).

Diagnosing sarcoptic mange is usually based on the clinical signs and favourable response to treatment. To make a positive diagnosis, skin scrapings may be needed to find the mites, although not finding them in a sample is not conclusive. Veterinarians scrape with a blunt scalpel or blade deep enough to draw blood. The edges of active skin lesions are the best places to look for mites. Oozing material from the outside of the ear is also a good place to look for mange mites (Jerome Goddard, 2017; Wittmann, *et al*., 2018).

Several treatment options are available for sarcoptic mange in pigs, including ivermectin, moxidectin, and selamectin. In addition, topical treatments such as lime, sulfur, but the efficacy of these products can vary depending on the mite population's susceptibility and the treatment regimen used (Miller, 2013). Control measures for sarcoptic mange in swine farms include improved biosecurity practices, such as quarantining new animals and disinfecting equipment, and regular monitoring and treatment of infested pigs (Miller, 2015). Where it is applicable, vaccination against sarcoptic mange has shown promise as a long-term control strategy, but more research is needed to develop effective vaccines for swine (Pasmans *et al*., 2021). Thus, the clinical signs, diagnosis, treatment,

and outcome of the treatment of Sarcoptic mange in swine have been discussed in this paper.

Case description

A phone call was received from a swine farm in Bishoftu, Ethiopia, in November 2022. The owner mentioned that he had 68 pigs. They were kept under intensive farming. The owner bought one new swine, whose history is not known, from the other farm five weeks ago and mixed them with the others on the farm. A week before, 15 swine died. He mentioned that there were also swine with skin rubbing against the sides of the pen and skin lesions on the ear at the time of his call. Up on clinical examination, ear shaking, severe rubbing of the skin against the sides of the pen, and skin lesions on the ear were observed.

Figure 54: Sarcoptic mange affected pig during clinical examination

Sample collection and Laboratory finding

A skin scraping was taken from the ear and skin digestions with hydroxide potassium were performed. Have sarcoptic mange through identification of S. scabiei on skin scrapes. Full-thickness skin samples were preferentially collected from areas of skin that were most severely affected, as evidenced by alopecia, skin crusting, thickened skin, and when possible, locations where mites were observed in initial skin scrapes. Samples were placed in individual sealable plastic bags and stored at −20°C until the digestion trials were performed. the skin scraping was in 10% potassium hydroxide (KOH) solution to facilitate identification Apply a cover glass to the scraping. Examine the preparation were under low power (10x) Sarcoptic mange mite were seen on the slide as indicated on figure as below.

Figure 55: Sarcoptic mange mite from the affected pig.

Treatment and management

Two injections of ivermectin (1 ml/50 kg) ten days apart and the owner was advised to keep the affected pigs out and spray the house and premises. Additionally, make sure the purchased pigs are also free from mange mite and quarantine them. The outcome of treatment was generally good, and the swine started to show improvement within a few days of starting treatment. The clinical signs cleared up within four weeks, although some animals may require additional treatments. After two months, the case was fully recovered, as indicated in the figure below.

Figure 56: Sarcoptic mange affected pigs after recovery :(A): during the first injection, (B& C): after treatments.

Discussion

Sarcoptic mange is a significant concern for swine producers worldwide due to its impact on animal welfare and economic losses. The disease can cause intense pruritus, hair loss, and thickening of the skin, leading to reduced weight gain, poor feed conversion, and increased susceptibility to secondary infections. In addition, infested pigs can serve as a source of infection for other animals and humans, emphasizing the need for effective control strategies (Song and Moon, 2020). The clinical signs observed in this case: nclude intense itching, hair loss, and crusting of the skin are all similar findings with the clinical signs of the disease written on literature Whitman, *et al*., (2018).

In literatures, Sarcoptic mange is usually diagnosed based on the clinical signs and physical examination of the animal.A skin scraping and/or biopsy may be done to confirm the diagnosis. In addition, a blood test may be done to identify the presence of antibodies to the mite used (Miller, 2013). But in this case diagnosis was performed based on the clinical signs, physical examination of the animal and skin scraping only due to lack laboratory reagent.

In literatures, the treatment of Sarcoptic mange in swine involves the use of antiparasitic drugs (Wittmann, *et al*., 2018). These include ivermectin, moxidectin, and selamectin. In addition, topical treatments such as lime, sulfur, and tar may be used to help control the mites. But in this case study treatment with injectable ivermectin only due to lack of the drug choice in the country. In conclusion, sarcoptic mange is a significant challenge for swine producers worldwide. The disease can cause substantial economic losses and impact animal welfare, emphasizing the need for effective control measures. Integrated control strategies that include improved management practices, early detection, and treatment of infested pigs, and the development of effective vaccines are necessary to reduce the impact of sarcoptic mange on swine production.

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5. CONCLUSION AND RECOMMENDATIONS

This paper presents conclusions and recommendations based on the findings from clinical case studies on major animal diseases. The case studies encompassed various diseases, including bacterial, viral, fungal, and parasitic infections, with their respective etiologies. Animals of different species, sexes, and ages were examined, and most cases were diagnosed based on clinical history and signs, and several were confirmed through laboratory tests. The study emphasized the importance of employing comprehensive clinical case handling methods, administering prompt and appropriate treatment, and ensuring diligent follow-up to mitigate the impact of diseases on animals, their owners, and the overall nation. Supportive therapy, in conjunction with antimicrobial treatment, proved effective in alleviating animal suffering and promoting their recovery but some of them was died due to delaying of the owners of the animal. However, certain challenges were identified during the study period in the area. These included the absence of adequate fluid therapy during treatment and a lack of follow-up care, resulting from owners bringing their sick animals when they were already weak or malnourished. The primary causes of these diseases were attributed to improper husbandry practices, insufficient nutrition, poor hygiene, overcrowding, and limited access to preventive healthcare services. Additionally, the study revealed that the lack of necessary laboratory reagents in functioning laboratories, such as the multipurpose laboratory in the Veterinary Teaching Hospital, and the shortage of trained personnel in molecular biology and virology laboratories, contributed to a lack of confirmatory diagnosis and potential misdiagnoses.

Based on the conclusion provided above, implementing the following recommendations will contribute to improving animal health, reducing disease burdens, and promoting the overall well-being of animals:

- ✓ Strengthen the capacity of Animal Health Facilities in Veterinary teaching hospital, by providing adequate resources and personnel training.
- ✓ Veterinary teaching hospital need inpatient care facilities should be needed

- ✓ Increase awareness on handling and management of diseases, regarding proper husbandry practices, nutrition, hygiene and preventive health care services among animal owners.
- ✓ Address the shortage of laboratory reagents in functional laboratories in CVMA
- ✓ Trained personnel in Molecular biology and virology laboratories should be there
- ✓ Confirmatory diagnosis by taking appropriate sample is recommended to reduce the drug resistance and risk of transmission of diseases to other animals and human being
- ✓ Educate animal owners about the early signs and symptoms of diseases, stressing the importance of seeking veterinary care promptly. Encourage them to bring their animals for check-ups at the earliest indication of illness to prevent further complications.
- ✓ Promote research activities and knowledge sharing within the veterinary community. Encourage clinicians and researchers to conduct more clinical case studies to further understand the diseases prevalent in the area and develop effective treatment strategies.

6. ANNEXES

Annex 1: Clinical Examination protocols

Recording of Rectal Temperature

Recording of body temperature of animal is most important in clinical diagnosis. Temperature should be recorded while the animal is at rest. Generally rectal temperature is recorded in animals by inserting the bulb of a clinical thermometer in the rectum, placed in contact of the rectal mucosae and keeping it for one to two minutes.

Recording of Pulse Rate

Usually the pulse rate is equal to the rhythmic contraction and expansion of heart. Increased pulse rate is common and occurs in most cases of septicaemia, toxaemia, circulatory failure, excitement and in pain striken condition. Marked slowing of heart beat (bradycardia) is common in traumatic reticuloperitonitis in cattle.

Site for Recording Pulse

- *Cattle*: Middle coccygeal artery, ventral coccygeal artery under the tail, facial artery, maxillary and median artery; femoral arteries (in case of calf).
- *Dog*: Femoral artery on the inner side of thigh.

Recording of Respiration Rate

In cattle average respiration rate per minute is 12-16. Variation occurs due to high ambient temperature, after exercise and it is normal. Respiratory rate is accelerated during fever and respiratory distress due to disease. Respiration rate should be noted when the animal is at rest. The type of respiration like costal, intercostal, abdominal, jerkey *etc*. are also to be noted. There is a ratio of 1:3 between respiration rate and pulse rate in healthy animals. Examination of respiration rate of animals is indicated for primary respiratory disease as well as secondary respiratory disease due to cardiac involvement, allergy and anaphylaxis.

Examination of Visible Mucous Membrane

This includes the examination of conjunctiva, buccal, nasal, vulval, vaginal and rectal mucosae. In normal and healthy condition of animals, the mucous membrane is moist and rosy in colouration. The following changes of mucous membrane are seen in unusual conditions of animals.

- *Congestion*: Signs of fever and inflammation, systemic diseases and allergic sensitization.
- *Paleness*: Revealing anaemia, internal haemorrhage, hypoproteinaemia, excessive blood loss and shock.
- *Yellow discolouration*: Signs of ecterus and hepatic disorder, jaundice.
- *Pin point/Petecheal haemorrhages*: Indicates septicaemia, surra, phosphorus and arsenic poisoning.
- *Cyanotic changes*: Bluish discolouration owing to dyspnoea, hypoxia, venous stasis, congestive cardiac failure, pleurisy, HCN and nitrate poisoning.
- *Ulcerations*: Typical ulcers on oral mucous membrane seen in FMD, PPR and RP.
- *Pinkish*: Equine infectious anaemia.

Examination of Eyes

Ophthalmic examination gives some clues in diagnosing some diseases.

- *Sunken appearance*: Indicates chronic wasting disease and dehydration.
- *Pupillary reflex*: Loss of pupillary reflex and pupillary response to light are seen in toxaemia and shock, poisoning and CNS disease.
- *Dilatation of pupil*: Seen in poisoning and shock.
- *Corneal opacity, ulcers*: Commonly occurs in mechanical injury or trauma. In canine it could also be due to canine distemper.

Normal Colour of Conjunctiva of Various Animals

Cattle and Buffalo – Pale pink

Horse – Pale roseate

Sheep and Goat – Pale pink

Pig – Reddish tinged

Dog – Roseate

Cat – Pale.

Palpation

Consistency of an organ or tissues or a part of the body can be felt by lying hand with gentle pressure. Tips of fingers and flat of the hand are mostly used for handling the tissues or organs. When tissue appears firm, hard, solid like muscle, that could be a neoplasm (tumour). When structure appears bone like consistency – it could be the exostosis or ossification of cartilage. Hot and painful swelling, hard or soft could be the abscess (hard in initial stage, soft in maturity/ripened abscess).

- *Doughy* – Where soft tissues retain finger points, or causes pits on pressure – oedema and impaction of rumen.
- *Cold and painless (fluctuating)* – could be the cyst distended with gas (bloat), distended with food (impaction), distended with fluid (ascites), crepitating sound (Black Quarter or Subcutaneous emphysema). Abnormalities of abdominal and urogenital organs can be felt by rectal palpation.

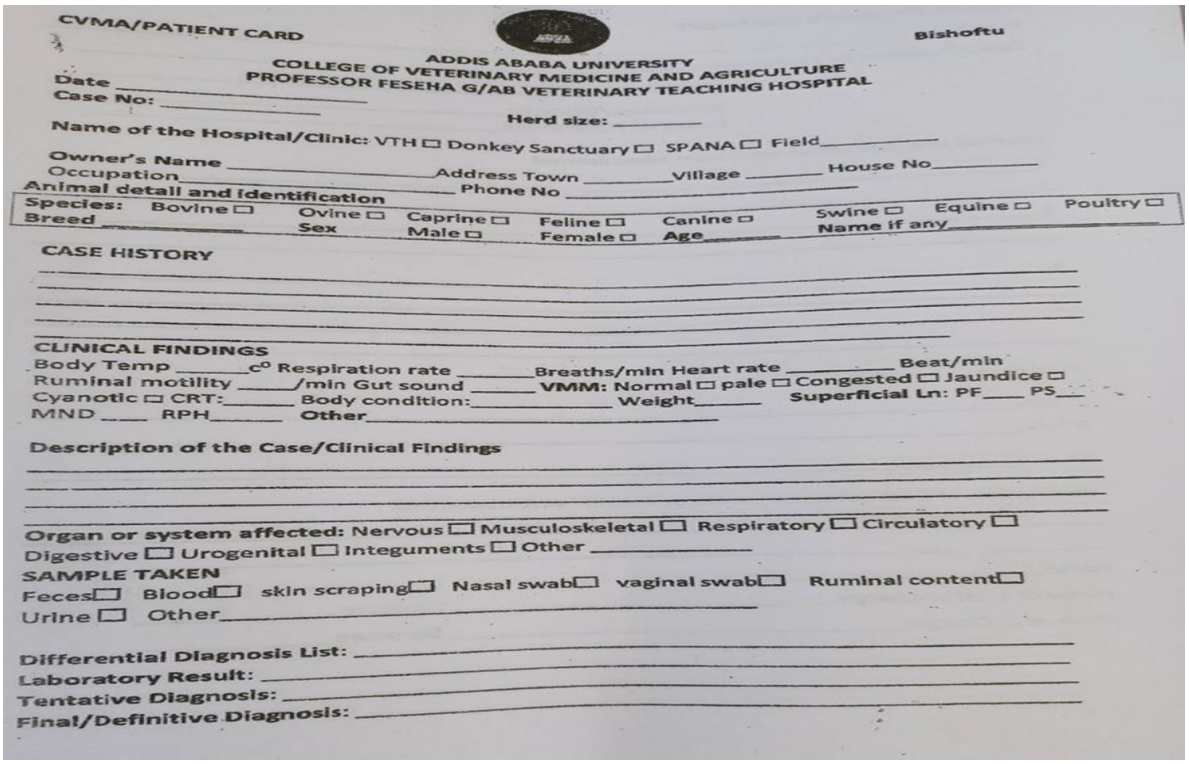
Percussion

Striking of any part of the body with a short, sharp blow that enables underlying organs to vibrate and generate an audible sound is called percussion. Drum like sound audible from rumen indicates tympanitis, dull resonance in impaction. Hyper resonant sound is observed while the lungs are filled with excessive air. Increased amount of gases will emit tympanic sound in abdomen. This method is useful in small animals than the large animals.

Auscultation

It means listening of various functional sounds produced by some thoracic and abdominal organs by use of stethoscope for ascertaining the pathological condition of lungs, pleura, heart and certain parts of alimentary tract. It is useful for hearing peristaltic sounds during ruminal and intestinal contractions, listening sounds produced in course of normal functioning of trachea and lungs (dry rales in congestion and moist rales in exudation), cardiac sounds like cardiac murmurs in valvular disease, splashing sounds in pericarditis and hydro pericardium.

Annex 2: Clinical Case Recording Form of VTH of Addis Ababa University



CVMA/PATIENT CARD

Bishoftu

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 _____
Occupation_____Address Town _____Village _____ House No_____
Animal detail and identification _____ Phone No _____

Species: Bovine ☐ Ovine ☐ Caprine ☐ Feline ☐ Canine ☐ Swine ☐ Equine ☐ Poultry ☐
Breed _____ Sex Male ☐ Female ☐ Age_____ Name if any_____

CASE HISTORY

CLINICAL FINDINGS

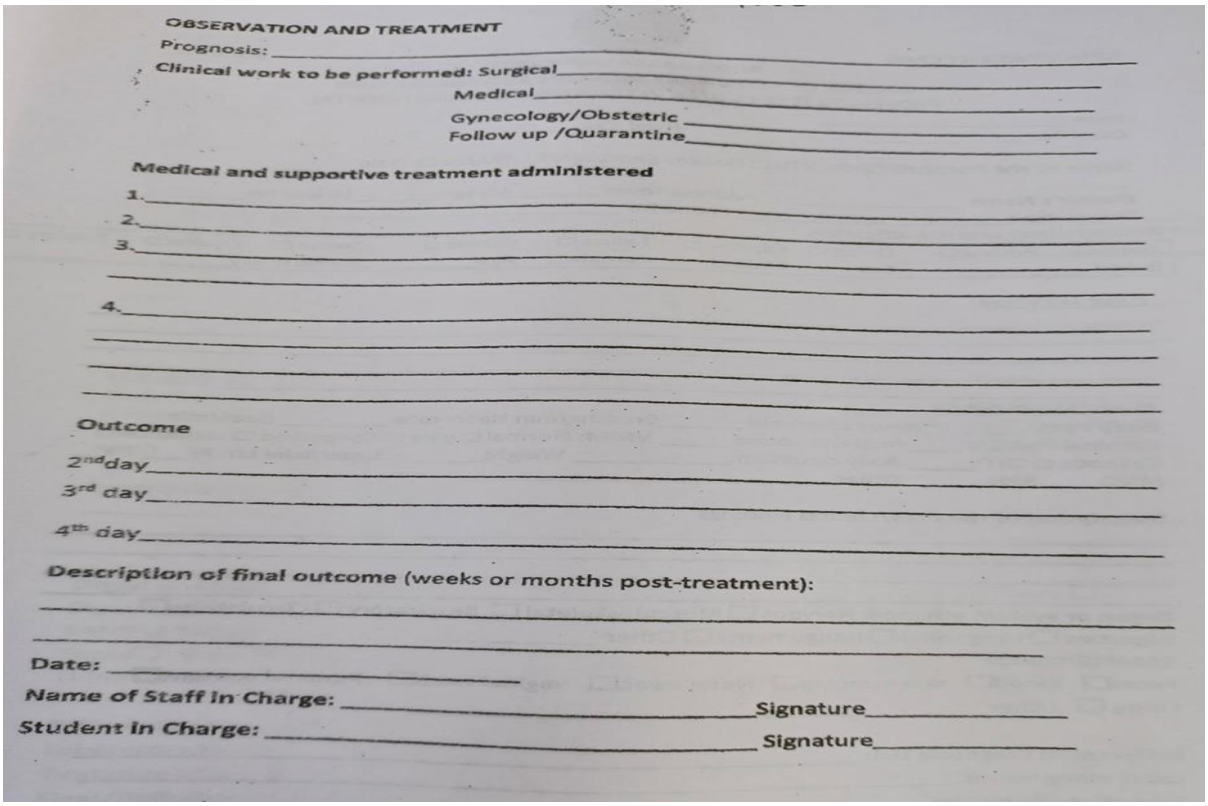
Body Temp _____c° Respiration rate _____Breaths/min Heart rate _____ Beat/min
Ruminal motility _____/min Gut sound _____ VMM: Normal ☐ pale ☐ Congested ☐ Jaundice ☐
Cyanotic ☐ CRT:_____ Body condition:_____ Weight_____ Superficial Ln: PF____ PS___
MND ___ RPH_____ Other_____

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: _____



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_____

Annex 3: Gram-staining procedure

1. Place slide with heat fixed smear on staining tray.
2. Gently flood smear with crystal violet and let stand for 1 minute.
3. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
4. Gently flood the smear with Gram‘s iodine and let stand for 1 minute.
5. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle. The smear will appear as a purple circle on the slide.
6. 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.
7. Immediately rinse with water.
8. Gently flood with safranin to counter-stain and let stand for 45 seconds.
9. Tilt the slide slightly and gently rinse with tap water or distilled water using a wash bottle.
10. Blot dry the slide with bibulous paper.
11. View the smear using microscope under oil-immersion.

Annex 4: Protocol of RT-PCR in Newcastle virus detection

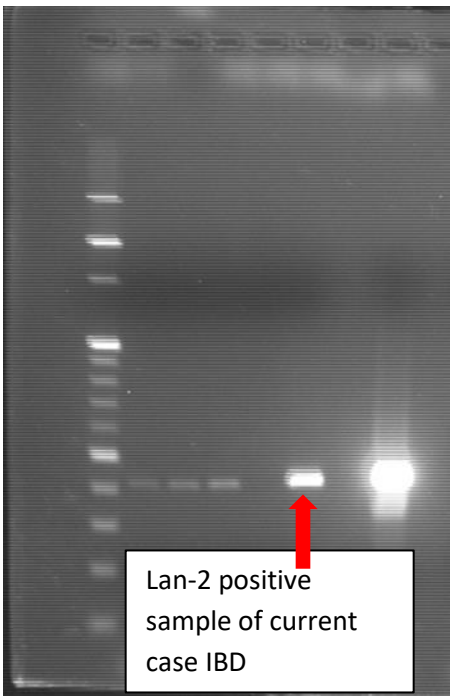
RNA extraction

Viral RNA extraction from one pools of tracheal, cloacal swabs and tissue were conducted using QIAamp viral RNA extraction kit according to manufacturer's instruction. The sample was centrifuged briefly in order to get cell free supernatant. The supernatant was lysed by adding 560 µl of prepared buffer AVL containing carrier RNA in to 1.5 micro centrifuge tube and 140 µl of sample was added to the buffer AVL carrier RNA in the micro centrifuge tube. The solutions were then mixed by pulse-vortexing and incubated at room temperature (15- 25°C) for 10 minutes. The tube was then briefly centrifuged to remove drops from inside the lid. Then equal 560 µl of 96% ethanol was added to filtrate and mixed thoroughly and washed with 500 µl washing 1 and 2 (500 µl buffer AW 1 and 500 µl AW 2), any unwanted protein and DNA were removed. Then, 60 µl of elute solution (AVE) was added to collect the RNA. Finally, the eluted RNA was kept at-20°C (Qiagen, Inc., Gaithersburg, MD, USA).

Real-Time Polymerase Chain Reaction (RT-PCR):

After RNA was extracted the Master Mix preparations were carried out in Ice bath. The reaction mixes were prepared by pipetting all the components of the Master Mixture in to 2 ml PCR tube according to the kit protocol. The reaction components were template RNA, primer solutions, dNTP Mix, and 5x QIAGEN One Step RT-PCR buffer, RNase-free water, probe and enzyme mix. Total RNA was extracted by scraping cells with RLT buffer and isolated according to manufacturers' instructions using the RNeasy Mini Kit (QIAGEN). The control that we used were NDV vaccine strain La Sotaa and RNase free water as positive and negative controls, respectively. Then Applied Bio-systems 7500 Fast RealTime PCR thermo cycler were used for amplification of the extracted RNA. A primer probe combination from a conserved region of the M gene APMVI F M+4100 5'-AGT GAT GTG CTC GGA CCT TC-3', APMV-I R M- 4220 5'-CCT GAG GAG AGG CATTTG CTA-3'and Probe APMV-1M+4169 5'-FAM TTCTCT AGC AGT GGG ACA GCC TGC TAMRA -3' was used to amplify all NDV isolates (Qiagen, Inc., Gaithersburg, MD, USA).

Annex 5: Positive PCR Result of the as infectious bursal disease



Sample allocation on the agarose gel lane from right to left

Lane -1 100 bp Marker,

Lane -2 to 6 suspected sample, Lan-2 was positive sample of current case IBD.

Lane -7 negative control,

Lane -8 was positive control

sample for the curren

5Pm IBD VP2 FW Primer sequence: 5'TCTTGGGTATGTGAGGCTTG -3'

5Pm IBD VP2 RV Primer sequence: 5'CCCGGATTATGTCTTTGA -3'

PCR condition

50oc for 30 min and 950c for 15 minutes for cDNA synthesis

95oc for 30 sec,

55 oc for 30 sec, for 35 cycles

720c for 30 se

72oc for 7 min for 1 cycle

PCR Master Mix components

Ranse free water….. 4ul

PCR buffer 5X……5ul

Q solution5x…….5x

Fw …… 2ul

Rv…….. 2ul

dNTP ……1ul

Enzyme ……..1un

Note: the above component in the master mix per reaction

7. APPENDIX

Plagiarisms check result.