Thesis Ref. No._____

CLINICAL CASE STUDIES ON MAJOR DISEASES OF VETERINARY IMPORTANCE IN BISHOFTU TOWN, ETHIOPIA

MVSc Thesis

By

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MVSc program in Clinical Medicine

June, 2018

Bishoftu, Ethiopia

CLINICAL CASE STUDIES ON MAJOR DISEASES OF VETERINARY IMPORTANCE IN BISHOFTU TOWN, ETHIOPA

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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June, 2018

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STATEMENT OF AUTHOR

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College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: June 15/ 2018

ACKNOWLEDGMENT

I am thankful to the almighty God, for His eternal mercy, love and protection in the ways and situations of my thesis work and my entire life.

I am highly indebted to my advisors Prof. Fekadu Regassa and Dr Reta Tesfaye for their insight guidance, technical advice, constructive criticism and excellent cooperation from the beginning of this work, which enabled me to complete the thesis work. My special appreciation also goes to my beloved and respected colleagues, the SPANA-Ethiopia project staffs, for their great support and encouragement during the study period.

I am also thankful to my families, especially to my husband, mom and dad. Had it not been with their great support, completion of this thesis work would have been a nightmare. I don't want to go without saying thank you to Manu who were taking care of my kids when I was highly obsessed with my thesis works.

Finally, I want to thank all veterinary professionals at the Veterinary Teaching Hospital of the College of Veterinary Medicine and Ada'a district veterinary clinic.

ABBREVIATIONS

AHS	African horse sickness
AHSV	African horse sickness Virus
CSA	Central Statistics Agency
CPV	Canine parvovirus infection
CVMA	College of Veterinary Medicine and Agriculture
EDTA	Ethylenediaminetetraacetic acid
EZL	Epizootic Lymphangitis
FAD	Flea allergy dermatitis
LSD	Lumpy skin disease
NVI	National Veterinary Institute
NSAID	Nonsteroidal Anti Inflammatory Drugs
OIE	World Organisation for Animal Health
PBS	Phosphate-buffered saline
SID	Single dose in a day
SPANA	Society for the Protection of Animals Abroad

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ABSTRACT

Ethiopia has huge number of livestock population but couldn't earn much from the sector. One of the bottlenecks, for the lesser degree of gain from the sector, is disease of animals. So this thesis was done with the objective of clinical case study on major diseases of veterinary importance in Bishoftu town Ethiopia. The study was conducted from September 2017 to June 2018 in clinics found in the College of Veterinary Medicine and Agriculture, and Ada'a district veterinary clinic. From the retrospective data of the aforementioned clinics, the most frequently occurred diseases during the past five years were first identified and patients that were coming to the clinics during the study period with the listed out type of disease were purposively sampled, diagnosed, treated and followed up for months until the final outcome of the condition. Twenty cases were studied and compiled following the scientific case publishing format where summary, literature review, case history, clinical examination and findings, tentative diagnosis, sample collection, laboratory diagnosis and result, treatment and outcome of the disease were briefly recorded. The findings of each case were finally discussed in relation to the findings on other literature and scientific publications. The twenty compiled case studies include seven cases on cattle (Actinomycosis, Blackleg, Colisepticemia, Navel ill, Lumpy Skin Disease, Mastitis and Parafilariosis), six cases on equines (African Horse Sickness, Epizootic Lymphangitis, Grain overload, Rabies, Strangles and Tetanus), two cases on small ruminants (Goat pox and Coccidiosis), three cases on poultry (Collibacillosis, Newcastle and Salmonellosis) and two cases on canine (Canine Parvovirus and Flea allergy dermatitis). So confirming diagnosis based on laboratory findings is recommended to initiate effective treatment and control measures which safeguards the health and welfare of treated animal and reduces the spread of infection to other animals or, in the case of zoonotic disease, to humans.

Keywords: case study, clinical examination and findings, treatment and outcome, discussion

1. INTRODUCTION

Agriculture is the basis of Ethiopia's economy and is the most important economic sector in terms of generation of foreign currency. The sector is the primary source of livelihood for more than 85 % of Ethiopian rural households who practice subsistence crop and livestock production. The livestock sector in Ethiopia contributes 12 and 33% of the total and agricultural Gross Domestic Product (GDP), respectively, and provides livelihood for 65% of the population. The sector also accounts for 12-15% of total export earnings, the second in order of importance (Jibat et al., 2015).

Animals provide highly nutritious foods, and provide draught power, transport, manure, hides and skins and they are companion to human beings. To ameliorate the development constraints and realize the benefits from the huge but untapped livestock resource, efforts have been made in various aspects to develop the livestock sector in Ethiopia. These efforts include the provision of input and services such as veterinary services, for through investigation of animal disease, as animal disease is one of the major bottlenecks of animal productivity in central Ethiopia including Ada'a Liben district (Jibat *et al.,* 2015).

In the investigation of any animal disease problem, the veterinarianmust, of necessity, undertake a careful and thorough clinical examination with the objective of recognizing the nature of the infection, so that effective treatment and, where practicable, control measures are adopted. The investigations of animal diseases are complex by the necessity to deal with a variety of species of domestic animals and birds; however, the same principles may be applied in all cases to deal with the diverse difficulties that clinical diagnosis presents (Duguma, 2016).

The organs or systems involved, the location, type of lesion present, the pathophysiological processes occurring and the severity of the disease can be deduced from the information gained during the clinical examination. Without a proficient clinical examination and an accurate diagnosis it is unlikely that the control, prognosis and welfare of animals will be optimized (Abdisa, 2016).

The success of clinical examination relies heavily on the knowledge of the clinician. Many clinicians begin their examination by performing a general examination which includes a broad

search for abnormalities (Ballard and Rockett, 2009; Frandson *et al.,* 2009). After the system or body part involved is identified, and is then examined in greater detail using either a complete or a problem oriented examination. For this, sound knowledge of Anatomy, Physiology, Pathology and Animal behavior, skills in the methods and techniques of clinical examination, knowledge of etiology, clinical sign and pathogenesis of the diseases are the basic requirements for clinician to make diagnosis (Duguma, 2016).

Confirmatory diagnosis by taking appropriate sample is necessary to select the appropriate drug of choice; as the primary purpose of veterinary drugs is to safeguard the health and welfare of animals (Cannavan, 2014). Appropriate use of antimicrobials will cure some sick animals and speed the recovery of others, and may improve the welfare of treated animals and reduce the spread of infection to other animals or, in the case of zoonotic disease, to humans (McKellar, 1998).

Previous researches done in the study area; College of Veterinary Medicine and AgricultureVeterinary Teaching Hospital (CVMA-VTH) and Ada'a district veterinary clinic showed that almost all patients (96.6 %), received drug therapy after they had been tentatively diagnosed without getting correct laboratory-supported diagnosis (Beyene *et al.,* 2015).This resulted in emergent of antimicrobial resistant disease causing organisms, and also could result in drug residue which had effect on the public health (Takele *et al.,* 2015). Therefore confirmatory diagnosis and appropriate prescription of drug is necessary to safeguard the health condition of both animals and human beings.

So the objective of these case studies was:

- To conduct clinical studies on major diseases of veterinary importancein Bishoftu town, Ethiopia

2. LITRATURE REVIEW

Literature of each case report is reviewed under the topic of each case.

3. MATERIALS AND METHODS

3.1. Study Area

The study was conducted from September 2017 to June 2018 at CVMA (College of Veterinary Medicine and Agriculture) clinics and at Ada district veterinary clinic, Bishoftu. CVMA clinics include Veterinary Teaching Hospital (VTH), Society for the Protection of Animal Abroad (SPANA) open air clinic and The Donkey Sanctuary open air clinic.Bishoftu town is found in central Ethiopia, which is located at 45 km South East of Addis Ababa. Bishoftu is situated at 9°N latitude and 4°E longitude and an altitude of 1850 meter above sea level in the central high lands of Ethiopia. Farmers near to Bishoftu town practice a mixed crop-livestock farming system (Zeleke *et al.,* 2005).

3.2. Study Design

A five year retrospective data of the frequently occurred diseases was taken from the case record books of the aforementioned clinics and patients that were coming to the clinics during the study period with the disease type similar to the frequently occurred diseases were purposively sampled for the case study. A case report, a descriptive study of individual patients was used to document cases and detailed report of the symptoms, signs, diagnosis, treatment, and follow-up of an individual patient was included in each case reports.

3.3. Study Population

Different animal species and phone call requests that were presented to the aforementioned clinics during the study period were included in the study (Cattle, goat, sheep, horses, donkey, dogs and chickens). Accordingly, twenty finally diagnosed cases were compiled and reported.

3.4. Case Handling Protocol

Individual case recording sheet was used (APPENDIX V). The species, breed, sex and age of the patient was recorded. The owner was asked about his/her complaints on the animals presented. Based on the owner complaints related to past (similar signs, previous medications, allergens…), present (duration of the disease, treatment give, number of animals affected …) and

environmental histories (housing, husbandry system, nutrition, stocking density…) were asked and relevant full history of the case was recorded.

The patient was observed from distance at its standing position for any abnormality including its gait, behavior and respiration rhythm. The patient was then observed from distance while walking for any abnormalities including the observational points at its standing position.

The patient was then restrained properly using either crush, lifting up the front one limb (for equines) or through verbal restraining (practiced by owner by massaging and calling the name of the animal).

Clinical examination of a patient continued by taking body temperature (through the rectum), respiratory rate (auscultation of the tracheal sound), heart rate/pulse rate (auscultation of the number of heart beatsor filling pulses from superficial blood vessels) and capillary refill time (compressing the mucosa of the mouth). The demeanor of the patient was also observed (bright, depressed, apathetic and anxious).

Regional clinical examination continued from head to tail with focus given to parts associated with the history of the patient. On the head examinations; checking mucous membrane (conjunctiva, nasal and muzzle), eyelids, eyes, nasal regions, mouth and tooth were done. On examination of skin and appendages; hoofs, hairs, horns, claws, nails, sebaceous glands and sweat glands were done. On examinations of the thoracic cavity examination; auscultations and percussions of the heart and lung were done. On clinical examinations of the abdominal and pelvic cavities; examination and checking the motility of the digestive tract parts and rectal examination (when necessary) of digestive and urinary organs were performed. All superficial lymphnodes (submandibular, prescapular and prefemoral) were also checked for any abnormality.

After compilation of clinical examination the case was tentatively diagnosed and empiric therapy was initiated. Appropriate sample was then taken, carefully labelled (label including; type of specimen, date of sampling, preservative used, identification of animals and name of the owner) and transported to laboratory in 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 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 done through phone communication to the owners and also by home visit.

4. COMPILED CASE REPORTS

In this thesis ‘Major diseases’ means diseases that are most frequently occurring in the study area. Accordingly 20 major diseases of veterinary importance in the study area were studied during the study period.

Each 20 definitively diagnosed clinical cases were compiled following the scientific case publishing format. summary, literature review, case history , clinical examination and findings, tentative diagnosis, sample collection, laboratory diagnosis and result, and treatment, outcome, discussion, acknowledgment and references were included for each individual case.

The brief statement of all the clinical diagnosis procedures and outcomes of the case were included under the ‘Summary’.

The patient Case history including relevant past, present and environmental histories were recorded followed by documentation of any abnormal findingsduring clinical examination. The body parameters were compared to the normal range of body parameter measurements listed under APPENDIX 4. Based on the history and clinical findings the best approaching tentative diagnosis was made followed by selecting appropriate sample with correct sample taking procedure and sample transporting system for the laboratory confirmation of the case. The Sample was processed in the laboratory based on the required laboratory procedure to confirm the diagnosis.

Based on the history, clinical signs and laboratory findings and sometimes based on the treatment outcome the diseases were confirmed. The drug of choice, the duration of therapy and the outcome of therapy were briefly discussed for each individual case.

The findings of the case were then discussed in relation to the findings on literature and scientific publications and references were written for each individual case.

4.1. Case Reports on Cattle

4.1.1. Actinomycosis

Summary

Actinomycosis (lumpy jaw) is a chronic infectious disease characterized by suppurative granulation of the skull, particularly the mandible and maxilla. This case report summarizes the case of Actinomycosis in two years Zebu calf presented to Ada'a distric veterinary clinic, Bishoftu, Ethiopia. Before a month of its presentation to the clinic, two swellings on the right mandible started to grow slowly and few weeks later these swellings started to discharge creamy pus. The calf reduced its feed intake. On clinical examination the body parameters were in normal range and the swellings were firm and painless. The Gram stain of the granules, taken from the pus, revealed Gram positive, rod and few filaments of the bacteria. The pus was surgically drained and flushed with 2% tincture iodine for seven days and the animal was also treated with systemic antibiotic. The swelling started to regress slowly and on the 60th day of visit it was changed to very small fibrous tissue. When compared with findings in literature treatment of this case took lesser time so further case studies should be done on the benefits of use of local tincture iodine rather than systemic iodides.

Key words: Actinomycosis, Gram stain, antibiotic, iodide

Literature Review

Actinomycosis (lumpy jaw) in cattle is a chronic infectious disease characterized by suppurative granulation of the skull, particularly the mandible and maxilla. It is mostly cause by bacteria under the genus Actinomyces. It is a normal inhabitant of the oral flora and upper respiratory and digestive tracts of most animals (Smith, 2002). The bacteria are non-motile, non-spore forming, non-acid fast, gram positive pleomorphic, anaerobic-to-microaerophilic filamentous bacterial rods (De Montpreville *et al.*, 1999).

The organism can access to the soft tissues through mucosal damage caused by sharp objects or erupting teeth. Following the infection, a proliferation of connective tissue, invasion with

leucocytes and the resulting formation of a walled tumor-like mass can be seen. The granuloma then invades the bones of the mandible or occasionally the maxilla (Eddy, 2004).

Clinically the involved parts of the jaws may be enlarged 2 or 3 times but the swelling is painless (Radostitis *et al.,*2007). Fistulas may develop from abscesses of the bones of the head and discharge the pus with characteristic sulfur granules. The palate and gums next to the bones often are swollen and inflamed. The teeth may loosen (Bertone and Rebhum, 1984; Raymond and Foglia, 1998; Van Metre *et al.,*2007) and, in most cases, osteomyelitis may develop arising from periodontitis (Palmer, 1993).

Presumptive diagnosis is often based on clinical signs. The diagnosis can be confirmed by culture of the organism from the lesion; however, this requires anaerobic conditions and is frequently negative. A Gram stain of purulent material will reveal gram-positive, club-shaped rods and filaments (sulfur granules) (Merck, 1995).

Treatment of bovine actinomycosis includes oral or intravenous dosing of iodides and/or antibiotics such as penicillin and streptomycin but with variable results (Radostitis *et al.,*2007; Brunton *et al.,*2005). Since the iodide dosing is time consuming and the antibiotics have poor penetration into the site of the infection (Radostitis *et al.,*2007), effective treatment of lumpy jaw is still awaited. Recent studies suggested that treatment of any sort is more likely to be of value when combined with surgical intervention (Melter *et al.,*2009).

A bovis is part of the normal oral flora in ruminants, so control should focus on avoiding coarse, stemmy feeds or feeds with plant awns that might damage the mucosal epithelium (Merck, 1995).

Case History

An owner presented a two years old calf to Ada'a veterinary clinic, Bishoftu in October, 2018. He mentioned that the calf was reared in extensive farming system and before a month, two swellings were seen on the right mandible. They started to grow slowly and one of the swellings started to discharge pus. The calf reduced its feed intake.

Clinical Examinations and Findings

The body parameters; temperature was 38.1^{o}c, respiration was 40 breaths/ minute and pule rate was 56 beats/ minute. All measured parameters were in normal range. The two swellings could be seen on the lateral surface of the mandible and from one swelling, creamy pus was discharging slowly. The other swelling, not discharging pus, was hard and painless. The non-discharging swelling was aspirated with 22 gauge needle and the content was pus.

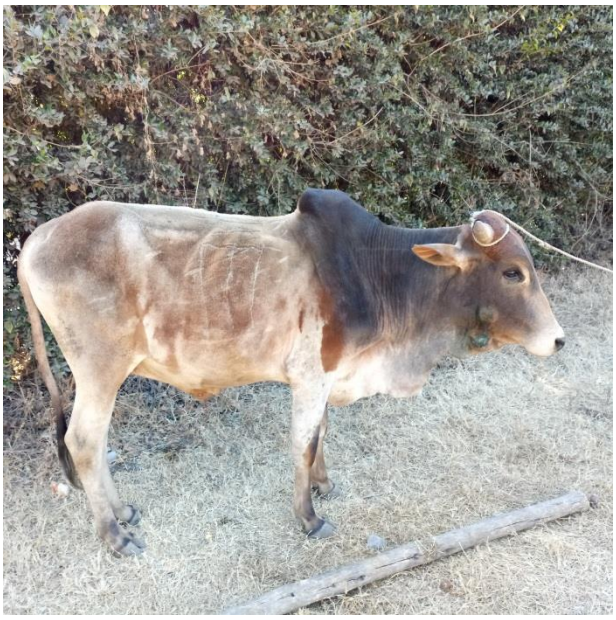

Fig. 1 Swellings on the lateral mandible

Fig. 2 Clinical examination

Differentila Diagnosis

Actinomycosis, local abscesses

Sample Collection

The non-discharging swelling was incised with surgical blade and the pus discharge was collected by sterile screwed bottle and transported to the lab with cold chain.

Laboratory Diagnosis and Result

A granule from the pus sample was crashed on a microscopic slide and air dried. After 30 minutes Gram staining (APPENDEX-1) was done 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. Gram positive, rod and few filaments of the bacteria were seen under the microscope.

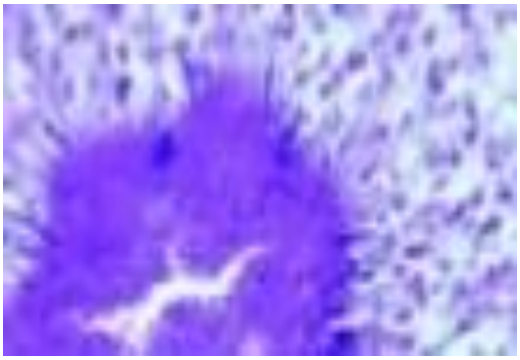

Fig. 3 Gram positive, rod shaped bacteria

Treatment

Following all aseptic techniques the both 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 a socked gauze continued for seven days with systemic injection of Penstrip-400 (1ml/20kg, IM, SID for seven days).

Outcome

There was no pus from the pouches after the end of treatment. After two months, when the calf was visited, the pouches regressed very considerably and they werechanged to fibrous tissue.

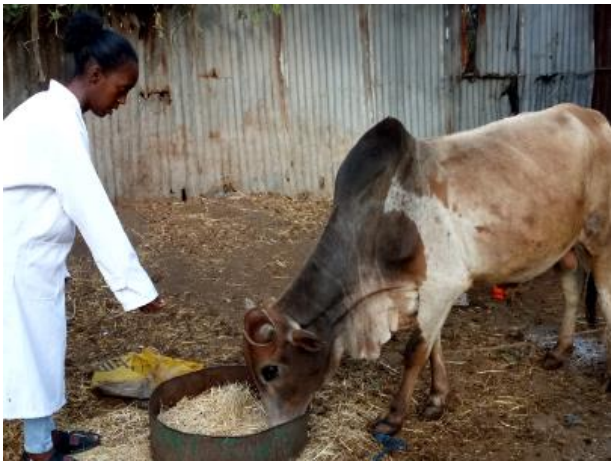

Fig. 4 The calf regains its appetite

Fig. 5 Fibrosis of the swelling (Day sixty)

Discussion

Actinomycosis is a chronic infectious disease, caused by Actinomyces. The bacteria are normal flora of the oral cavity but it can access to the soft tissues through mucosal damage caused by sharp objects or erupting teeth. The disease is characterized by suppurative granulation of the skull, particularly the mandible and maxilla.

The clinical signs observed in this case; slowly growing painless and firm swellings, discharging of pus are all similar findings with the clinical signs of the disease written on literatures (Bertone and Rebhum, 1984; Van Metre *et al.*,2007; Palmer, 1993; Merck, 1995). Unlike other findings the calf has lost its appetite and it could be due to the mechanical pressure of the swelling when the calf tried to open its mouth.

In literatures, treatment of bovine actinomycosis includes oral or intravenous (IV) dosing of iodides and/or antibiotics such as penicillin and streptomycin but with variable results. And oral or IV dosing of iodides needs longer period of administration (Radostitis *et al.*,2007; Brunton *et al.*,2005). But in this case study treatment with penstrip (combination of penicillin and streptomycin) and local dressing of the swellings with 2 % tincture iodine took only seven days. So further studies should take place with local application of tincture iodine rather than systemic iodides.

In this case report it was unable to culture the causative organism because of lack of anaerobic transport medium to the pus sample. And also due to lack of diagnostic imaging equipment the extent of the surrounding tissue damage and the content of healed granulomatous tissue were not confirmed.

Acknowledgment

I want to thank Dr Naol Mengesha (veterinarian at Ada'a district veterinary clinic) and Mrs Tsedale Teshome (Vet technician at CVMA) for assisting in the diagnosis and treatment of this case.

4.1.2. Blackleg

Summary

Blackleg is an acute, febrile, highly fatal disease of cattle caused by *Clostridium chauvoei*. This case report summarizes the case study of Blackleg in a six years ox presented to Ada'a district veterinary clinic Bishoftu, Ethiopia. The ox had lost its appetite completely since a day ago and it was very difficult for the ox to walk. The ox was living with one other ox, which was healthy. The owner was using his oxen for ploughing. On clinical examination the ox was very depressed, the muzzle was dry and the ox had difficulty to move its limbs. Around thigh muscle on the right limb there was swelling which had crepitation sound during palpation. All the body parameters; temperature, respiratory rate and pulse rate were above the normal range. And there was no rumen motility. With needle fluid from the swelling area was aspirated and smear was prepared immediately. Gram staining of the smear revealed Gram positive rod bacteria. For further confirmation of the case blood sample was sent to NVI for PCR test. The result given from NVI confirmed the presence of *Clostridium chauvoei* in theblood sample. Even though the ox started to be treated with Pencillin, it died after few hours. The apparently health ox took prophylactic Pencillin. Vaccination of all susceptible animals before anticipated danger period is recommended.

Key words: Blackleg, ox, Gram positive, rod

Literature Review

Blackleg is an acute, febrile, highly fatal disease of cattle caused by *Clostridium chauvoei.* The bacterium is a Gram-positive, rod shaped, spore-forming and toxin-producing anaerobe. The spores of the bacterium are very resistant to adverse environmental stress. It can withstand high temperature and desiccation and are resistant to disinfectants. In soil, the spores can persist for a number of years (Sultana et al., 2010).

Most cases of black leg occur in the warm months of the year. Outbreaks can occur following excavation of soil, which can expose and activate latent spores. Also the disease is enzootic in

areas with a history of flooding. It is common for a number of animals to be affected within a small time frame (Veterinary Manual, 2018a).

In cattle, blackleg infection is endogenous. Lesions develop without any history of wounds, although bruising or excessive exercise may precipitate disease in some cases. Commonly, the animals that contract blackleg are of the beef breeds, in excellent health, and gaining weight. Most cases are seen in cattle from 6–24 months old, but thrifty calves as young as 6 week and cattle as old as 10–12 year may be affected (Veterinary Manual, 2018a).

The pathogenesis of blackleg is still not completely understood, but certain aspects of disease progression have been confirmed. Cattle are exposed and become infected with *Clostridium chauvoei*through ingestion of spores from pasture.Spores, either those directly ingested or those formed after germinating in the gut, are then carried across the intestinal mucosa. There they aretaken up by macrophages that distribute the spores throughout the body. *Clostridium chauvoei*spores can be found in many tissues throughout the body of normal animals,including muscle, where they are stored for long periods of time in phagocytic cells. Activation of latent spores resulting in infection and disease is an area of conjecture, but evidence suggests that conditions resulting in reduced local oxygen content or muscle damage may enable the spores to germinate. These conditions could include muscle bruising, such as with transport, handling, intramuscular injections, or strenuous exercise (Van Vleet and Valentine, 2007; Songer, 2004).

Clinical signs of blackleg have been well-documented, but due to the sudden onset and often peracute nature of the disease, are not often observed in field cases.The course of clinical disease is rapid, and most animals die within 12 to 36 hours of the onset of clinical signs. When a limb is involved, as is commonly the case, acute lameness and pronounced muscle swelling in the upper limb may be noted. However, if lesions are deep within the muscle group, swelling may not be evident externally. In some cases, the swelling may be present in the back, brisket, neck, or elsewhere. Regardless of the site, the swollen area is initially hot and painful, but becomes cold, insensitive, and crepitates as the condition progresses. Edema and emphysema are commonly present in the tissue, and the overlying skin may become dry and cracked. Regional lymphadenopathy may be observed. Other signs observed in affected animals include depression, complete anorexia, rumen stasis, tachycardia, and tachypnea. Fever is present initially, but body temperature quickly drops to normal or subnormal levels as other signs progress. Terminally,

signs of ataxia, tremors, and dyspnea quickly progress to recumbence, coma, and death (Radostits and Done, 2007).

Diagnosis of blackleg couldbe done on the basis of clinical history, ante mortem findings and laboratory examinationof clinical specimens. Laboratory confirmation requires muscle sample from the affected tissue or it could be done from impression smears or fluids taken from the surface of freshly cut lesions using anaerobic culture, FAT or PCR (Veterinary Manual, 2018).

Vaccination is the best method of disease control. Calves 3–6 months of age should be vaccinated twice, 4 weeks apart, followed by annual boosters before the anticipated danger period. In an outbreak, all susceptible cattle should bevaccinated and treated prophylactically with penicillin (10,000 IU/kg, IM) to prevent new cases for as long as 14 days (Veterinary Manual, 2018a).

Case History

An owner presented a six years ox to Ada'a distric veterinary clinic on May, 2018; where there was unusual heavy rain that resulted in flooding. The owner mentioned that the ox hadlost its appetite completely since a day ago and it was very difficult for the ox to walk. The owner mentioned that he had one other oxat home, which was healthy. The owner was using his oxen for ploughing.

Clinical Examination and Findings

It was very depressed, the muzzle was dry and the ox had difficulty to move its limbs and around thigh muscle on the right limb there was swelling. The swelling has crepitation sound during palpation.

The body temperature was 39.2^{o}C, respiratory rate 56 breaths/ minute and pulse rate was 72 beats/ minute. The ox was febrile and both respiratory rate and pulse rate were higher than the average value. Up on auscultation there was no ruminal motility detected.

Fig. 6 The depressed ox

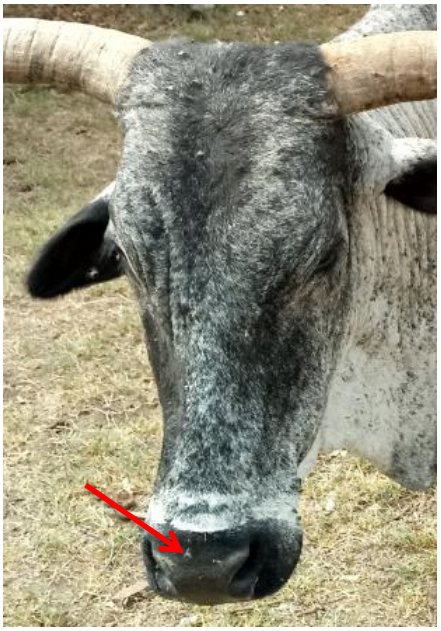

Fig. 7 Dry muzzle of the ox

Fig. 8 Clinical examination of the swelling area

Differential Diagnosis

Blackleg, local trauma, edema

Sample Collection

With needle only small amount of fluid was found inside the swollen area and it was immediately transferred on microscopic slide and smear was prepared

The ox was kept in crush to restrain it. The jugular furrow was located and the jugular vein was identified by applying thumb pressure on the base of the jugular groove. The proximal third of the neck, over the jugular vein, was cleaned with alcohol. The vacutainer needle was screwed on the needle holder and the EDTA vacutainer tube was preloaded on the needle holder. Care was given not to puncture the vacutainer tube. The jugular vein was distended by applying thumb pressure over the vein. The vacutainer needle was inserted in to the engorged vein and the vacutainer tube was inserted in to the vacutainer needle that was protruding through the holder. About 8ml of blood was collected and the needle was taken out of the vein. The venipuncture area was disinfected. And the blood sample was transported to NVI in cold chain for PCR technique.

Fig. 9 Blood Sample Collection

Laboratory Diagnosis and Result

The prepared smear was stained with Gram staining procedure (APPENDIX 1). And a drop of immersion oil was added on the slide and observed under 100X binocular microscope. Gram-positive and rod shaped bacteria were observed.

The sample sent to NVI was cultured on selective media and on Gram staining the bacteria were rod and Gram positive.

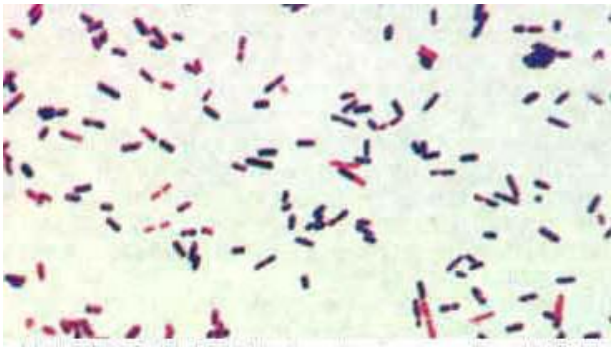

Fig. 10 Gram-positive and rod shaped bacteria (from Culture)

The National Veterinary Institute had done PCR on the blood sample and *Clostridium chauvoei* was detected on the result (APPNDIX VI).

Treatment

Procaine Penicillin 22,000IU/ kg was given IM SID.

Outcome

The ox died after 10 hours of treatment (confirmed through phone communication with the owner). The other ox, which was living together with the dead ox, had been given with prophylactic dose of Pencillin (10,000IU/ kg, IM, SID for 7 days).

The outcome of the laboratory result was communicated with the district Veterinary office so that the susceptible animals living in that area would be vaccinated.

Discussion

Blackleg is an acute, febrile, highly fatal disease of cattle caused by *Clostridium chauvoei.* The disease is known to affect mostly calves but in this case study the disease was found in a 6 years ox. This could be due to the anaerobic environment that may be occurred due being hit around the thigh muscle during ploughing.

The clinical signs observed in this case report; sudden onset of the disease, depression, anorexia, lameness and crepitation sound of the swelling area were similar finding of the disease with the signs of the disease mentioned on literatures (Merck Veterinary Manual, 2005; Radostits and Done, 2007; Veterinary Manual, 2018a).

The appropriate sample that should be sent to laboratory was tissue sample from the affected area kept in screwed bottle but the owner refused that. The fluid that was accumulated in the infected area was very minimal (that was only enough to do the smear) so since the animal was febrile (that could be due to bacteremia), blood sample taken in vacutainer tube (anaerobic environment) was used for confirmation of this case.

Intravenous preparation of Penicillin G was preferable for fast action but due to unavailability of the drug on the market intramuscular preparation of Procaine penicillin was used.

Acknowledgment

I thank Mr Belay Temesgen (senior vet technician at Ada'a district veterinary clinic) and Dr Liyu (senior microbiologist at NVI) for their help in the diagnosis of this case.

4.1.3. Colisepticemia

Summary

Colisepticemia is caused by specific invasive serotypes of *E coli* that possess virulence factors enabling them to cross mucosal surfacesand produce bacteremia and septicemia. This case report summarizes the case management of Colisepticemia in six months old calf presented to Ada'a veterinary clinic, Bishoftu, Ethiopia. The calf was reared under intensive farming system and the calf was living with its dam and other five cattle sharing the same shade. The calf became weak, inappitant and had diarrhea stained with blood for three consecutive days. The calf was only taking a mixture of wheat bran and water. The calf strained at defecation and sometimes blood is seen with its faeces. The body temperature of the calf was 41.2^{o}C (febrile) and the pulse was 64 beats/ minute, evident of rapid beats. The hair coat was rough and the conjunctiva was pale. For laboratory confirmation blood sample and faecal sample were taken and transported to lab with separate cold chain. Isolation and identification of the bacteria from the blood sample confirmed the presence of E. coli. No parasitic egg detected from the faecal sample. Gentamycin was given for five days. The calf completely returned to its normal condition after the fifth day of treatment. Since Colisepticemia could occur in older calves subjected to stress, keeping calves in hygienic and if possible in a separate shed is recommended.

Key words: E. coli, calf, diarrhea

Literature Review

Escherichia coli, causative agent of Colisepticemia, are widely distributed in nature; being present in soil, surface water, animal and human feces. The disease is caused by specific invasive serotypes of *E coli* that possess virulence factors enabling them to cross mucosal surfaces, overcome the bactericidal plasma factors, and produce bacteremia and septicemia. The main determinant of the disease is deficiency of circulating immunoglobulins as the result of a failure in passive transfer of colostrum immunoglobulin.

E. coli is a natural inhabitant of the intestines. However, some types of E. coli bacteria are capable of causing disease. The bacterium, E. coli, is the primary cause of diarrhea in calves one to two days old. It is usually associated with inadequate intake of the mother's colostrum, unhygienic conditions and stress. It may also occur in older calves subjected to stress (Health Management and Disease, 2010).

It is assumed that the primary source of the infection is the feces of infected animals, including the healthy dams and neonates, and diarrheic newborn animals, which act as multipliers of the organisms. Invasion occurs primarily through the nasal and oropharyngeal mucosa but can also occur across the intestine or via the umbilicus and umbilical veins. The organism is excreted in nasal and oral secretions, urine, and feces; excretion begins during the preclinical bacteremia stage. Initial infection can be acquired from a contaminated environment. In groups of calves, transmission is by direct nose to nose contact, urinary and respiratory aerosols, or as the result of navel sucking or fecal-oral contact (Gruenberg, 2014).

The pathogenesis of Collibacillosis is related with fimbria (some species of E. coli) adhesion and enterotoxin production of the bacteria, E. coli. The fimbrial adhesion F5 (K99) promotes the adhesion of bacterial cells to glycoproteins on the epithelial surface of the jejunum and/or ileum, and bacterial enterotoxin also causes damage to the epithelial cells, resulting in fluid secretion and diarrhea (Acres, 1985). This diarrhea results in loss of water and electrolytes from the body of the animal. This loss of water and electrolytes creates dehydration and alteration of the acid-base balance of the body fluids. Inflammation of the intestinal lining impairs the calf's ability to digest nutrients, creating weight loss and the potential for hypoglycemia (Cho, 2010).

Collibacillosis signs are nonspecific and vary widely among different hosts. Morbidity and mortality are very variable depending on which infection/infections the E. coli strain causes in a particular flock of animals (Ahmed *et al.,* 2013). But generally, Collibacillosis can be detected in livestock with signs of severe diarrhea caused by enteritis, lameness, stunted growth inactivity, lack of appetite and water consumption, and unresponsiveness (Ahmed *et al.,* 2013; Nolan, 2013).

Diagnosis depends on an accurate history, clinical signs, and culture and biochemical tests of the bacteria from blood or internal organs and serotyping of the organism (Parr *et al.,* 1980; Barry *et al.,* 1962).

Parental antibiotics can be useful if given early, but not without rehydration therapy (Constable, 2004). Treatment requires aggressive antimicrobial, fluid and anti-inflammatory therapy. Although blood cultures are recommended to retrospectively confirm the diagnosis, antimicrobial therapy must be initiated immediately in any animal suspected of being septic. Because there is no time for sensitivity testing, the initial choice should be a bactericidal drug that has a high probability of efficacy against gram negative organisms. Administration IV of large volumes of balanced electrolyte solutions over several hours is essential to correct hypovolemia and assure adequate peripheral tissue perfusion; fluids should include glucose to correct hypoglycemia (Gruenberg, 2014).

Several of the agents that produce diarrhea in calves can also produce diarrheal disease in people. These organisms could also present as subclinical infections in the gut of calves and lambs; immune compromised people should avoid contact with young ruminants (Radostitis *et al.,* 2000).

Case History

An owner presented a six month calf to Ada'a distric veterinary clinic. The owner mentioned that the calf was reared in intensive farming system and had diarrhea for the last three days. It strains at defecation and sometimes blood is seen with its faeces. It could not suck milk from its mother and also lost interest for the wheat bran that it used to eat. The owner mixed a little wheat bran with water and the calf was only taking that. The dam of this calf had no history of diarrhea. The dam gives only about 3 liters of milk per day. Starting from birth, the calf is allowed to suck its dam after she is milked. The colostrum was not fed to the calf as the owner thought that the colostrum could cause diarrhea to the calf. The dam and the calf were sharing a shade with other five cattle.

Clinical Examinations and Findings

The body temperature of the calf was 41.2^{o}c. The pulse was 64 beats/ minute, evident of rapid beats. The calf usually wanted to lie down. The hair coat was rough and the conjunctiva was pale. But the skin resumed its place with less than 2 seconds on skin tenting test.

The hind quarter was soiled with foul smelling faecal material, evidence of diarrhea. The calf strained while passing faeces. The faeces, when taking sample, was having little blood stains.

Fig. 11 Weak calf (Day one)

Fig. 12 Soiled hind quarter; diarrhea (Day one)

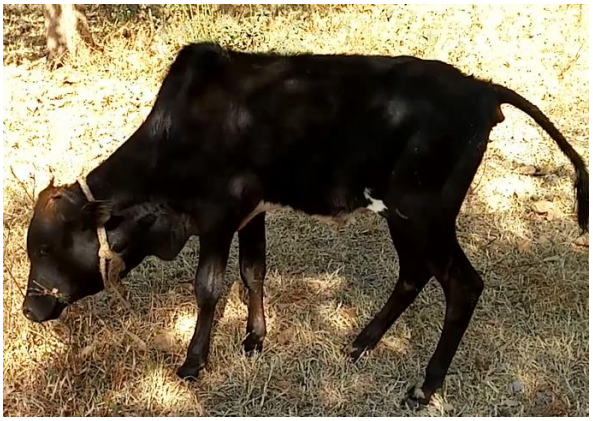

Fig. 13 Straining during passing faeces (Day one)

Fig. 14 Mucoid and slightly blood stained faeces (Day one)

Differential Diagnosis

Colisepticemia, coccidiosis, calf scoure

Sample Collection

The jugular farrow was located and the jugular vein was identified by applying thumb pressure on the base of the jugular groove. The skin over the jugular vein was cleaned with alcohol. The vacutainer needle was screwed on the needle holder and the EDTA vacutainer tube was preloaded on the needle holder. Care was given not to puncture the vacutainer tube. The jugular vein was distended by applying thumb pressure over the vein. The vacutainer needle was inserted in to the engorged vein and the vacutainer tube was inserted in to the vacutainer needle that was protruding through the holder. About 3ml of blood was collected and the needle was taken out of the vein. The venipuncture area was disinfected and the blood in the EDTA vacutainer tube was slowly rotated to mix the anticoagulant with the blood. The sample was transported to lab with cold chain and kept in $+4^{o}C$ until processed.

Faecal sample was taken directly from rectum with arm length and immediately transported.

Laboratory Diagnosis and Result

For determination of packed cell volume two plain capillary tubes were used (as the blood was collected in EDTA vacutainer tube). From the EDTA vacutainer tube blood was filled up to its ¾ level of capillary tubes by the capillary action. The ends of the capillary tubes, in contact with the blood, were sealed with clay up to 2 mm depth. The two capillary tubes were placed in to two opposite grooves of hematocrit centrifuge. They were centrifuged at 15,000 rpm (resolution per minute) for one minute. The capillary tube was removed from the centrifuge and it was read using hematocrit reader. The reading of the packed cell volume was 45%, in the normal range.

3 gm of the faeces was measured in a beaker (no need to crush it with pestle and mortal because it was fluidly). 50ml of floatation fluid was added. The faeces and water was mixed thoroughly. The solution was strained through tea strainer. The filter material was filled in three test tubes (to increase the chance of getting parasite egg). The test tubes were placed in a test tube rack. The test tubes were gently topped up with the filter solution until convex meniscus formed. Gently cover slip was placed on top of the test tubes. The test tubes were left for 20 minutes. Carefully the coverslip was lifted off from one test tube, together with the drop of fluid adhering to it, and it was placed immediately on a microscope slide. The same procedures were done for the

remaining two test tubes. The slides were then observed under microscope but no coccidian egg was detected.

A loop of blood sample was streaked on Blood agar and incubated for 24 hours. Big, circular and non-hemolytic colonies were observed. A colony was cultured on MacConkey agar plate and incubated for 24 hours. Circular, smooth, pink and lactose fermenting bacteria were observed. And a biochemical test done was Catalase test only.

For gram staining*;* with a sterile cooled loop, a drop of sterile water was placed on a clean labeled microscopic slide. 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 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 technique (APPENDIX 1).

A drop of oil immersion was added on the stained slide and observed under 100X objective lens of binocular microscope. Gram negative rod bacteria were observed.

All the culture, biochemical tests and Gram staining done on the sample confirmed the presence of E. coli in the blood sample taken.

Treatment

Gentamycin 10mg/kg IM SID was given for 5 days.

Outcome

After the second day of treatment the diarrhea stopped and the calf started to suck up milk and took little amount of feed. After the fifth day of treatment the calf regained its normal condition.

At the 30^{th} day of follow up the calf was grown up and healthy.

Fig. 15 Normal, pink conjunctiva color

Fig. 16 Clean hind quarter, smooth & shiny hair coat (Day sixty)

Discussion

Colisepticemia is a disease condition that is caused by pathogenic *E. coli.* The disease affects most animals but calves are the most susceptible (Radostitis et al., 2000). Not getting enough colostrum and stress are among predisposing factors to the disease.

The clinical signs observed in this case report; fever, increased pulse, diarrhea and weakness, are similar findings with literatures (Ahmed et al., 2013; Nolan et al., 2013). But blood stained faeces and pale conjunctiva found in this case could be due to enteritis that may arise from E.coli infection or secondary complication due to other parasites (though not detected on feacal examination that could be due to uneven distribution of parasites egg in faeces).

Antimicrobial sensitivity test should have to be done before treatment of *E. coli* cases, as most *E. coli* species develop drug resistance, but due to lack of facilities it was not done in this case.

The calf could probably be exposed to this disease because it was not fed with the colostrum or there could be any stressful condition, like unhygienic environment, since it shared a shed with other cattle.

Colisepticemia and Collibacillosis could occur in older calves subjected to stress (Health Management and Disease, 2010), keeping calves in hygienic and if possible in a separate shed is recommended.

Acknowledgment

I want to tank Mr Misgana Tefera (Veterinary Laboratory Technician at Adiss Ababa University, College of Veterinary Medicine) for the isolation and identification of the causative agent of the case.

4.1.4. Navel ill

Summary

Navel ill is a condition characterized by inflammation, as a result of infection, in the umbilicus and its associated structures. This case report summarizes the diagnosis, treatment and follows up of Navel ill in three months old Holstein calf presented to Ada'a veterinary clinic, Bishoftu, Ethiopia. The calf lost its dam since birth.The calf had slowly growing swelling around the umbilicus and gradually the calf started to lose its appetite. The body temperature, respiration and pulse rate of the calf were in normal range but there was firm, painless swelling around the umbilicus. Aseptically, the swelling was aspirated with needle and the content was pus. The pus sample was taken to lab with ice cold and immediately inoculated to nutrient broth media that was incubated aerobically at 37^{o}c for 24 hours. For isolation of the causative bacteria, a loop of sample from the nutrient broth, that was incubated for 24 hours, was streaked on blood agar and incubated for 24 hours. Mannitol salt and MacConkey agar were used as selective media. Gram staining was done on isolates from each selective media. All the culture characterstics and Gram staining confirmed the presence of*Staphylococcus aureus* and *Escherichia coli*. Systemic antibiotic (Penstrip) and local dressing of the swelling with 2% tincture iodine were used for five days to treat the calf. The owner washed the wound area with homemade saline for additional one week. At the 30^{th} day of follow up the wound had totally healed. Calves which lost their mother are more susceptible to Navel ill so thorough cleaning of their environment and disinfection of the umbilical region is recommended.

Keywords: Calf, Navel ill, Staphylococcus, Escherichia coli

Literature Review

Navel ill is a condition characterized by inflammation, as a result of infection, in the umbilicus and its associated structures. It occurs commonly in neonatal farm animals and appears to be particularly common in calves delivered in dirty environments (Radostits *et al.,* 2007; S.G *et al.,* 2011).

Anatomically the umbilicus and its associated structures comprised of the amniotic membrane, umbilical vein, umbilical arteries and the urachus. At birth the amniotic membrane of the umbilical cord breaks followed by gradual closure of the umbilical vein and the urachus (Abdullah *et al.,* 2015).

At birth the smooth muscles of the umbilical arteries contracts thereby forcing the umbilical arteries retract as far back as the top of the bladder (Radostits *et al.,* 2007). The umbilical cord normally dries up within a week, usually 1 to 8 days, after parturition (Hides and Hannah, 2005).

Navel ill may manifest in any of the clinical entities or combination of omphalitis, omphalophlebitis, omphaloarteritis or infection of the urachus (S.G *et al.,* 2011). In can be caused by Gram positive and/or Gram negative bacteria and the common ones are Staphylococcus, Streptococcus, Escherichia coli and Klebsiella (Smith, 2015).

The clinical findings in navel ill are usually the enlargement of the umbilicus with purulent material, chronic toxaemia, and unthriftiness (Radostits *et al.,* 2007). If this condition is left untreated it can lead to reduced growth, joint ill and other sequelae (Mee, 2008).

The occurrence of this condition is mostly associated with poor hygienic maintenance of maternity pen, prolonged residency of new born calf in unhygienic maternity pen, lack of adequate and early intake of good quality colostrum and immediate navel antisepsis after parturition (Mee, 2008). It has been observed that in new-born calves that previously had failure of transfer of maternal immunity during fetal life, navel infection may act as source of infection leading to septicaemia (S.G, *et al.,* 2011).

In the treatment of umblical cord infection, intravenous injection of antibiotics such as pencillin against Staphylococcus and aminoglycosides is effective against Gram negative bacteria and for anaerobic bacteria metronidazole can be effective (Abdullah, *et al.,* 2015).

In the prevention of this disease, disinfect the umblical cord after birth with iodine, bacitracin, and silver sulfadiazine can be useful (Abdullah, *et al.,* 2015).

Case History

An owner presented a three months old calf to Ada'a veterinary clinic, Bishoftu in November 2018. The owner mentioned that the mother of the calf died at the birth of this calf. The calf had slowly growing swelling around the umbilicus. Gradually the calf started to lose its appetite.

Clinical Examinations and Findings

The body temperature was 38.2^{o}C, respiration was 32 breaths/ minute and pulse rate was 60beats/ minute. All the measured parameters were in normal range. There was firm, painless swelling around the umbilicus. Aseptically, the swelling was aspirated and the content taken out was pus.

Fig. 17 The calf at its presentation (Day one)

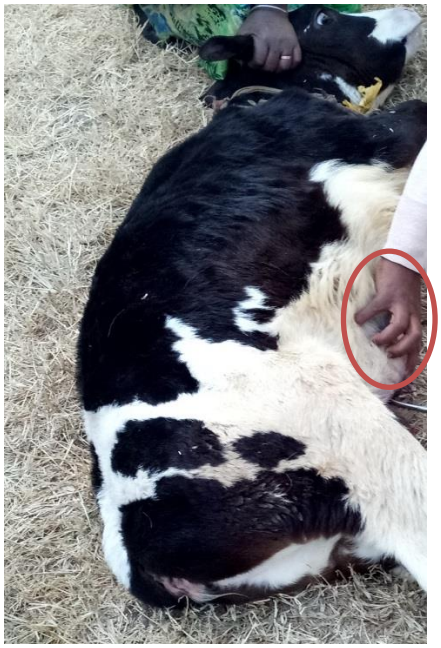

Fig. 18 Swelling around the umbilicus (Day one)

Differential Diagnosis

Navel ill, edema, local abscess

Sample Collection

After the swelling area was aseptically prepared, a puncture was made with 22 gauge needle and pus sample was taken. The sample was immediately transferred to a sterile screwed bottle and transported to lab with cold chain.

Laboratory Diagnosis and Result

A loop of sample was inoculated in to 9 ml nutrient broth media and incubated for 24 hours. After the 24 hours again a loop of sample from the nutrient broth was taken and streaked on blood agar plate and incubated for 24 hours. Staphylococcus (golden in color and zone of hemolysis) suspected colonies were further sub-cultured on mannitol salt agar plate and incubated aerobically for 24 hours. *Escherichia coli*(big, circular and non-hemolytic) suspected colonies were further sub-cultured on *MacConkey* agar plate and incubated aerobically for 24 hours.

The colonies that grew on Mannitol salt agar were yellow colonies with yellow zones, and the colonies that grew on MacConkey agar were Circular, smooth, pink and lactose fermenting bacteria.

For Gram staining of a colony that grew on Mannitol salt agar*;* with a sterile cooled loop, a drop of sterile water was placed on a clean labeled microscopic slide. 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 (APPENDIX 1).

For Gram staining of a colony that grew on MacConkey agar; with a sterile cooled loop, a drop of sterile water was placed on another clean labeled microscopic slide. The loop was again sterilized over Bunsen burner flame and allowed to cool. With the cooled 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 (APPENDIX 1).

A drop of oil immersion was added on the two stained slides and each were observed under 100X objective lens of a binocular microscope. Gram positive bunch of Cocci were observed from the stained sample taken from Mannitol agar plate. Gram negative rod bacteria were observed from the stained sample taken from the MacConkey agar plate.

The culture characteristics and Gram staining done confirmed the presence of Staphylococcus species (that grew on Mannitol salt agar) and Escherichia coli (that grew on MacConkey agar) in the pus sample taken.

Treatment

The soft part of the swelling area was aseptically prepared and incised with number No 24 scalpel blade. The pus was drained with the help of forceps and the pouch was flushed with 2% tincture iodine. Cleaning with tincture iodine continued for 5 days.

Penstrip (1ml/ 10kg IM, SID) was given for 5 days (1ml contains Procaine Pencillin G 200,000IU and Dihydrostreptomycin sulphate 200mg).

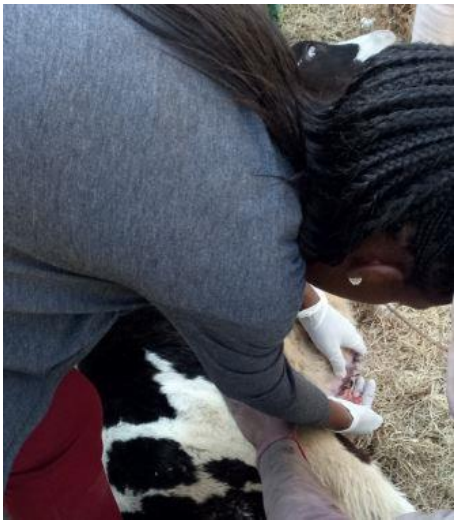

Fig. 19 Removing the pus (Day one)

Fig. 20 Cleaning the pouch with Iodine.

Outcome

After the fifth day of treatment the owner was advised to keep the environment clean and wash the area of the wound with saline daily for a week. At its 30^{th} day follow up, there was no swelling and the wound had healed completely.

Fig. 21 The calf at its home

Fig. 22 No umbilical abscess (Day thirty)

Discussion

Navel ill is a condition characterized by inflammation, as a result of infection, in the umbilicus and its associated structures (Radostits *et al.,* 2007). The disease is mostly seen in calves that are not fed with quality colostrum (S.G, *et al.,* 2011).

The clinical findings in navel ill are usually the enlargement of the umbilicus with purulent material and unthriftiness (Radostits *et al*., 2007).

In this case report the navel ill might arise from the lack of colostrum fed to the calf because its dam died at birth or it might be due to the unhygienic environment that the calf may be kept in.

Staphylococcus and *Escherichia coli* were the identified causes of the navel ill of this calf which is consistent with the findings in literature (Smith, 2015).

As calves which lost their mother are more prone to Navil ill, because of lack of maternal colostrum, special care has to be given to cleaning their environment and disinfecting the navel area atleast until the navel dried up.

Acknowledgment

I thank the owner of the calf, for implementing all management advises that were given to her.

4.1.5. Lumpy skin disease

Summary

Lumpy skin disease is a pox disease of cattle. This case report summarizes the case management of lumpy skin disease in ten months old Zebu calf presented with its dam to Ada'a veterinary clinic, Bishoftu, Ethiopia. The calf and the dam were living with other three bulls. The calf had fever (41.6^{o}c), generalized skin nodules and lameness In addition prescapular and prefemoral lymphnodes were swollen. The dam and the other three bulls were healthy with no nodular skin lesion observed. After a selected nodular area is prepared and infiltrated with 2% lidocaine, nodular skin biopsy was taken in a sterile universal bottle with Phosphate-buffered saline (PBS) and transported with cold chain to National Veterinary Institute (NVI) for confirmatory diagnosis. The polymerase Chain Reaction (PCR) result, done at NVI, confirmed the presence of Lumpy Skin Disease virus in the tissue sample. The calf was treated with antibiotic for five days to reduce the risk of secondary bacterial complication. To help improve the appetite of the calf one dose of injectable multivitamin was given. After the fifth day of the commencement of treatment the calf returned back to its healthy condition. At its fourth month visit, there was no single nodule visible on the skin of the calf. Vaccination of newly born calf, from vaccinated dam, between the ages of three to six month is recommended.

Keywords: Calf, Lumpy skin disease, PCR

LiteratureReview

Lumpy skin disease (LSD) is a pox disease of cattle (OIE, 2014; ESFA, 2015). The causative agent is a pox virus that is called by the same name, Lumpy skin disease virus. LSD virus belongs to the genus *Capripoxvirus* within the family *Poxviridae* (Buller *et al.*, 2005). The prototype of LSD virus is Neethling strain (Alexander, *et al.*, 1957). Pox viruses are highly resistant and can remain viable in infected tissue for more than 120 days or probably longer time.

Most of LSD virus infections are thought to be transmitted through insects. The incubation period is ranged between 2 to 5 weeks (Haig, 1957).

After the virus enters the body of the animal, through abraded tissues, it starts to replicate locally in that tissue that will result in local swelling. After 1 week the local swelling will be followed by enlargement of the regional lymph nodes, while generalized eruption of skin nodules usually occurs 7-19 days after injection (Barnard *et al.,* 1994).

Host susceptibility, dose and route of virus inoculation affect the severity of disease (OIE, 2010). Skin nodules about 0.5-5 cm in diameter in whole skin or subcutaneous tissue and swollen superficial lymph nodes especially subscapular and precrural lymph nodes are the main symptoms of LSD infection in most animals. These nodules can also affect the nasal, oral, ocular, and genital mucosa. Their number may range from a few to several hundred (Barnard *et al.,* 1994).

Cutaneous lesions may resolve rapidly or may indurate and persist as hard lumps, or become sequestrated to leave deep ulcers partly filled with granulation tissue, which often suppurates (Wainwright *et al.,* 2013). Papules most easily seen in hairless areas of perineum, udder, inner ear, muzzle and eyelids (Babiuk *et al.,* 2008) which leads to the development of ulcerative lesions with excessive salivation, lacrimation and nasal discharge that may contain LSD virus. Some of the infected cattle may develop edematous swelling of one or more legs and show lameness.

At present time, no commercial diagnostic test kits for LSD virus detection are available yet (Tuppurainen and Oura, 2012). Thus, the tentative diagnosis of LSD is usually based on the characteristic clinical signs. And confirmatory diagnosis is by laboratory tests using conventional polymerase chain reaction (PCR) techniques (OIE, 2011).

As LSD is a viral disease there is no specific antiviral treatment. Sick animals should be removed from the herd and follow supportive treatment such as antibiotics, anti-inflammatory drugs, and vitamin injections. These therapies are usually given to avoid chances for the development of secondary bacterial infections, inflammation and fever, and thus improving the appetite of the animal (Capstick *et al.,* 1959).

As biting flies and certain tick species are probably the most important method of transmission of the disease, control by quarantine and movement control is generally not very effective. In

endemic areas, like Ethiopia, control is therefore essentially confined to immunoprophylaxis (Coetzer and Tuppurainen, 2004; OIE, 2011).

Case History

An owner presented a ten months old calf, with her dam to Ada'a veterinary clinic, Bishoftu in November 2018. The owner mentioned that the dam and the calf were living with three other bulls under extensive farming system. The bulls and the dam were taking vaccine against lumpy skin disease every year and the last year's vaccination was when the dam was about 9 month pregnant with this calf. The history of the calf was inappetance, generalized skin nodules and lameness. The lesions were seen only in the calf; but not in the dam and the other three bulls.

Clinical Examinations and Findings

On clinical examination the calf was having temperature 41.6^{o}c, respiratory rate 24 breaths/min and pulse rate 72 beats/min. Skin nodules with average diameter of 0.4 cm were seen all over the skin. The calf was depressed and the limbs were swollen and pits on palpation. Prescapular and prefemoral lymphnodes were swollen and hot.

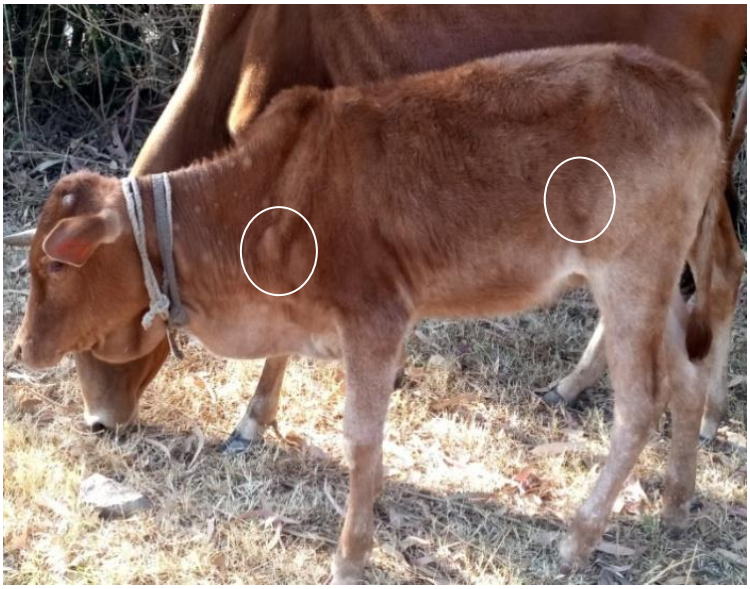

Fig. 23 Skin nodules & swollen prescapular and prefermoral lymphnodes (Day one)

Differential Diagnosis

Lumpy skin disease, Ring worm, Dermatophilosis

Sample Collection

A nodule on the left front limb was selected and the hair over the nodule was clipped off. With waterproof marker the area of the nodule was marked and with 25 gauge needle 5 ml of 2% lidocaine was infiltrated around the nodule. Using scalped blade No. 24 and thumb forceps, the marked nodule was surgically removed and the tissue sample was kept in sterile universal bottle that contain Phosphate-buffered saline (PBS). On the calf the area where the sample was taken, was cleaned with 1% iodine and covered with gauze for three days. The sample was then transported with cold chain to NVI for molecular identification of antigen.

Laboratory Diagnosis and Result

National Veterinary Institute had done Polymerase chain reaction (PCR) test and confirmed the presence of Lumpy skin disease virus in the tissue sample.

Treatment

Oxytetracycline (short acting) 15 mg/ kg/ day, IM, SID for 5 days was given to prevent secondary bacterial complications.
Multivitamin 10ml, IM SID was given to boost the appetite of the calf

Outcome

After five days of follow up, the calf started suckling and eating its food. At its one month visit, the calf was back to her normal condition unless the nodules. And at its 4 month checkup, there was no nodule on the calf and the calf was healthy.

Fig. 24 The calf free from the nodular lesion (Day one hundred twenty)

Discussion

Lumpy Skin Disease (LSD) is a pox disease of cattle. The clinical findings in this case report; skin nodules, swollen lymphnodes and swollen limbs are all in consistent with literatures (OIE, 2010; Wainwright *et al.*, 2013; Babiuk *et al.*, 2008).

As Lumpy Skin Disease is endemic in Ethiopia, annual vaccination is the practice for control of the disease. In this case report all the cattle that were living with the calf, including the dam were vaccinated when the dam was 9 month pregnant with the calf; it was before 10 months. So there were additional two months remained for the next vaccination time.

Calves born to naturally infected or vaccinated cows are protected by passive maternal immunity for three months (FAO, 2011). But they should be vaccinated against lumpy skin disease between their three to six months. The calf in this case report, even though it was 8 months old, it did not get the vaccination. So transmission of the disease by insects could be from distant area, and not getting vaccine at the age between three to six months of the calf could be the reasons that predispose the calf to the disease.

So in endemic areas vaccination of newly born calf, from vaccinated dam, between the ages of three to six month should be considered.

Acknowledgment

I thank the owner of the calf for giving all the information on every phone communication and I thank also Mr Belay Temesgen (senior Animal Health professional at Ada'a district veterinary clinic), for his helping hand during sample taking and treatment of the calf.

4.1.6. Mastitis

Summary

Mastitis is the inflammation of the mammary gland and udder tissue. This case study summarizes the management of mastitis in a Holstein cow from one of the dairy farm found in Bishoftu, Ethiopia. The farm had 40 dairy cows. A history of reduced milk production (on average 2 liters less/ day) in three cows (one cow gave birth before two months and the other two gave birth before a year and half) was reported. There was no visible abnormality observed on the quality of the milk. On clinical examination the body temperature of the cows was in normal range (37.5^{o}C, 37.8^{o}C, and 37.3^{o}C); all the three cows were bright and had smooth and shiny hair coat and there were no abnormal swelling and inflammatory signs on the udder of all the three cows. California Mastitis Test (CMT) was performed for all the quarters of each three cows but the result was positive on a quarter of one cow (that gave birth before two months). Milk sample from the affected quarter was taken with a sterile screwed bottle to isolate the causative agent. The characteristics of the bacterial colony on blood agar and characteristics of the Gram stained bacteria and again the growth on selective, Mannitol Salt agar confirmed the causative agent as Staphylococcus species. Disk diffusion susceptibility method was used to select the antibiotic of choice for treatment of the case, and the Staphylococcus species was susceptible to Cotrimoxazole. The treatment was given for five consecutive days and milk was withdrawn for 72 hours after the last treatment. After the end of treatment and milk withdrawal period, CMT was again performed on cow that was initially positive for the test and the result was negative. As there was no evidence of systemic disease and mastitis on the other two cows, pregnancy diagnosis was done (because pregnancy could also reduce milk yield) and both cows were pregnant. Use of dipping, regular screening of mastitis, isolation of positive cases and proper treatment of cases was recommended for the farm for better control of contagious mastitis especially on dams that gave recent birth and on those that are pregnant.

Key words: mastitis, Staphylococcus aureus, CMT, disk diffusion susceptibility

Literature Review

Mastitis is the inflammation of the mammary gland and udder tissue. It is also known as udder inflammation, is a common problem in dairy herds causing increase in costs of milk production (Bramley *et al.,* 1996) and also have negative impact on milk composition

Somatic cells count (SCC) is an indicator of the udder health problems. Milk from cows with healthy udder should contain less than 200,000 SCC in 1 ml of milk. Most somatic cells are white blood cells (e. g. macrophages and neutrophils) that influx mammary gland tissue from blood during inflammation (Akers and Nickerson, 2011). Conditions decreasing the resistance and susceptibility of cow udders to inflammations can be e.g. prolonged intra-udder antibiotics administration, increased incidence of udder mycosis results from mineral-vitamin deficiencies, antioxidant deficiencies, imbalanced diet, poor environmental conditions and even weather changes (Wawron *et al.,* 2010).

Based on the clinical manifestation, mastitis is divided as Clinical and Subclinical mastitis. There are no visible signs in case of sub-clinical mastitis but decrease in milk production and increased somatic cell count are evident. Abnormal quarters contain somatic cell count above 300,000 somatic cells/ml of milk while uninfected quarters contain below 200,000 somatic cells/ml of milk (Jones, 2006). Clinical mastitis is characterized by the sudden onset, swelling, pain, decline in the milk quantity and quality. Animal may show the signs of fever, depression and anorexia accompanied by altered milk quality in the form of flakes, clots or watery appearance (Hillerton, 1999).

Many approaches have been made to determine milk somatic cell counts both in the field and laboratory including California Mastitis Test (CMT, a cow side test). The California Mastitis Test (CMT) is a simple indicator of the Somatic Cell Count (SCC) of milk. It works by using a reagent which disrupts the cell membrane of somatic cells present in the milk sample; the DNA in those cells to reacting with the test reagent. It is a simple but very useful technique for detecting subclinical mastitis on-farm. A four-well plastic paddle is used, one well being used for each quarter of the cow to be tested. The foremilk is discarded, and then a little milk drawn into each well. An equal volume of CMT test reagent is added and then the sample is gently agitated. The reaction is scored on a scale of 0 (the mixture remaining unchanged) to 3 (an almost-solid

gel forming), with a score of 2 or 3 being considered a positive result. This result is not a numerical result but is an indication as to whether the cell count is high or low; the CMT will only show changes in cell counts above 300,000 (Dairy.ahdb.org.uk, 2018).

Mastitis is mostly caused by two types of pathogens, one are contagious while others are environmental. *Staphylococcus aureus, Streptococcus agalactiae* and *Mycoplasma* are major contagious pathogens which are transmitted from diseased to healthy animals during milking process while *Streptococcus disagalactiae, Streptococcus uberis* and *Escherichia coli* are the major environmental pathogens responsible for this disease and are present in beddings and animal surroundings. Contagious bacteria mostly get transferred to teat due to unsanitized milking instruments (Bramely, 1992).

About 90% of pathogens responsible for udder inflammations are environmental pathogens which presence is common in cow-barn environment. Increase in animal amount, humidity and pollutions in cow environment increase also bacteria and other pathogens present on animals (Lassa *et al.*, 2013).

Mastitis is a multifactorial disease and its prevention requires proper management on both stages (clinical and subclinical), but should concentrate on mastitis prevention (Oliver and Murinda, 2012). Clean and dry bedding, clean and dry udders at the time of milking, and lack of teat-end lesions all have a positive effect on control. The single most important management practice to prevent transmission of new infections is the use of an effective germicide as a post-milking teat dip. These products should be applied as a dip (rather than a spray) immediately after milking. Other practices that augment teat dipping include use of individual towels for drying teats, gloves for milkers' hands, use of a pre-milking germicide (spray or dip), attachment of units at the proper time after teat stimulation (60–120 sec), cleaning milking units after an infected cow has been milked, or segregation of infected cows into a separate milk group. This last option may be unrealistic for cattle in free housing that are normally segregated for nutritional or reproductive reasons. Routine milking equipment evaluations should be conducted to ensure that the teat-end vacuum is operating at a proper level and remains stable during milking. Proper pulsator function should be maintained, and liners and rubber air hoses should be replaced as needed (Veterinary Manual, 2018d).

Ethiopia has one of the largest livestock populations in Africa (CSA, 2013; Tilahun and Schmidt, 2012). The livestock subsector has an enormous contribution to Ethiopia's national economy and livelihoods of many Ethiopians, and still promising to rally round the economic development of the country. Livestock plays vital roles in generating income, creating job opportunities, ensuring food security, providing services, contributing to asset, social, cultural and environmental values, and sustain livelihoods (Metaferia *et al.,* 2011). But mastitis is one of the major and expensive diseases in terms of production losses in dairy production (Abebe *et al.,* 2016; Bardhan, 2013).

Case History

A report was received from one of the dairy farm in Bishotu, Ethiopia. The owner of the farm mentioned that he had 20 cows, kept in intensive farming system and among them three cows had reduced milk production. There was no gross change on the milk quality (on change color, consistency and odor). There was no change of feed and the animals didn't show any reduction in feed intake. From the three, one had given birth before two months. The other two gave birth before a year and half. From the record book of the farm the three cows gave on average 2 liters less per day for fifteen consecutive days.

Clinical Examinations and Findings

The body temperature of the cows was in normal range (37.5^{o}C, 37.8^{o}C, and 37.3^{o}C). All the three cows were bright and had smooth and shiny hair coat.

General examination of the udder was done but there were no abnormal swelling and inflammatory signs on the udder of all the three cows.

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 other two cows 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.

Fig. 25 Performing CMT test (Day one)

Fig. 26 CMT test showing one positive quarter (Day one)

Differential Diagnosis

Subclinical mastitis, early pregnancy, systemic disease

Sample Collection

For isolation of the causative agent 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.

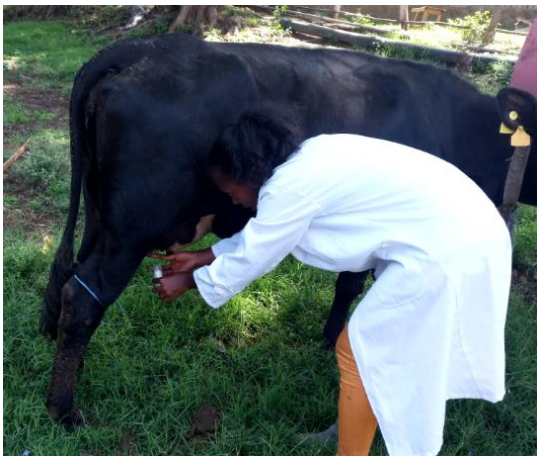

Fig. 27 Milk Sample Collection from affected quarter (Day one)

Laboratory Diagnosis and Result

A loop of milk sample was inoculated on blood agar plate and incubated for 24 hours. Colonies that were golden in color and with zone of hemolysis 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 (APPENDIX 1).

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; 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.

Antimicrobial sensitivity test was done on disk diffusion susceptibility method. A selective media for *Staphylococcus aureus*, Mannitol salt agar was prepared on Mueller-Hinton agar plate. After the media cooled the pure colony of Staphylococcus species, isolated from the case, was inoculated all over the surface of the media. Cotrimoxazole, Pencillin, Methclin and Tetracyclin commercially-prepared, paper antibiotic disks were placed on the inoculated agar surface and plates were incubated for 24 h at 35°C. The result was read against black background. The zones of growth inhibition around each of the antibiotic disks were measured to the nearest millimeter. Accordingly the zones of inhibition were 36.13mm, 20.18mm, 23.71mm and 15.23mm respectively. The Staphylococcus species were found to be susceptible to both Methcilin and Cotrimoxazole. And Cotrimoxazole was used for the treatment of the case.

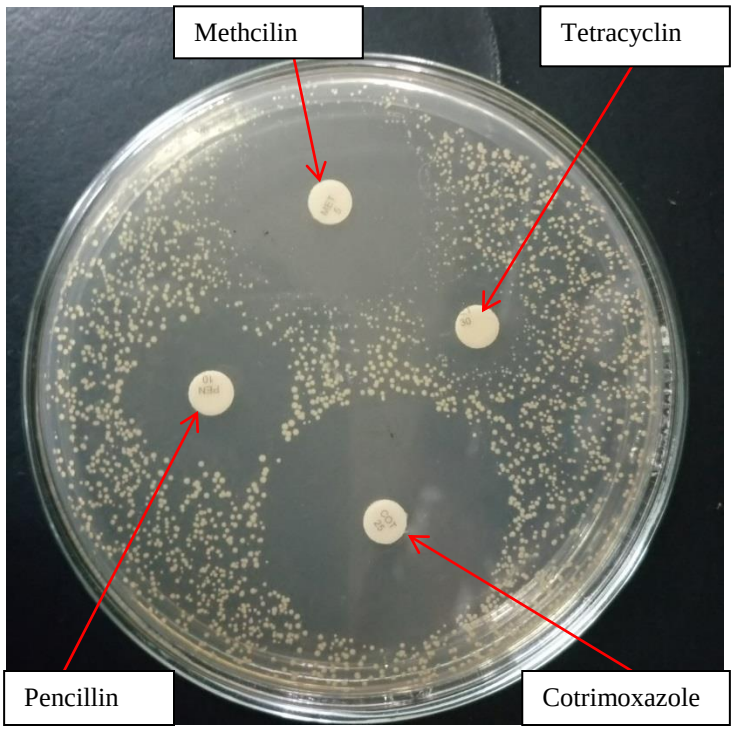

Fig. 28 Disk diffusion susceptibility test

Treatment

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.

Cotrimoxazole 15mg/ kg body weight, IM, SID for five days was used. The milk was withdrawn for 72 hours after the last treatment.

Outcome

After the end of the treatment, the milk was withdrawn for 72 hours. The cow, with mastitis was again rechecked with CMT test and the result was negative.

The two cows that had history of reduced milk production but showed negative result for CMT and had normal body parameters were examined for pregnancy and they were 3 & 4 months pregnant.

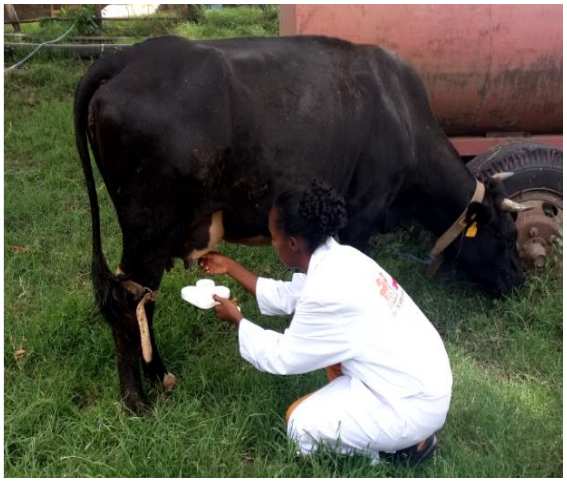

Fig. 29 Milk sampling for CMT (Day nine)

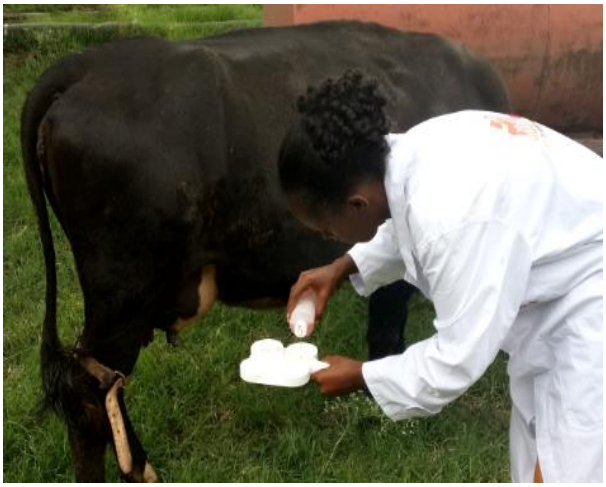

Fig. 30 Adding CMT reagent (Day nine)

Fig. 31 Negative result on CMT (Day nine)

Discussion

Mastitis is the inflammation of the mammary gland and udder tissue. Based on the clinical manifestation, mastitis is divided as Clinical and Subclinical mastitis. There are no visible signs in case of sub-clinical mastitis but decrease in milk production and increased somatic cell count are evident (Hillerton, 1999). The history and clinical signs of this case study were similar with the signs of subclinical mastitis.

California Mastitis Test (CMT) could be used to determine Somatic Cell Count (Akers and Nickerson, 2011). So in this case study CMT was used to determine the Somatic Cell Count, which showed negative result for two cows and strong positive result for one quarter of the third cow.

The causative agent identified, Staphylococcus species is one of the major causes of mastitis and is responsible for about one-third of cases of clinical and subclinical mastitis (Hillerton, 1999; Akers and Nickerson, 2011; Veterinary Manual, 2018d; Topuzoğlu, 2015; Li T, 2017).

The antibiotic sensitivity test result for Staphylococcus species indicated high resistance of the bacteria to different antibiotics (Nibret *et al.,* 2011; Amanu *et al.,* 2016). So disk diffusion antibiotic sensitivity test with the available four drugs (Cotrimoxazole, Tetracyclin, Methicilin and Pencillin) was done and Cotrimoxazole was selected as the Staphylococcus species identified were susceptible to it.

As subclinical infection of one quarter of the udder may not cause that much recognizable reduction in milk production, there might be any uninvestigated underlying additional cause reduction in milk production.

On the two cows even though there was a history of reduced milk production, there was no evidence of systemic disease or mastitis. But both cows were pregnant on pregnancy diagnosis done and pregnancy has been reported to have a negative effect on milk yield of dairy cows due to hormonal changes, causing regression of the mammary gland (Akers, 2006), and nutrient requirements of the fetus, reducing available nutrients for milk production (Bell *et al.,* 1995).

To prevent the possible occurrence of this type of contagious mastitis in the farm the following recommendations were given; to regularly do CMT test and isolate positive cases. Cows with contagious mastitis should be milked last or a separate milking claw used for the infected cows. Milking claws should be flushed with hot water or germicide after milking infected cows. Individual towels should be used to wash/dry teats. Milkers should have clean hands and wear latex gloves.

Acknowledgement

I want to thank Mr Getahun (microbiologists at CVMA) for his help in all the laboratory works of this case study. I also want to thank Dr Geda Regassa (for performing pregnancy diagnosis in the two cows.

4.1.7. Parafilariosis

Summary

Bovine parafilariosis is a seasonal vector-borne parasitic disease caused by the cutaneous filaria, *Parafilaria bovicola.* This case report summarizes the case management of Parafilariosis on a six year Zebu cattle presented to College of Veterinary Medicine open air clinic, Bishoftu, Ethiopia. The ox was always had focal bleeding especially after rainy season. When the area bled, the owner used to wash it with salty water and the bleeding would stop for few days but would start again after few days. Antibiotics were given but could not help it. On clinical examination the body parameters were in normal range. Oozed blood was collected with sterile test tube. Smear was prepared from the sample, stained with Wright stainand examined under microscope which revealed many eosinophils. With sedimentation techniques, the oozed blood was also checked for parasite egg but no parasite egg was detected; this could be due to uneven distribution of parasitic egg. Single dose of Ivermectin was given subcutaneously and the focal lesions were washed with chlorhexidine. After fifteen days the focal bleedings had stopped and the sites healed.

Key words: parafilariosis, focal bleeding, Ivermectin

Literature Review

Bovine parafilariosis is a seasonal vector-borne parasitic disease caused by the cutaneous filaria *Parafilaria bovicola*. This is a nematode parasite whitish in color; adult females are 50–65 mm long, and males 30–35 mm. It is a parasite of the subcutaneous and intermuscular connective tissue of the skin which causes local mechanical lesions manifested as the “bleeding spots” (Caron *et al.,* 2013).

The incubation period ranges between 7-10 months and the only external signs of infection in cattle are focal cutaneous hemorrhages (“bleeding spots”) that may ooze for some hours before clotting and drying in the matted hair of the coat. Bleeding spots are induced by the female worm, which causes the formation of a small nodule, perforates the skin, and oviposits in the

blood dripping from the central wound. The tiny eggs contain the first larval stage (microfilariae) of the parasite (Worms, 1972).

The development cycle is indirect with flies of the genus *Musca* as intermediate vectors. Muscid vectors become infected with nematode microfilaria when they feed on sero–haemorrhagic exudates from skin wounds of infected cattle. Then, L3 infectious larvae develop from microfilaria in vectors (the incubation period of the larvae in the vector varies depending on the suitable conditions for hatching). Infection occurs when vector as intermediate host, again feeds on cattle, and in this cases L3 larva can penetrate into dermis and migrate to different locations (subcutaneous and intermuscular tissue of neck, rump etc.) of the body. After development of adults from L3 larvae, female nematode laid eggs which can be found in exudates or blood (Nevill, 1980).

The seasonal bleeding spots are sometimes confused with those caused by thorns, wire, ticks, or biting insects. For differentiation, either fresh or dried blood should be mixed with water in a test tube and centrifuged. The characteristic eggs are found on microscopic examination of the sediment. Carcass lesions can be differentiated from bruising by the presence of numerous eosinophils in Giemsa-stained impression smears made from the lesions. In addition, affected tissue has a characteristic, disagreeable, metallic smell. Usually, only small numbers of worms are present in affected carcasses and are often difficult to find because of their color and the accompanying inflammatory reaction. 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 Ivermectine group, subcutaneously with 0.2 mg/kg moxidectin has shown good effect in the treatment of Parafilariosis (Deprez *et al*., 2010).

Case History

An owner presented an ox to the Veterinary Teaching Hospital of College of Veterinary Medicine with history of local bleedings on September 2017. The owner mentioned that the ox always had bleeding in these focal sites especially after rainy season. When the area bled, the owner used to wash it with salty water and the bleeding would stop for few days but would start

again after few days. Antibiotics were given but could not help it. The ox had been used for ploughing but due to its age and this disease the owner wanted to fed it and sell it to slaughter houses.

Clinical Examinations and Findings

The body temperature of the ox was 37.3^{o}C, respiratory rate 24 breath/ minute and pulse rate was 52 beats/ minute; all parameters were in normal range. There were three focal cutaneous sites (one on the right thoracic area, one on the right neck area and one on the left lumbar area) which oozed blood. On palpation the bleeding areas were nodular.

Fig. 32 Cutaneous focal bleeding sites, right side (Day one)

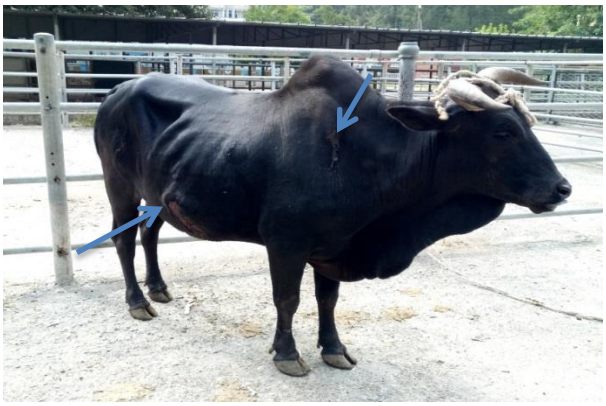

Fig. 33 Cutaneous focal bleeding sites, left side (Day one)

Differential Diagnosis

Parafilariosis, local trauma

Sample Collection

About 3ml of oozed blood, from the three bleeding sites, was collected in a sterile test tube.

Laboratory Diagnosis and Result

Immediately after collection of sample, small amount of blood was put on one end of microscopic slide and another spreader slide was kept at 45^{o} 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^{o}, it was pushed forward rapidly and smoothly. The smear was allowed to air dry for 5 minutes. The dried smear was stained with Wright stain (APPENDEX 2). 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 eosinophils.

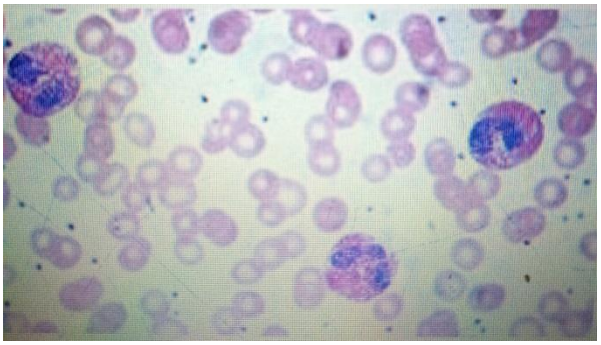

Fig. 34 Eosinophils in the smear made from a drop of oozed blood

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 but no parasitic egg was detected.

Treatment

Ivermectin 1ml/50kg was given S/c and the local bleeding and the bleeding was washed off with water and chlorhexidine.

Outcome

After 15 days of treatment when the owner was communicated through phone, the cutaneous wounds of the ox dried up and it was back to its normal condition.

When the ox was visited after three months the ox was in a good health condition the local bleeding never reappeared after the treatment.

Fig. 35 No bleeding spot, right side (Day ninety).

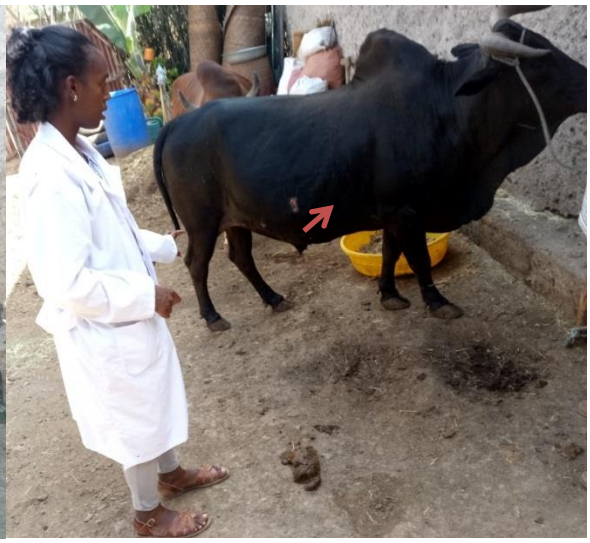

Fig. 36 Bleeding spot formed dry scar on the left side (Day ninety)

Discussion

Bovine parafilariosis is a seasonal vector-borne parasitic disease caused by the cutaneous filaria *Parafilaria bovicola.*

The only clinical sign observed in this case was focal cutaneous bleeding spots; were inconsistent with other findings (Abebe, 2017; Worms, 1972). Bleeding spots are induced by the female worm, and ovipositional bleeding was strongly seasonal with blood spots first appearing

in June, reaching a peak in September-November (Abebe, 2017); inconsistent with this finding the case of the ox in this case report came to clinic on September.

The characteristic eggs could not be found on microscopic examination of the sample sediment but this could be due to the uneven distribution of the parasite egg in the oozed blood. But in the stained sample relatively increased number of eosinophils were detected; indicator of parasitic inflammation.

History, presence of increased eosinophils and response to Ivermectin treatment were used for confirmation of the case. Tissue biopsy, which could reveal the parasite's egg, was not done due to lack of pathologist.

Acknowledgment

I thank Mrs Tsedale Teshome (Vet technician at CVMA) for her help in the laboratory works.

4.2. Case Reports on Equines

4.2.1. African horse sickness

Summary

An African horse sickness is a highly fatal insect born viral disease of equids. This case report summarizes a case of African Horse Sickness in a nine years horse presented to Society for the Protection of Animals Abroad (SPANA) open air clinic Bishofu, Ethiopia. The horse was newly bought with unknown previous history. The horse was depressed and had swelling over both side of the supraorbital fossa. On clinical examination the horse had high body temperature, 40.7^{o}c, and the pulse quality was very weak. There was also hemorrhage on the conjunctiva and the ventral surface of the tongue. For confirmation of the disease, whole blood sample was collected in EDTA vacutainer tube and transported in cold chain to the National Veterinary Institute (NVI) lab for virus neutralization test. The lab result confirmed the presence of African Horse Sickness Virus (AHSV) in the blood sample. Diuretic and antipyretic were given but could not help the case and the horse died after two days of its stay in SPANA stable. The gross post mortem findings showed hydrothorax and hydropericardium and these further confirmed the disease as the cardiac form of African horse sickness. As the disease is acute and has no treatment, asking the history of horses before buying and seasonal vaccination of horses are recommended.

Key word: African Horse Sickness, virus neutralization, hydrothorax, hydropericardium

Literature Review

An African horse sickness (AHS) is a highly fatal insect born viral disease of equids, where horses are found more susceptible. It is caused by a virus of the Reoviridae family, of the genus Orbivirus (Bouayoune *et al.,* 1998). A virus is double stranded, viscero- tropic (Radostits *et al.,* 2007) RNA virus. There are nine antigenic strains of the virus.

AHS transmitted indirectly to equidae by the way of haematophagus arthropods. The principal vector of African horse sickness virus (AHSV) worldwide is Culicoides imicola (Bouayoune *et al.,* 1998). Transmission of the virus to areas where it does not usually exist occurs both by

movement of infected animals and transportation of midges by wind. Mechanical transmission of the virus on contaminated surgical instruments and needles should be considered a possibility (Radostits *et al.,* 2007).

The incidence of the disease is often seasonal because of the seasonal variations in the number of Culicoides species present. The areas most affected are low lands and swampy areas (Radostits *et al.,* 2007).

AHS takes on four different clinical forms in infected equids: sub clinical (horse sickness fever), sub-acute (cardiac), acute (respiratory or pulmonary) and mixed forms. The sub-acute (cardiac) form exhibits mortality rates of 50% and higher, the mixed form has mortality rates at 70-80% or higher and the pulmonary form is almost always fatal. Horse sickness fever is rare, if ever, non-fatal. This form is often the result of infection with less virulent strains of AHS virus or the existence of previous immunity (Vanniekerk *et al.,* 2001). But in general, morbidity and mortality are dependent on the species of animal, previous immunity acquired and the form of the disease (Vanniekerk *et al.,* 2001). Horses are more susceptible to the severe (pulmonary and mixed) forms of disease than other equids (AHCF, 2005).

Despite the distinct differences in the clinical severity of AHS infection in different equids, the typical pattern of pathogenesis is similar (Coeter and B.G, 1994). Infection results in damages to the circulatory and respiratory systems resulting in serious effusion and hemorrhage in various organs and tissues. After susceptible equid is bitten by an infected midge, there will be replication of the virus in regional lymph nodes that leads to primary viremia. And this primary viremia will lead to the development of infection of target organs (endothelial cells and mononuclear cells of the lung, spleen, and lymphoid tissue) and resulted in secondary viremia. This virally induced endothelial cell damage and activation of infected macrophages with subsequent cytokine production increases vascular permeability and there will be development of edema. Distinct serotypes demonstrate individual tropisms for pulmonary and cardiac endothelial cells and account for the four, frequently over lapping, clinical forms of AHS (Carrasco *et al.,* 2000).

In natural infections, the incubation period appears to be approximately 5-7 days (OIE, 2004). In per acute (pulmonary) form the clinical signs may develop so rapidly that an animal can die without previous indication of illness. Usually there will be marked depression and fever (39-41°C) followed by the onset of severe respiratory distress. Coughing spasms may occur, the head and neck are extended and severe sweating develops (Radostits, 2007).

In sub-acute (cardiac) form fever may last for 3-6 days and, as the temperature falls, characteristic edema appears, involving the supra orbital fossae and eye lids, sometimes with accompanying congestion and hemorrhage in the conjunctiva and ventral tongue. Subcutaneous edema may also track down the neck toward the chest (Radostits, 2007).

Mixed form is evident as an initial sub-acute cardiac form that suddenly develops acute pulmonary signs. This is not common in field out breaks (Knipe and P.M, 2007). This form is the most frequently observed presentation in horses (Boinas *et al.*, 2002).

Horse sickness fever is the mildest form of the disease that is characterized by a 10 day incubation period, with high temperature (40.5°C for 1 to 3 days) but 100% recovery (Knipe and P.M, 2007). The disease occurs in horses with some immunity or infection by serotypes of low virulence. This is the only form of the disease that occurs in zebras (Radostits, 2007).

For diagnosis African horse sickness virus (AHSV) may be isolated from spleen, lungs, heart or from the blood of a viraemic animal (blood collected in EDTA stored at 4°C) (Lefevre, 2010). Serological diagnosis of the acute disease may be difficult because many horses die before they produce a detectable antibody response. In horses that survive for at least 10 day, AGID, VN and ELISA tests are all effective in detecting antibody to the virus (Radostits, 2007). The gold standard for the identification of AHS serotypes present in suspicious samples is virus neutralization (Boinas *et al.*, 2002).

Gross post mortem findings in acute cases include severe hydrothorax and pulmonary edema and moderate ascites. The liver is acutely congested and there is edema of the bowel wall (Radostits, 2007).It is possible to find 3-5 litter of fluid in the chest cavity (Bouayoune, 1998). In addition, there is marked hydro pericardium, endocardial hemorrhage, myocardial degeneration and anasarca, especially of the supra orbital fossa (Radostits, 2007). In necropsy of per acute form of

the disease, the lungs are distended and heavy and frothy fluid may fill the trachea, bronchi and bronchioles. This frothy exudate may ooze from the nostrils (Radostits, 2007).

There is no treatment that has been shown to have any effect on the course of the disease but careful nursing and symptomatic treatments are not without value (Hirsh and Y.C, 1999). Vaccination and vector control are the current strategies for prevention of the disease (Lefevre *et al.*, 2010).

Case History

An owner presented a nine years horse to Society for the Protection of Animals Abroad (SPANA) open air clinic, Bishoftu on September 2017. He mentioned that the horse was newly bought, one week ago, from the nearby rural town, Chefe. The horse refused to eat and had bilateral supraorbital fossa swelling for two days before its presentation to the clinic.

Clinical Examinations and Findings

The body temperature was 40.7^{o}c, fever developed. The respiratory rate, 28 breaths/ minute, was in normal range and pulse rate was, 48 beats/ minute, but the quality of the pulse was very weak. Both supraorbital fossa (right & left) were swollen. There was hemorrhage on the conjunctiva and the ventral surface of the tongue.

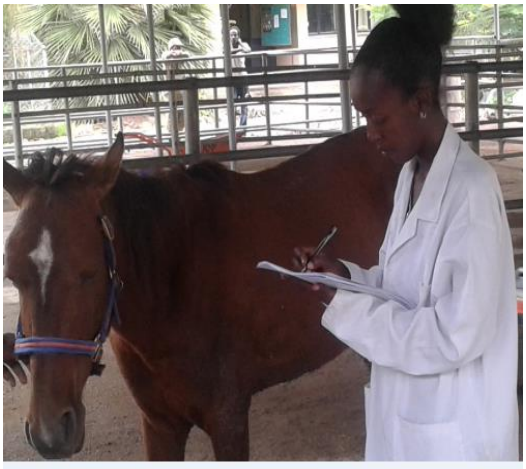

Fig. 37 Depressed horse (Day one)

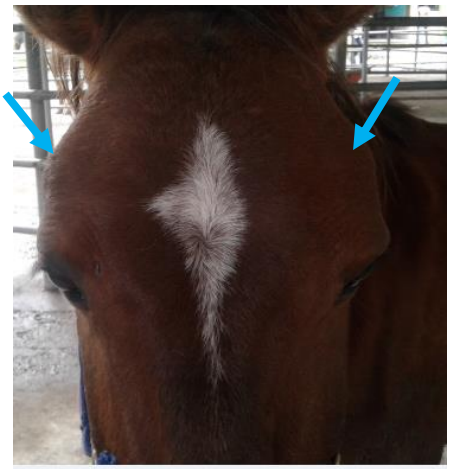

Fig. 38 Swollen supra orbital fossa (Day one)

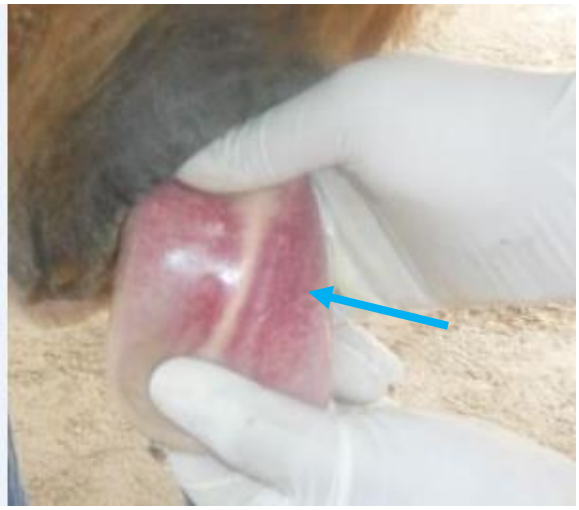

Fig. 39 Hemorrhage on the ventral surface of tongue (Day one)

Differential Diagnosis

African horse sickness, Hypoprotenimea

Sample Collection

The horse was kept in crush to restrain it. The jugular furrow was located and the jugular vein was identified by applying thumb pressure. The jugular furrow, inn proximal third of the neck, was cleaned with piece of cotton socked with 70% alcohol. The vacutainer needle was screwed on the needle holder and the EDTA vacutainer tube was preloaded on the needle holder. Care was given not to puncture the vacutainer tube. The jugular vein was distended by applying thumb pressure over the vein. The vacutainer needle was inserted in to the engorged vein and the vacutainer tube was inserted in to the vacutainer needle that was protruding through the holder. About 8ml of blood was collected and the needle was taken out of the vein. The venipuncture area was disinfected and the blood in the EDTA vacutainer tube was slowly rotated to mix the anticoagulant with the blood. The whole blood sample was transported to NVI in cold chain.

Laboratory Diagnosis and Result

National Veterinary Institute has done virus neutralization test and confirmed the presence of African Horse Sickness virus in the blood sample.

Treatment

Flunixin meglumine (1.1 mg/kg IV SID for three days) was given as antipyretic. And Furosimeide (0.8mg/kg IM BID) was given to reduce tissue fluid (edema).

Since the horse refused to eat, it was intubated (with nasogastric tube) and given fluid based feed.

Outcome

The condition of the horse got worsened on the second day. The development of edema prolongs to the face and neck area also. The horse had difficulty in breathing, cannot hold is neck up and finally died after few hours. And post mortem lesions showed hydrothorax and hydro-pericardium.

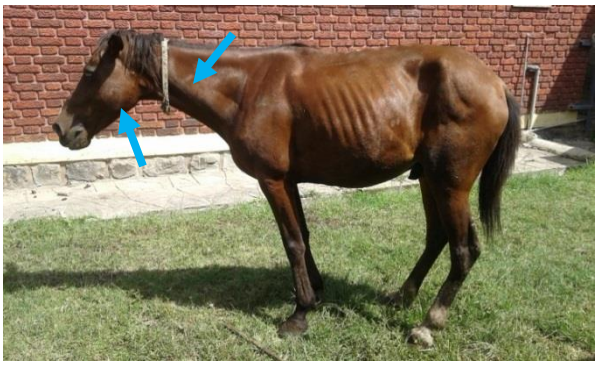

Fig. 40 Edema progressing to face & neck (Day two)

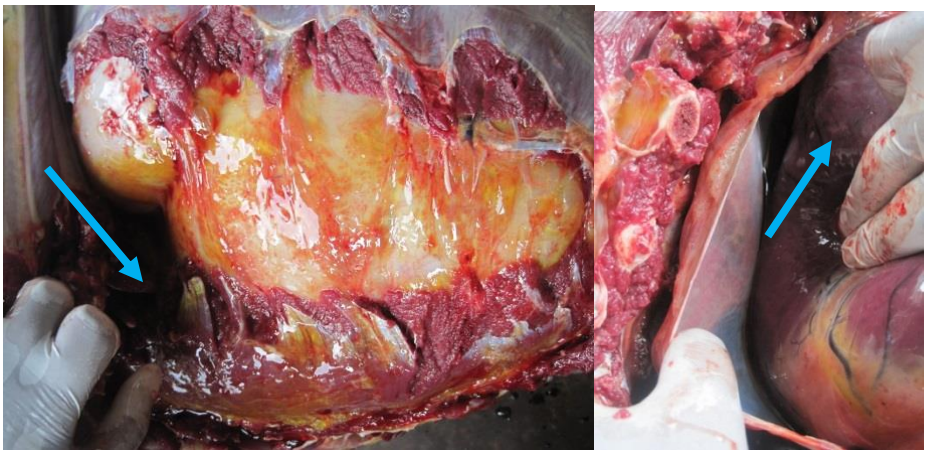

Fig. 41 Hydrothorax (post mortem)

Fig. 42 Hydro-pericardium (post mortem)

Discussion

An African horse sickness (AHS) is a fatal viral disease of equids. AHS takes on four different clinical forms in infected equids: sub clinical (horse sickness fever), sub-acute (cardiac), acute (respiratory or pulmonary) and mixed forms (Vanniekerk *et al.,* 2001).

In this case report the laboratory result (findings of AHSV in blood sample), clinical signs observed (fever, development of edema on different parts of the body) and the post mortem finding (hydrothorax and hydropericardium) confirmed the disease was the cardiac form of African Horse Sickeness.

There is no treatment that has been shown to have any effect on the course of the disease but in this case report Flunixin and Furosime were given as symptomatic treatments; as antipyretic and diuretic respectively.

Even though all the symptomatic treatments were given to this horse, the final outcome was death. This could be due to the species of animal (horses are very much susceptible) (Radostits *et al.,* 2007) or it could be due to lack of immunity of the horse as it might not be vaccinated (newly bought horse and the previous history was not known).

As African Horse Sickness is acute disease of equines and has no treatment, knowledge of vaccination history of newly bought horses and seasonal vaccination of all susceptible horses are recommended.

Acknowledgment

I thank SPANA-Ethiopia and its staffs for covering all the treatment costs and helping hands on the treatment of this horse.

4.2.2. Epizootic lymphangitis

Summary

Epizootic lymphangitis is a chronic disease of horses that is caused by dimorphic fungus, *Histoplasma capsulatum.*This case report summarizes the case study of cutaneous form of Epizootic lymphangitis (EZL) presented to Society for the Protection of Animals Abroad (SPANA). The horse initially had one small swelling on its left forelimb before a month. Few days' later a swelling line had appeared staring from the pastern joint to the shoulder area on the same limb and again day later small swellings had appeared along the swollen line and the swellings started to use creamy like pus. On clinical examination there were about 20 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 in normal range. The infected area was cleaned with water and chlorhexidine and all the hairs along the affected lymphatic vessel were shaved. Using syringe and needle pus was aspirated from a nodule that was not ruptured and smear was immediately prepared on a microscopic slide. The smear was stained with Gram stain and observed under microscope. The result showed Gram-positive a halo, unstained capsule-like structure which confirmed the yeast form of *Histoplasma capsulatum*. The horse was treated with oral Potassium iodide and local flushing of the nodules with 4% tincture iodine for six weeks. After the end of treatment all the nodules were changed in to scars. Cost of the treatment was very high so focus has to be given in the prevention of the disease, like prevention of wounds as the fungus cannot penetrate intact skin.

Key words: Epizootic lymphangitis, dimorphic, nodules, Potassium iodide

Literature Review

Epizootic lymphangitis is a chronic, contagious, disease of horses, donkeys and mules that is caused by fungus, *Histoplasma capsulatum* var. farciminosum (Al-ani, 1999). It is a thermally dimorphic fungus where the mycelial form is present in soil, while the yeast form is usually found in lesions (Ameni, 2006).

The incubation period (IP) of the disease is from 3 weeks to 12 months. It causes considerable debility but low mortality that doesn't usually exceed 10% to 15% and the main loss results from the inability of animals to work for several weeks because of extremely painful lesions (Jubb *et al.,* 2006).

The mode of transmission of EZL (Epizootic lymphangitis) includes transmission by direct or indirect contact of HCF with traumatized skin, by biting flies, by ticks or by inhalation of HCF (Morrow and Sewell, 1990). As previous studies showed, the wounds which caused by harness are the major predisposing factors of Epizootic Lymphangitis in carthorses in Ethiopia (Asfaw, *et al.,* 2012)

Clinical signs of Epizootic lymphangitis are described based on the pathological lesions; otherwise the body temperature and general character of the animals are not changed. There are three clinical forms of epizootic lymphangitis in horse; cutaneous, ocular and pulmonary form. The cutaneous form of the disease is the most common (Radostits, *et al.,* 2006; Ameni, 2007).

The initial lesion of the cutaneous form is an open granulomatous wound along the course of a lymphatic vessel, rope like, which has a tendency to ulcerate, or to undergo alternating periods of discharge and closure for some weeks before healing with residual scar formation. Repeated cycles of ulcerating and healing nodules occur. Lesions are most common in the forelimbs, the chest wall, and the neck. In severe cases, skin over the entire body may be affected (Ameni, 2007; Kharter *et al.,* 1998).

Diagnosis of epizootic lymphangitis depends on the clinical sign, history of animals and laboratory confirmation. Laboratory confirmation of the disease is by stained smears of the cutaneous exudate based upon demonstration of the typical yeast-like, double-contoured cells in pus collected aseptically from the lesion and confirmed by culturing the pathogen. Microscopic examination of Giemsa or Gram-stained smears of pus aspirated from a not raptured nodule reveal Gram-positive yeast forms with a halo (unstained capsule-like) structure (Asfaw, *et al.,* 2012).Culture of HCF, on special media such as Sabouraud dextrose agar, from body fluids or tissues is the "gold standard" for confirming the diagnosis but the procedure is time-consuming to grow and sometimes false-negative results may occur (Radostits *et al.,* 2006).

The disease does not have effective treatment so far. Many treatment types have been tried, but it was without success. Parenteral amphotericin B and iodides have been reported as effective. Even though Epizootic lymphangitis is highly prevalent and economically important in Ethiopia, the treatment options have not been employed because of the cost of the drugs and their absence in the market (Ameni, 2006).

The unpublished ongoing research that is being done in the Society for the Protection of Animals Abroad (SPANA) with oral dosing of Potassium Iodidie (KI) and local infusion of 4% tincture iodine is giving promising results in the treatment of early cases (less than 20 nodules and only one part of the body affected) but the treatment does not gave success rate in the treatment of severe cases of the disease.

Case History

An owner presented a horse to SPANA open air clinic. The owner mentioned that his horse had one small swelling before a month. Few days' later a swelling line had appeared staring from the pastern joint to the shoulder area. Small swellings had appeared along the swollen line and the swellings started to use creamy like pus.

Clinical Examination

There were about 20 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^{o}C, respiratory rate 24 breaths/ minute and pulse rate 44 beats/ minute. All were in normal range.

The infected area was cleaned with water and chlorhexidine and to help in the proper cleaning of the area, all the hairs along the affected lymphatic vessel were shaved.

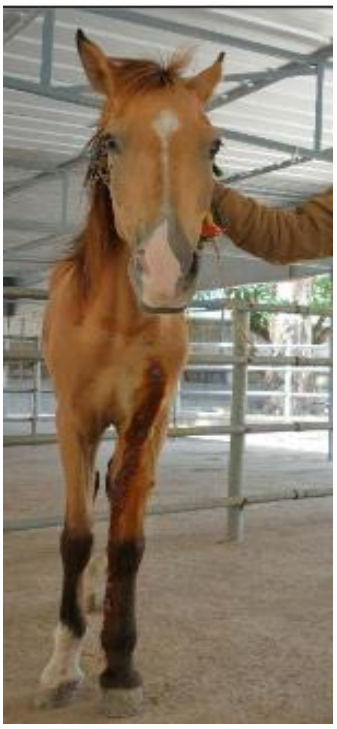

Fig. 43 Nodules along the lymphatic vessels

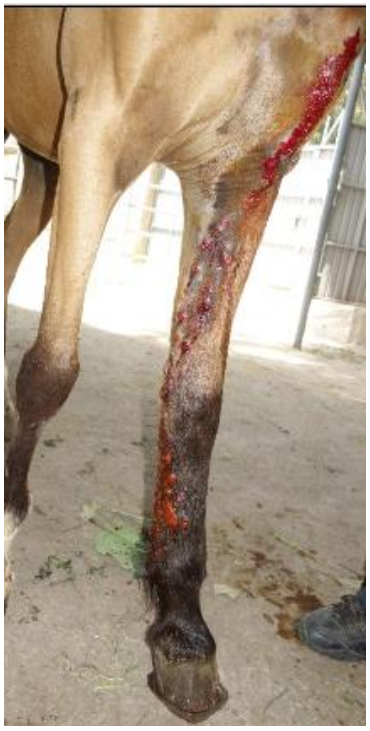

Fig. 44 Shaved infected area (Day one)

Differential Diagnosis

Epizootic lymphangitis (EZL), Ulcerative lymphangitis

Sample Collection

Using syringe and needle pus was aspirated from a nodule that was not ruptured.

Laboratory Diagnosis and Result

Immediately smear was prepared from the pus sample and allowed to air day. The smear was stained with Gram stain (APPENDIX 1) 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 Gram-positive a halo, unstained capsule-like structure which confirmed the yeast form of *Histoplasma capsulatum.*

The remaining pus sample was cultured on Sabouraud dextrose agar (SDA) and kept at room temperature. It took two months for the fungus to grow. Dry, grey, white, granular with wrinkled/lined, cerebriform, convoluted mycelia colonies were observed.

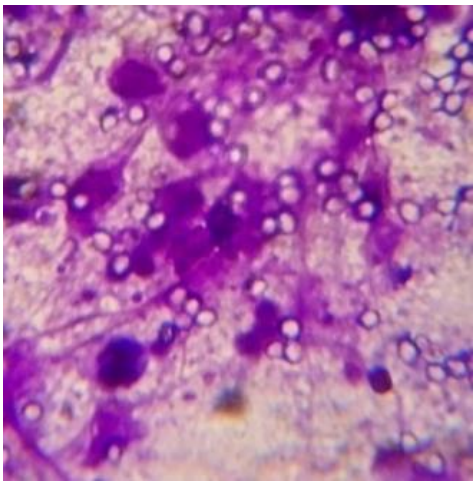

Fig. 45 Gram-positive a halo structure; yeast form of HCF

Fig. 46 Grey, white, granular with wrinkled, convoluted mycelia colonies on SDA

Treatment

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.

Potassium Iodide (KI) 25gm SID, with feed was given for consecutive 5 days and then after 30gm of KI SID, with feed was given every other day for 5 weeks.

The owner was strongly advised to clean the infected area with soap and water every day.

Outcome

There were no new nodules emerged after the 2 weeks of treatment. All the nodules were changed in to scar at the 6 weeks of therapy so treatment stopped.

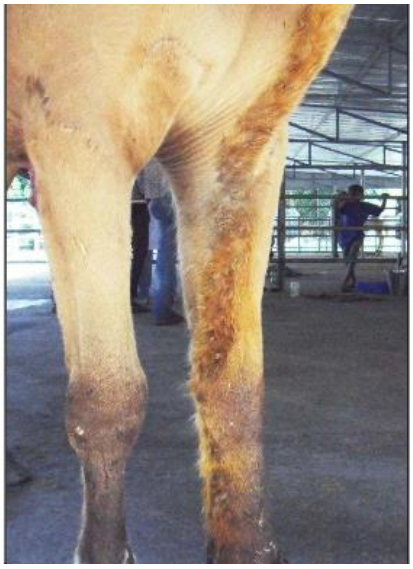

Fig. 47 After six weeks of therapy

Discussion

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 (Radostits, *et al.*, 2006; Veterinary Manual, 2018b).

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 treatment of one EZL case reaches up to 2,500 ETB, which if not been covered by the project, would have been difficult for owners to pay this much cost for the treatment of their horse. As treatment is costly and in not effective in advanced cases focus should be given to control measures of the disease like prevention of wound as the fungus cannot penetrate through intact skin (Morrow and Sewell, 1990).

Acknowledgment

I thank Mr Kefyalew Mideksa (Vet Microbiologist at SPANA) for doing all the laboratory procedures in the diagnosis of this disease.

4.2.3. Grain overload

Summary

Grain over load is a disease condition that occurred due to excessive intake of carbohydrate rich feeds. This case report summarizes case report of symptomatic grain overload in horse, presented to Society for the Protection of Animal Abroad (SPANA) open air clinic Bishoftu, Ethiopia. The horse ate fine wheat bran that was given for milking cows and a day later its flank got distended and the horse started to roll on the ground and listen to its belly. On clinical examination the flank was distended on both sides, the horse had severe sweating and it was frequently listening to its abdomen and also rolling on the ground. All body parameters; temperature, respiratory rate and pulse rate, were in above normal range. There was no peristaltic movement noted and on rectal examination colonic distension with tight bands were palpated. Nasogastric tube was passed to the stomach to check the presence of reflex but there was no fluid reflex. Blood sample was taken with EDTA vacutainer tube and transported to lab immediately. Blood smear was prepared and stained with Wright stain where the result showed toxic changes in neutrophils. Flunuxin meglumine was given intravenously with large volume of ringer lactate fluid to facilitate the removal of the toxins from the blood stream but the prognosis of the condition was poor and the horse died three hours later.

Key words: grain overload, colic, colonic distension, tight bands

Literature Review

The horse is classified as a *hind-gut fermenter.* The "back end" of the digestive system is housed within an enlarged colon and caecum, and breakdown of nutrients here is accomplished almost exclusively by microbial fermentation (similar to the processes in the rumen of a cow). The foregut (stomach and small intestine) has a small capacity (about 38% of the total) relative to the capacity of the hind gut. This would suggest that the horse is not well suited to large single meals, but rather to continuous intake of a high fiber diet. The hind gut contains microbes which break down and utilize the substrate provided by the diet. Any feed that passes through the stomach and small intestine undigested, will be subjected to microbial fermentation in the hind

gut. The end-products of the fermentation process are mainly volatile fatty acids, heat and gas (Blog.vumafeed.co.za, 2018).

However, horses need high energy diets in order to perform, and grains form the mainstay of such diets. The problem with high grain diets results from the disruption of the sensitive pH balance in the hind gut. A diet high in carbohydrate and low in fiber will favor the microbial population with the capabilities of utilizing these substrates, to the detriment of others. The health of this microbial population is essential to the health of the horse. Sudden changes in diet will cause a radical die-off of segments of the microbial population that are not able to survive the new gut conditions. These dying microbes that are toxins and the end product of carbohydrate fermentation (which include volatile fatty acids, lactic acid and gas) together cause damage to the gut lining and enter the bloodstream, causing distended abdomen, colic, and laminitis in acute cases (Briggs, 2015).

The clinical signs most frequently noted in symptomatic grain overload include: colic, marked abdominal distension, severe lameness trembling, sweating, polypnea and less frequently diarrhea. Clinical findings include bright red to purple membrane, tachycardia, abcense of intestinal sound. Gastric reflux and colonic distension with tight bands palpated on rectal examination (Orsini and Divers, 2003).

Numerous clinical signs are associated with colic. The most common include pawing repeatedly with a front foot, looking back at the flank region, curling the upper lip and arching the neck, repeatedly raising a rear leg or kicking at the abdomen, lying down, rolling from side to side, sweating, stretching out as if to urinate, straining to defecate, distention of the abdomen, loss of appetite, depression, and decreased number of bowel movements. It is uncommon for a horse with colic to exhibit all of these signs. Although they are reliable indicators of abdominal pain, the particular signs do not indicate which portion of the GI tract is involved or whether surgery will be needed (My Horse University Online Horse Management, 2018; Veterinary Manual, 2018e).

A diagnosis can be made by complete blood cell which usually reveals severe polycythemia, neutropenia with left shift, and vacuolization of neutrophils; toxic changes (Orsini and Divers, 2003).

Treatment should be with IV fluid therapy. Hypertonic saline initially, but this must be followed within 1-2 hours by administration of 1-2 hours by administration of polyionic fluid at 2-4 litre/ hour for adult horses. The prognosis with marked clinical signs is poor. If severe abdominal pain and marked abdominal distension are present, a very poor prognosis should be given, and affected individuals generally die within 24 to 48 hours even with aggressive therapy (Orsini and Divers, 2003).

Case History

An owner presented a 13 years old male horse to Society for the Protection of Animals Abroad (SPANA) open air clinic, Bishoftu. The owner mentioned that his horse ate fine wheat bran that was given for milking cows. After a day its abdomen got distended and the horse aggressively started to roll on the ground and listen to its belly. The amount of the fine wheat bran eaten by the horse was not known.

Clinical Examinations and Findings

The horse had distended flank that was observed on both sides and the horse had severe sweats all over its body. The horse repeatedly was frequently looking at its belly and was also rolling on the ground.

Body parameters were; temperature 39.6^{o}C, respiratory rate 40 breaths/ minute and pulse rate 80 beats/ minute. All were above the normal range. The visible mucous membranes; conjunctiva and gingiva were congested. On auscultation of the gut motility, there was no peristaltic movement noted and on rectal examination colonic distension with tight bands were identified. A nasogastric tube was passed to the stomach but there was no reflex.

Fig. 48 The horse listening to its belly

Fig. 49 The horse rolling on the ground(Day one)

Fig. 50 Checking rectally (Day one)

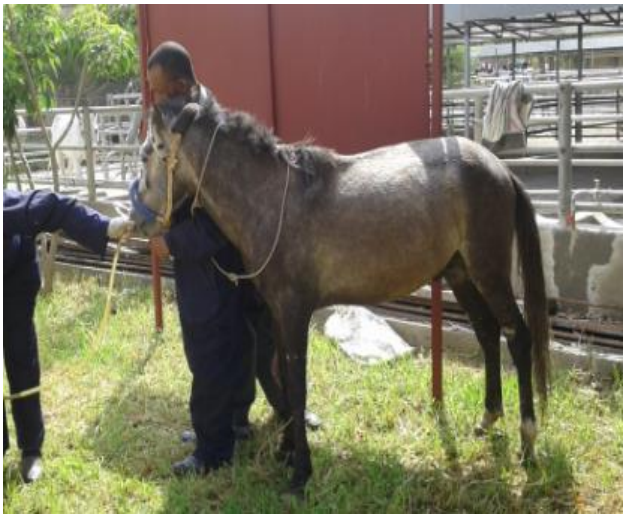

Fig. 51 No reflex on nasogastric intubation (Day one)

Differential Diagnosis

Grain overload, obstructive colic,

Sample Collection

The horse was kept in crush to restrain it. The jugular furrow was located and the jugular vein was identified by applying thumb pressure on the base of the jugular groove. The proximal third of the neck, over the jugular vein, was cleaned with alcohol. The vacutainer needle was screwed on the needle holder and the EDTA vacutainer tube was preloaded on the needle holder. Care was given not to puncture the vacutainer tube. The jugular vein was distended by applying thumb

pressure over the vein. The vacutainer needle was inserted in to the engorged vein and the vacutainer tube was inserted in to the vacutainer needle that was protruding through the holder. About 8ml of blood was collected and the needle was taken out of the vein. The venipuncture area was disinfected and the blood in the EDTA vacutainer tube was slowly rotated to mix the anticoagulant with the blood.

Laboratory Diagnosis and Result

A drop of blood sample was added on frosted end of a microscopic slide. Other slide (spreader) was kept with 45^{o}, with the blood sample on the first slide. 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, it was pushed forward rapidly and smoothly. The smear was allowed to air dry for 5 minutes. The dried smear was stained with Wright stain (APPENDIX 2). A drop of oil immersion was added on top of a stained slide and it was observed under 100x oil objective lens of binocular microscope. Cytoplasmic vacuoles were observed in neutrophils that were suggestive of toxic changes in neutrophils.

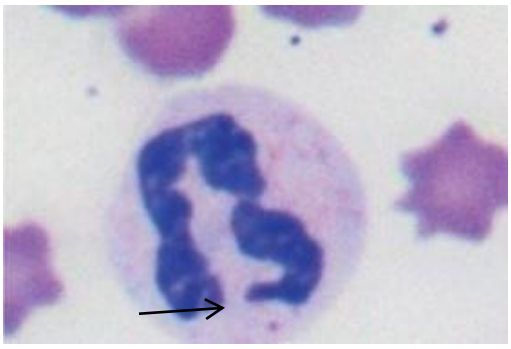

Fig. 52 Cytoplasmic vacuoles in neutrophils

For determination of packed cell volume two plain capillary tubes were used (as the blood was collected in EDTA vacutainer tube). From the EDTA vacutainer tube blood was filled up to its ¾ level of capillary tubes by the capillary action. The ends of the capillary tubes, in contact with the blood, were sealed with clay up to 2 mm depth. The two capillary tubes were placed in to two opposite grooves of hematocrit centrifuge. They were centrifuged at 15,000 rpm (resolution per minute) for one minute. The capillary tube was removed from the centrifuge and it was read using hematocrit reader. The reading of the packed cell volume was 53%, higher than the normal range.

Treatment

Flunixin meglumine (1.1mg/kg SID) was given IV.

Ringer lactate five bags (5 liters) were given IV/ two hours.

Outcome

The condition of the horse was getting worse with severe sweating and severe abdominal pain (continued to roll even after the IV injection of a strong analgesic; Flunixin).

The horse died three hours later.

Discussion

Grain overload is a condition that resulted after excessive intake of carbohydrate rich feeds. The history and clinical signs observed in this case; taking of carbohydrate rich feed (fine wheat bran), increased body parameters, severely distended abdomen, signs of colic (Veterinary manual, 2018e) and laboratory finding of toxic changes in neutrophils were confirmatory diagnosis for grain overload.

During passing of the nasogastric tube there was no reflex observed this could be due to the further progression of feed materials to the intestines as the stomach of the horse is very small.

Laminitis was not observed in this case and it could be due to the obscured clinical signs due to the severe abdominal pain or it could be due to the lesser period for the clinical signs to be shown.

Acknowledgment

I thank SPANA-Ethiopia project for giving free veterinary care and also the veterinary team members, with special thanks to Mr Gizaw Gemechu, for their helping hands in the management of the case.

4.2.4. *Rabies*

Summary

Rabies is an acute fatal zoonotic disease that is caused by caused by a neurotropic Lyssavirus. This case report summarizes the case management of rabies in an 11 years old horse. The horse was bitten by a dog one month before. The dog was having some strange behavior; it was drooling out its saliva, barking on inanimate objected and refused drinking water. After it bit the horse, the dog disappeared and never returned back. Starting from a day before its presentation to clinic, the horse started showing strange characteristics; restlessness, biting a tree, biting itself aggressively and it also bit the other horse living with it. On examination, the horse was severely biting itself and the tree, on which it was tied up. It was very difficult to take body parameters as the horse was trying to bit everything when it was approached. Based on the evidence of history and clinical signs the case was tentatively diagnosed as rabies. The horse was euthanized, using Pentobarbital, and the head was transported to Pasteur Institute Ethiopia for confirmatory diagnosis. The institute had done Florescent Antibody Test (FAT) and confirmed the presence of Lyssa virus in the brain tissues. Vaccination of dogs, primary host, against rabies is recommended.

Key words: rabies, horse, FAT, euthanasia

Literature Review

Rabies is an acute fatal zoonotic disease, with a very wide global distribution and wide host range and it is one of the oldest infectious diseases that were known to medical science. Rabies is originally derived from the Latin word "*rabere"* which means "*madness*" (Frederik, 2007).

Rabies is caused by a neurotropic Lyssavirus (Rhabdoviridae family) bullet-shaped viruses, which have a single-stranded RNA genome and includes six genotypes that infect the nervous system and salivary glands (Radostits, 2000; Long and Sellon, 2014).

An animal can become infected if the saliva from an infected mammal contaminates an open wound or a mucous membrane and any form of skin injuries (bite, scratch, or cut). Time between

bite and the appearance of clinical signs can be long (usually 2-9 weeks) depending on the wound severity, wound site and its distance from the brain, amount and strain of virus, however, it can be longer than 6 months so the incubation period can vary from several days to many years (Bishop *et al.,* 2003).

Rabies virus enters the body through wounds or by direct contact with mucosal surfaces. It cannot cross intact skin. Rabies virus replicates in the bitten muscle (local viral proliferation in non-neural tissue) and gains access (viral attachment) to motor endplates and motor axons to reach the central nervous system (Ugolini, 2008). Virions are carried in transport vesicles (Klingen *et al.,* 2008) and travel to the central nervous system (CNS) exclusively by fast retrograde transport along motor axons, with no uptake by sensory or sympathetic endings (Hemachudha *et al.,* 2013). Following centrifugal transport along efferent cranial nerves, the salivary glands become infected and virus particles are shed in the saliva. Infection of the brain commonly leads to behavioural changes that induce the host to bite other animals, thereby transmitting the virus (Bishop *et al.,* 2003; Shite *et al.,* 2015).

The clinical picture can be highly variable between different species, individuals of the same species, and even within the course of the disease in a particular individual (Blackmore, 2014). During this first, prodromal, stage which usually lasts for about 1-3 days minor behavioral changes might occur, i.e. aggressiveness in tame animals, daytime activities in nocturnal animals, no fear of humans in wild animals or abnormalities in appetite (WHO, 2013). The prodromal stage is followed by a period of severe agitation and aggressiveness; characterized by restlessness, wandering, howling, polypnea, drooling and attacks on other animals, people or inanimate objects. The last phase, Paralytic or "dumb" form of rabies is characterized by the inability to swallow, leading to a typical sign of foaming saliva around the mouth. Some animals may develop paralysis beginning at the hind extremities. Eventually, complete paralysis is followed by death (WHO, 2013).

Infection with rabies virus can be difficult to diagnose ante-mortem. Although hydrophobia is highly suggestive, no clinical signs of disease are pathognomonic for rabies. Historical reliance on the detection of accumulations of Negri-bodies is no longer regarded as suitable for diagnostic

assessment because of low sensitivity and alternative laboratory-based tests based have been developed to conclusively confirm infection (Abera *et al.*, 2015).

Most diagnostic tests for rabies virus in animals need brain material for diagnosis and as such are often only possible post mortem (Fooks, 2012). There are many diagnosis methods for detection of rabies in animals like; direct florescent antibody, mouse inoculation technique, tissue culture infection technique, and polymerase chain reaction (Yousaf, *et al.*, 2012).

There is no certain cure for rabies except supportive care. Rabies can be prevented before the latent symptoms can develop, consists of giving an injection of rabies immune globulin and another injection of rabies vaccine as soon as possible after the bite or exposure to saliva from an infected animal (Yousaf *et al.*, 2012).

In horses, as in other warm blooded animals, rabies is a severe and rapidly progressive neurological disease (Wilkins and Del Piero, 2007). Although rabies in horses is low and relatively uncommon, the potential for human exposure makes it important to be considered (Singh, 1990).

Case History

A phone call was received from one horse owner living around Bishoftu, Ethiopia. The owner mentioned that his horse was bitten by a dog one month before. The dog was having some strange behavior; it was drooling out its saliva, barking on inanimate objected and refused drinking water. After it bit the horse, the dog disappeared and never returned back.

Starting from a day before the horse started showing strange characteristics; restlessness, biting a tree, biting itself aggressively and it also bit the other horse living with it.

Clinical Examinations and Findings

The horse was severely biting itself and the tree, on which it was tied up. It was very difficult to take body parameters as the horse was trying to bit everything when it was approached.

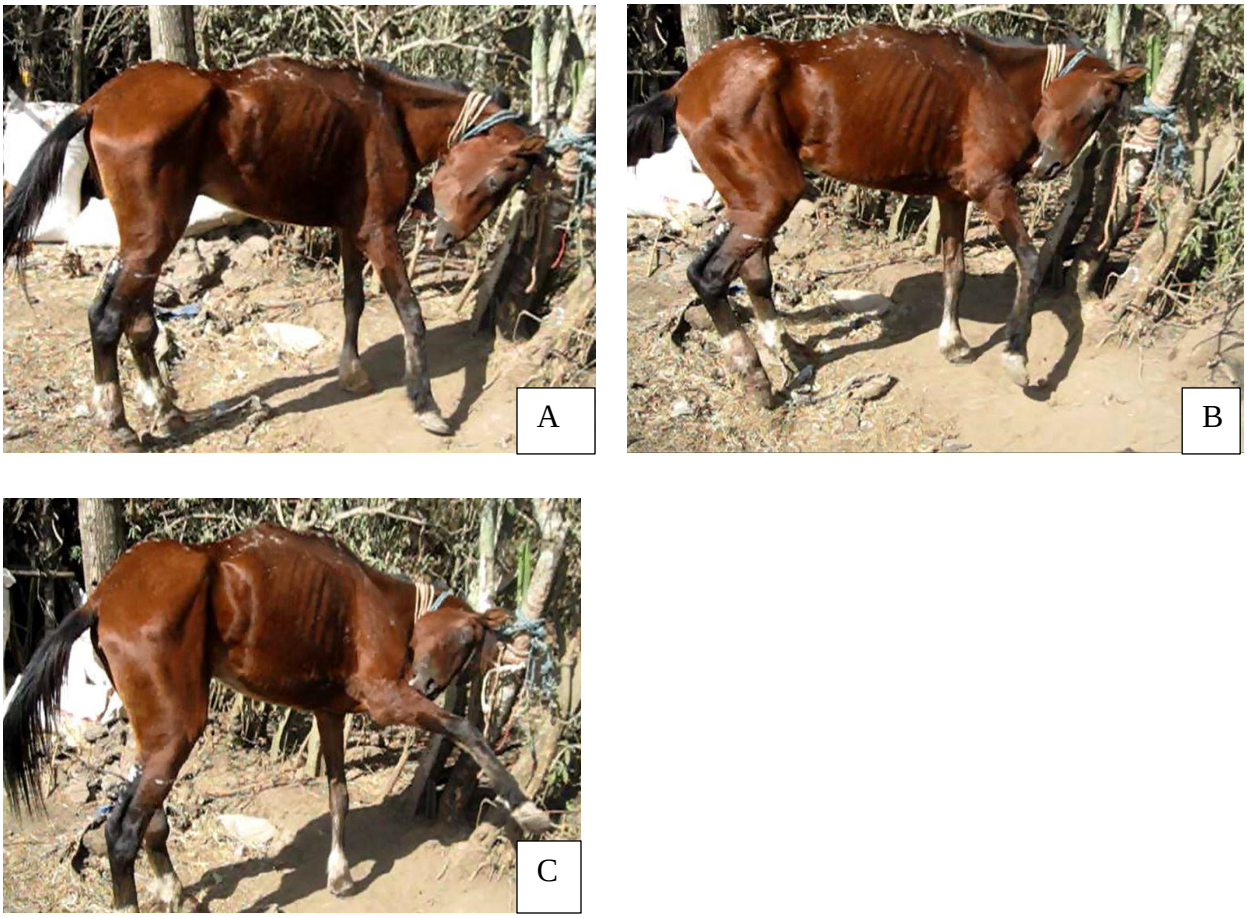

Fig. 53 (A, B & C). The horse biting itself aggressively

Differential Diagnosis

Rabies, Canine encephalomyelitis

Sample Collection

The horse was euthanized and the head was transported to Pasteur Institute Ethiopia, the only institute in Ethiopia to confirm rabies cases.

Laboratory Diagnosis and Result

Pasteur Institute Ethiopia had done Florescent Antibody Test (FAT) and Lyssa virus was detected in the brain tissue.

Treatment

Rabies has no curative drug so both horses were euthanized with rapid intravenous injection of Pentobarbital Sodium (20%) at a dose of 0.35ml/kg body weight.

Discussion

Rabies is an acute fatal zoonotic disease that is caused by caused by a neurotropic Lyssavirus (Radostits, 2000; Long and Sellon, 2014).

In this case report the horse was bitten by a dog that had change in behavior. Even though the dog could not be found for diagnosis, in general any animal bitten by, scratched by, or having direct contact with a wild mammal that is not available for rabies testing should be regarded as having been exposed to rabies(CDPH, 2012).

The clinical picture can be highly variable between different species, individuals of the same species, and even within the course of the disease in a particular individual (Blackmore, 2014). The clinical signs observed in this case; restlessness, biting itself, biting the other horse and biting inanimate objects was similar clinical findings of rabies in literatures and other research findings (WHO, 2013; Radostits, 2000; Yousaf, *et al.*, 2012).

The diagnostic test used in this case report, Florescent Antibody Test is the most widely used primary diagnostic test for rabies in animals and humans. This test is based on antigen detection and is recommended by both WHO and OIE as the gold standard for rabies diagnosis (WHO, 2013).

Rabies has no curative drug ones the clinical sign started so the horse with the clinical sign was euthanized.

And the bitten horse was also euthanized immediately because unvaccinated livestock bitten by or exposed to a rabid or suspect rabid animal should be euthanized (CDPH, 2012).

Prevention of rabies is only possible through pre-exposure vaccination of succeptible livestocks specially dogs. Depending on the vaccine manufacturer's instructions, the vaccination should be repeated in the timely interval (Murray et al., 2009).

4.2.5. Strangles

Summary

Strangles is a highly contagious and serious disease of equids caused by *Streptococcus equi sub spp. Equi.* This case report summarizes case management of Strangles in two years, female donkey presented to The Donkey health and welfare project open air clinic, Bishoftu. The donkey was kept in extensive farming system. The donkey became depressed and had nasal discharge, coughing since a day before its presentation to the clinic. On clinical examination the body temperature, respiratory rate and pulse rate of the donkey were high, and submandibular lymphnodes were swollen, hot and painful. For confirmation of the disease, nasal swab was taken and transported to microbiology lab with amies charcoal transport media. The bacteria was cultured on blood agar and incubated for 48 hours. Medium sized, mucoid and hemolytic colonies were observed. On Gram staining of the colony; Gram positive Cocci, arranged in chain were observed under microscope. Culture and Gram staining of the bacteria confirmed the presence of *Streptococcus* species. Procaine pencillin was given for five days as antimicrobial therapy and flunixin meglumine was given for three days to alleviate the pain. After the therapy, the donkey recovered from the clinical signs. Some research findings do not recommend the use of antibiotics for strangles as it hinders the development of immunity against reinfection. But as it is indicated in this case report antibiotic was used as the donkey could be source of infection for other equids that graze together.

Key word: Streptococcus equi, Strangles, Donkey

Literature Review

Strangles is a highly contagious and serious disease of equids caused by the gram positive, pyogenic and hemolytic bacterium *Streptococcus equi sub spp. equi (S. equi equi). Streptococcus equi (S. equi) is* an obligate bacterium of equids' upper respiratory tract (Pritchard *et al.,* 2005; Lefevre *et al.,* 2010).

S.equi equi is spherical in shape and 0.8 to 1mm in diameter. It has the appearance of chain during division and it is encapsulated. Culturing of *S.equi equi,* needs media containing blood or

serum. After 24 hours of incubation at 37^{o}c, it produces clear and mucoid colonies usually less than 4mm in diameter. *S.equi equi* is catalase (Jorm, 2012).

Strangles occurs in equids worldwide. The disease affects mostly young equids (Three months to five years). Outbreak can occur at any time of the year but most likely to happen in cold weather. The incubation period usually ranges between 7 to 14 days (Faiefield, 2013).

The bacteria, *S. equi*, enters via the mouth or nose and attaches to tonsil crypt cells and adjacent lymphoid nodules (Amanda, 2011). Adherence triggers internalization and subsequent localization in the subepithelial spaces. The bacteria produce pyogenic exotoxins that initiate an acute inflammatory response, resulted in neutrophils migration to the site. This migration of neutrophils in to lymph nodes causes swelling and abscessation (Newton *et al.,* 1997).

Transmission occurs through contact with purulent nasal discharge from horses with active and recovering strangles. Direct or indirect transmission can occur. Direct transmission involves horse to horse contact, nose to nose touching or coughing of infected secretions into the next stall (Stringer, 2009). Indirect transmission involves sharing of contaminated housings, water sources, feed or feeding utensils, tack, or handlers and their clothing. Apparently, healthy animals may transmit the disease either during the incubation phase or during the recovery phase (Quinn, 2002).

The early clinical signs of the disease are fever, nasal discharge and lymph node swelling (specially retropharyngeal and submandibular lymph nodes). The lymph nodes become enlarged, hot and painful. As these lymph nodes abscessate, they may begin to ooze creamy pus and eventually rupture and drain externally or internally (Smith, 2009). Chronic infection of the guttural pouches can lead to drying of the pus and formation of solid, concretions called chondroids (Kahn, 2005). In complicated cases of strangles, several conditions can be developed including pneumonia, guttural pouch empyema, bastard strangles and purpura hemorrhagica (Small hemorrhage in the skin). In addition, in complicated cases of strangles, the lymph node swelling may exert pressure on the esophagus and pharynx which results in difficulty swallowing. If the lymph node swelling obstructs the airway, the horse will develop difficult respiratory and require emergency attention (Quinn *et al.,* 2002).

Isolation of the bacteria from nasal discharges, pus from abscess and lymphoid tissues by culture or PCR is the most effective diagnosis for strangles. Detection of the serum protein, antibodies by ELISA is also possible to diagnose strangles (Lefevre *et al.*, 2010)

Strangles is treated with penicillin although treatment is rendered by the stage, feature and severity of the disease. Supportive treatments with penicillin are effective when the disease is painful and complicated (Sweeney *et al.*, 2005). Nonsteroidal Anti Inflammatory Drugs (NSAID), are used as supportive treatment to reduce swelling and to provide pain relief (Songer and Post, 2005).

Control and prevention of strangles can be achieved by reducing the movement of horses in and out of the herd, quarantine of the newly introduced horses, vaccination, cleaning and disinfection of housings, utensils, pastures and grooming brushes. Personnel should wear protective boots and clothes to avoid contamination (Radostits *et al.*, 2007).

Case History

An owner presented a two years old female donkey to the Donkey Sanctuary open air clinic, Bishoftu, on March, 2018. The owner mentioned that the mother of this donkey died 2 months ago. This donkey would stay grazing outside, with cattle and donkeys of neighbors when not at work. But since a day ago, the donkey refused to go out and it was depressed and had bilateral nasal discharge and coughing.

Clinical Examinations and Findings

The body temperature was 39.7^{o}c, above the normal range. The respiratory rate was 24 breaths/ minute and pulse rate was 60 beats/ minute. Both respiratory and pulse rates were above normal range. The submandibular lymphnodes in both sides (right and left) were swollen, hot and painful. Mucoid to purulent bilateral nasal discharge was evident on both nostrils.

Fig. 54 Depressed donkey with ears drop down

Fig. 55 Bilateral mucoid to purulent discharge (Day one)

Fig. 56 Swollen submandibular lymphnodes (Day one)

Differential Diagnosis

Strangles, Equine influenza (with secondary bacterial complication)

Sample Collection

The outside of the nostril was cleaned with tap water. Nasal swabs of 20 cm long, with 2 cm cotton tip were introduced deep via nostrils and were gently rotated for 30 seconds. The swab was removed and placed into Amies charcoal transport media and transported to laboratory.

Fig. 57Sample Collection (Day one)

Laboratory Diagnosis and Result

The nasal swab was cultured on to blood agar and it was incubated at 37^{o}c for 48 hours. After 48 hours, there were medium sized, mucoid and hemolytic colonies on the culture. A colony was taken for Catalase test and the bacteria were Catalase negative.

For gram staining; with a sterile cooled loop, a drop of sterile water was placed on a clean labeled microscopic slide. 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 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 technique (APPENDEX 1).

A drop of oil immersion was added on the stained slide and observed under 100X objective lens of binocular microscope. Gram positive Cocci, arranged in chain were observed.

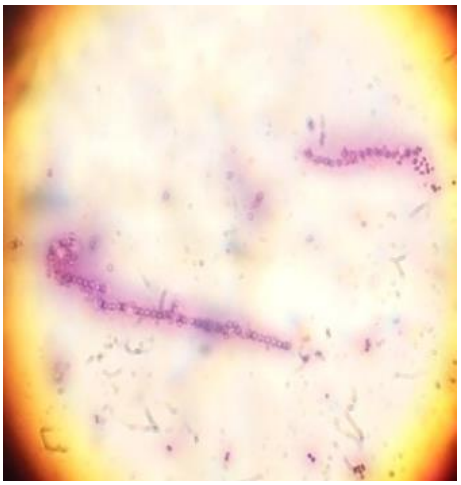

Fig. 58 Gram positive Cocci arranged in chains

Treatment

Procaine pencillin (22,000IU/kg IM, SID) was given for consecutive 5day as antibiotic therapy. And flunixin meglumine (1.1 mg/kg IV SID) was given for three days to alleviate the pain.

The owner was also advised to keep this donkey in isolated area at least until it recovery, to reduce the risk of disease transmission to the other donkeys.

Outcome

At the fifth day of treatment the swollen lymphnodes were found to be remarkably reduced in size. There was no hotness and pain on the lymphnodes. The donkey stopped coughing.

On the 30^{th} day of follow up, the donkey was completely free from any clinical signs of strangles.

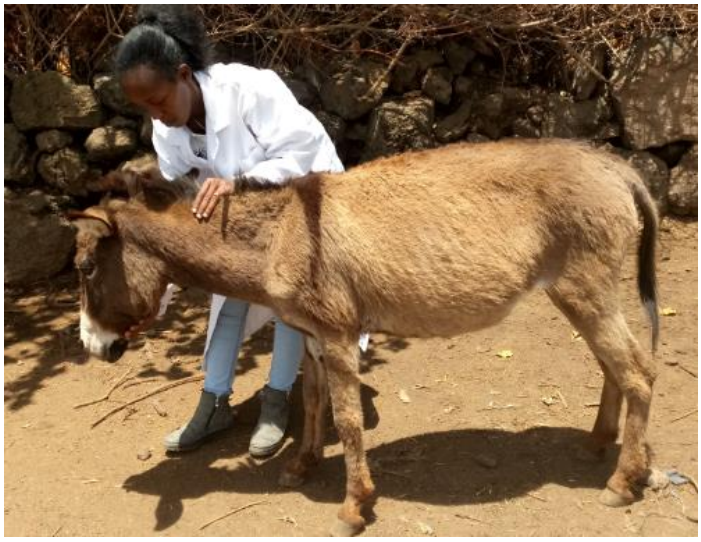

Fig. 59 No Swollen lymphnode (Day thirty)

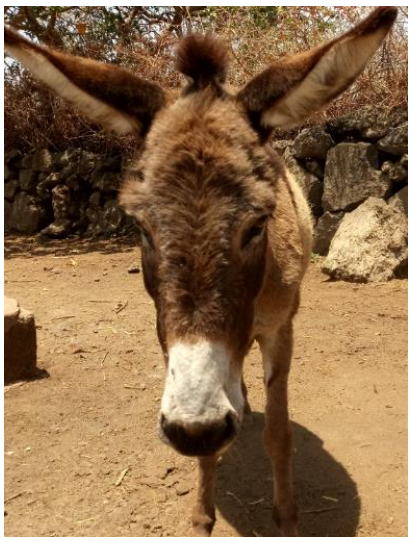

Fig. 60 No nasal discharge (Day thirty)

Discussion

Strangles is a highly contagious and serious disease of equids caused *Streptococcus* Species. The disease affects mostly young equids (Faiefield, 2013).

The clinical signs observed in this case report; fever, nasal discharge, swollen, hot and painful submandibular lymph nodes, were in consistent with clinical findings in literatures (Smith, 2009; Kahn, 2005; Radostits *et al.*, 2007).

Antimicrobial therapy remains controversial for the treatment of strangles. Some literatures suggests as uncomplicated cases of submandibular lymph node abscessation do not require antimicrobial therapy (Sweeney *et al.*, 2005). But antibiotic is used in this case report, because the donkey was febrile (that indicates septicemia) and it was living in extensive system so if not treated it could be a source of infection for equids that graze together.

Acknowledgment

I want to thank The Donkey Sanctuary vet staffs for supplying treatment drugs free from charge.

4.2.6. Tetanus

Summary

Tetanus is an acute noncontiguous infectious disease caused by *Clostridium tetani* exotoxins. This case study summarizes the case follow up and treatment of tetanus in nine years old horse presented to Society for the Protection of Animals Abroad (SPANA) open air clinic Bishoftu, Ethiopia. When the horse was shod, the nail got in to the sensitive part of the hoof; the horse became lame. After five days of being shod, the horse started to drool out excessive salivation, unable to take its feed and was also reluctant to move. On clinical examination all the body parameters were higher than the normal range (temperature of 38.8^{o}C, respiratory rate of 28 breathes/ minute and pulse rate 56 beats/ minute), the third eye lid was prolapsed, the horse was hypersensitive and had extended head, raised tail and the jaws were partially locked. The history and the clinical signs were confirmatory for Tetanus. Procaine pencillin, acepromizine and tetanus anti-toxin were used to treat the horse. For 25 days, the horse was kept in a dark stable of SPANA with its ears plugged with cotton. At the end of the 25th day there was no prolapsed third eye lid, the horse could easily move its neck and limbs. Since treatment and management of tetanus took long period, production of the vaccine and prophylactic equine tetanus antitoxin in the country is recommended.

Key words: horse, deep puncture wound, tetanus, prolapsed third eye lid, hypersensitive

Literature Review

Tetanus is an acute noncontiguous infectious disease caused by *Clostridium tetani* (*C. tetani*) exotoxins that affects many animal species and humans (Radostitis *et al.*, 2007). In unfavorable conditions the bacterium produces highly resistant spores that can survive for years in soil, and it is sometimes also found in the gastrointestinal tract and feces of healthy horses and humans (Radostitis *et al.*, 2007).

Tetanus is still associated with a high mortality rate, ranging from 58 % to 80 % in equidae (Reichmann, 2008)

The organism gains entry to the body via wounds. Although deep, penetrating wounds, such as punctures of the hoof capsule, are more liable to permit proliferation of *C. tetani*, even superficial wounds can provide suitable anaerobic conditions (Kay *et al.*,2007) required for their transition to the vegetative form and replication at the site of infection.

Exotoxins are produced, the most important of which are tetanolysin and tetanospasmin (Turton *et al.*,2002). Tetanolysin causes damage to viable tissue, lowering its redox potential and creating favorable conditions for the spread of anaerobic infection (Cook *et al.*,2001) whereas tetanospasmin enters the circulation and binds irreversibly to receptors on the motor nerve endings (Attygalle and Rodrigo, 2004).

The activity of these toxins leads to spastic paralysis (Smith, 2002). Among animal species, the horse is considered most susceptible to tetanus toxin (Mackay, 2007). The disease incubation takes 3-28 days (Green *et al.*,1994). The initial clinical signs manifest as spasm of the head muscles, resulting in trismus and lockjaw (Beronza, 1980).

In the initial stage of the disease, touching the eyeball can cause prolapse of the third eyelid, which then returns slowly to its natural resisting position (Radostitis *et al.*, 2007), whereas in the later stage the third eyelid protrusion may be permanent (Vangalen, 2008). With progression of the disease, spasms involve the neck and the esophagus, making swallowing difficult (Brook, 1970). Ears stand erect and immobile, and the tail-head is held elevated. In very severe cases, the horses adopt a saw horse stance with serious dyspnea, inability to ingest food, stiff neck, before becoming recumbent (Johnson, 1987).

Signs of the general infection syndrome and spasms of the extremity musculature develop concurrently, making movement difficult or impossible (Radostitis *et al.*, 2007). Death is due to spasm of the respiratory musculature (Johnson, 1987).

The diagnosis of tetanus is not usually difficult and can be done based on the clinical sign only (Sedrish, 1996).

Acute treatment of tetanus is based on wound cleaning and antibiotic eradication of Clostridium tetani, e.g., with intravenous metronidazole, 500 mg three times daily, or penicillin, 100,000–200,000 IU/kg/day (Ganesh *et al.*, 2004; Campbell *et al.*, 2009). Treatment is continued for

seven to ten days. Tetanus antitoxin is given once intramuscularly; doses of 500 IU, 3000 IU, or higher have been used, but it is debatable whether the higher doses are more effective (Blake, 1976). The antitoxin is given to inactivate any free tetanus toxin. The toxin that has been taken up into nerve terminals is probably not available to the antitoxin. Therefore, muscle symptoms may develop further, although the clostridia have been eradicated and antitoxin has been given, because tetanus toxin continues to be transported axonally and trans-synaptically (Kabura, 2006).

Treatment of the muscular rigidity and spasms in tetanus is of vital importance, since this feature of the disease often interferes with respiration and is a likely cause of death (Bleck, 2005). Rigidity and spasms also cause severe pain, which stimulates muscle activity.

Tetanus patients should be in a calm environment to avoid the triggering of spasms by noise or other sensory stimulation so placing cotton in the ears can help with respect to sound-induced spasms. Stabling or hospitalization in a dark, quiet stall with as little handling and disturbance as possible is desirable. Good footing is very important. If an affected horse manages to lie down, it can have extreme difficulty getting back up; slippery footing can result in needless trauma and, at worst, a fracture (Turton, 2002).

Prophylaxis against tetanus consists of immunization with formaldehyde-inactivated tetanus toxin (toxoid) (Turton, 2002).

Case History

A owner presented a nine years old horse to SPANA open air clinic, Bishoftu, Ethiopia in October, 2017. The owner mentioned that his horse has been shod one week before and just after it is shod, the horse became lame. The owner removed the newly shod shoes but the horse continued to be lame. Five days later the horse started to have excessive salivation and difficulty in chewing up its food. The horse was reluctant to walk and had abnormal posture when passing urine.

Clinical Examinations and Findings

The horse had a temperature of 38.8^{o}C, respiratory rate of 28 breathes/ minute and pulse rate 56 beats/ minute. The horse was hypersensitive, had extended head, stiffened neck, erected ear and

raised tail, prolapsed third eye lid and had long and inspiration, with evident of flaring up of the nostrils.

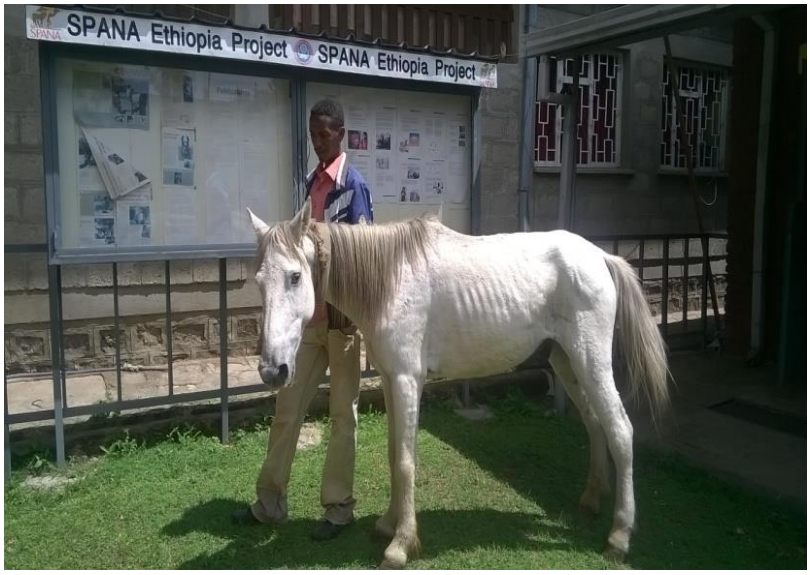

Fig. 61 Extended head, stiffened neck and raised tail (Day one)

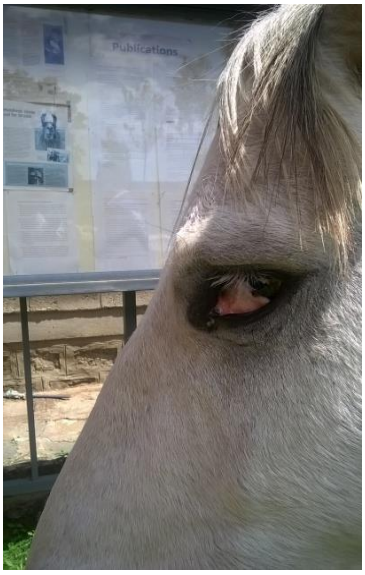

Fig. 62 Prolapsed third eyelid

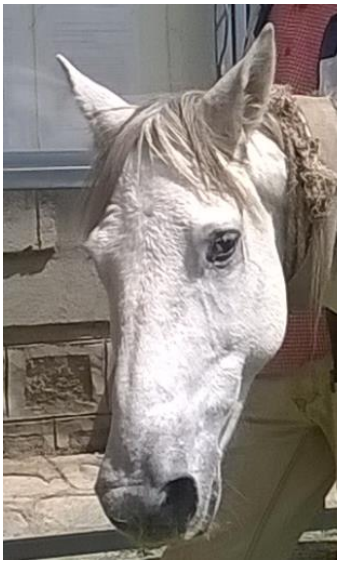

Fig. 63 Erected ear (Day one)

Differential Diagnosis

Tetanus

Confirmatory Diagnosis

It was diagnosed based on history and pathognomonic clinical signs.

Treatment

The hoof was tested, with hoof tester, and there was a pain reflex. Using hoof cutter and hoof knife the sole was slowly opened over the site that had pain response. Pus was detected while opening the sole, so about 0.5 cm in diameter opening was made and the pus was drained, the area was flushed with 2% iodine and the opening was left open (which was cleaned daily) to create aerobic condition which is lethal to *Clostridium tetani*.

Procaine pencillin (22,000IU/kg IM, SID) was given for consecutive 7 days as antibiotic of choice. Acepromazine (2mg/kg IM, BID) was given for 3 consecutive days as muscle relaxant. Tetanus antitoxin (10ml, SID) was given to neutralize the unbound circulating toxin.

After the end of treatments, the horse was kept in a dark stable of SPANA for additional 18 days (total of 25 days including treatment days) being given palatable feeds and water.

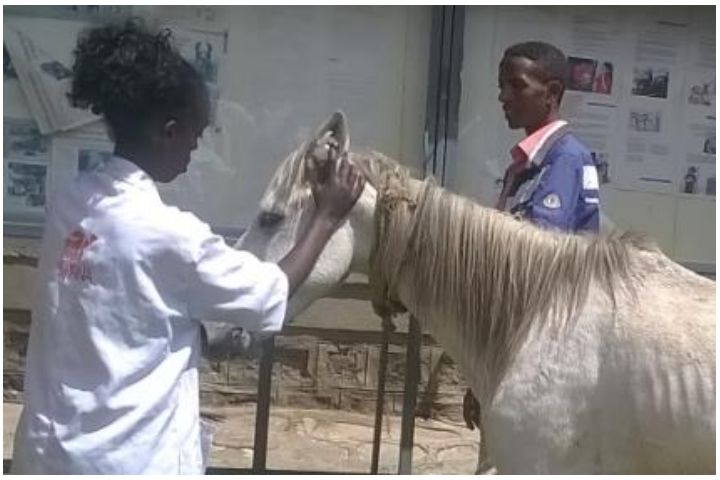

Fig. 64 Plugging the ear with cotton (Day one)

Fig. 65 The horse in SPANA stable (Day two)

Outcome

After the 25th day in the stable, the stiffness of the muscle and the prolapse of the third eye lid returned normal. The horse had normal posture where there was no head extension and neck

stiffness. The wound in the sole dried out and it was covered with very thin and soft layer of the sole.

The owner was advised to rest the horse until the soft layer of the sole hardened. And give it palatable feed and also to take his horse to vet clinics whenever they got deep wounds.

The owner, with phone communication, told us that the horse needed additional 1 month rest to have hardened sole to put on the shoe.

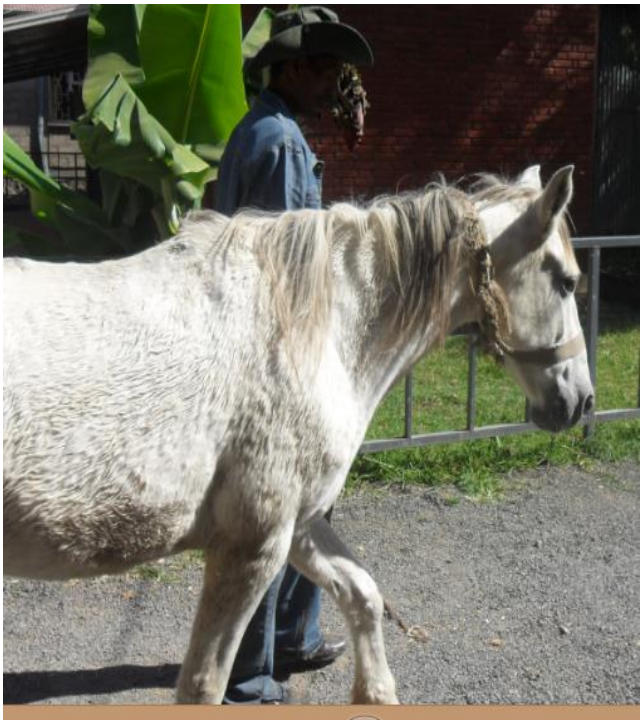

Fig. 66 No extended head & stiffened neck

Fig. 67 No difficulty on walking

Fig. 68 No prolapsed third eyelid and no erected ear **(**Day twenty five)

Discussion

Tetanus is an acute noncontiguous infectious disease caused by Clostridium tetani (C. tetani) exotoxins. The clinical signs which include prolapse of the third eyelid, spasm of muscles of the neck and the esophagus, erecting of Ears, elevation of the tail-head is held of the horse in the case report are all similar with the clinical signs listed in the literatures (Radostitis *et al.,* 2007; Van Galen *et al.,* 2008; Brook, 1970; Johnston, 1987).

The body parameters, measured were higher than the normal range; this could be due to the development of muscle rigidity so that the horse's body metabolism should increase rate of action to deliver the normal energy requirement to the horse.

Diagnosis was done based on history and clinical signs that in in agreement with literatures (Sedrish *et al.,* 1996).

Even after the end of therapy follow up and management of the case took long period because the toxin that has been taken up into nerve terminals could not probably be available to the antitoxin. Therefore, muscle symptoms developed further, although the clostridia had been eradicated and antitoxin had been given (Kabura, 2006).

Generally treatment of tetanus in equids aimed at neutralizing the circulating toxins, killing the disease causing organism, reliving the muscle spasm and calming the environment; which usually took very long period. So to protect equids from tetanus, production of the vaccine and also production of prophylactic equine tetanus antitoxin in the country is best recommended so as to reduce the suffering of equids and also to reduce the economic loss occurred in treatment of such kinds of cases.

Acknowledgment

I thank SPANA-Ethiopia project veterinary team members for their help during treatment and management of this case

4.3. Case Reports on Small Ruminants

4.3.1. Goat pox

Summary

Goat pox is serious, fatal viral systemic diseases characterized predominantly by skin lesions. This case report summarizes the case management of goat pox in a flock of goats presented to veterinary teaching hospital at the College of Veterinary Medicine, Bishoftu, Ethiopia. One of the goats was newly bought. The lesion were first seen on the newly bought goat and on the others followed. The goats had generalized skin lesions, lacrimal and nasal discharge, coughing, anorexia, depression and also swollen limbs. On clinical examination body temperature and respiratory rate of all the four goats were higher than the normal range. The lesion area was aseptically prepared and surgically tissue sample was taken from the representative goat with distinct clinical lesion. The tissue sample was preserved in Phosphate-buffered saline (PBS) and sent to National Veterinary Institute (NVI) for molecular test. NVI has done Polymerase Chain Reaction (PCR) test on the sample and confirmed the disease as goat pox. The goats were treated with Flunixin meglumine (1.1 mg/kg/day IV) for 3 days and Oxytetracycline (15 mg/kg/d IM) for 5 days. The case was followed for 60 days, during which two of the goats recovered and two others died. A newly bought goats should be quarantined at least for three weeks before introduced to a flock was recommended.

Keywords: Newly bought goat, Goat pox, PCR

Literature Review

Goat pox is serious, fatal viral systemic diseases characterized predominantly by skin lesions extending all over the skin, but are most obvious on face, eyelids and ears, perineum and tail and internal lesions (Bhanuprakash, 2005).

The disease is result from infection caused by sheep pox virus (SPV) or goat pox virus (GPV), of family poxoviaride. The poxviruses have prolonged survival in environment and inactivated by drying, freezing, thawing, and remain viable for months in the lyophilized state. But it is

sensitive to 1% of formalin and extreme PH. The virus can remain infectious for up to six months in sheep and goat pens (Sharma *et al.,* 1988).

Transmission is mostly by aerosol, through contact with infected animal or fomite. Vectors like, stomoxys calcitrans and tsetse fly can transmit the virus mechanically (Webbs, 1980). So transmission is fast in environmental conditions favoring the multiplication of Stomoxys calcitrans and the tsetse fly (Webbs, 1980).

Incubation period mostly varies between four to twenty one days. After the virus enters the body of the animal, through abraded tissues, it starts to replicate locally in that tissue. After 5- 7th day post-inoculation, where the virus titer reached to its peak, the virus will spread to the regional lymph nodes. After 3-4 days of replication in these lymph nodes viremia will develop. The viremia spread in the body, and affect spleen, lungs, liver and skin. Disseminated infections, papules, of the skin are the results of viraemia (Afshar *et al.,* 1986; Davies and Otema, 1978). Within 24 hours of the appearance of generalized papules, affected animals develop conjunctivitis, rhinitis and enlargement of all the superficial lymph nodes, in particular the superficial lymph nodes.

There are five stages in the development of pox lesions on the skin of affected animals. Lesions first start as small red spots that after 3 days will be changed to papules (hard during palpation). These papules after 5-6 days will be changed to vesicles, which will later on change to pustule. Finally the pustules will be changed to scab (Davies and Otema, 1978).

The clinical sign of Goat pox can be either malignant or benign. The malignant form is mostly common in kid where the mortality rate could reach 100%. Affected kids may die without observable pox lesion. But sometimes fever which peak at 40-42°C, dyspnea, and occulo-nasal discharge and pox lesion on skin are manifested in malignant form of the disease. In benign form, only skin lesions occur particularly under the tail. This form of the disease is common in adults. In benign form there is no systemic reaction and the animal recovers in 3-4 weeks. Abortion and secondary pneumonia are rare complications (Animal Health Australia, 2018).

If lesion is present in the lung acute respiratory distress occurs. Animals with lung lesions may have respiratory signs; coughing, nasal discharge and dyspnea. Nodules in the digestive system can cause diarrhea. Depression and emaciation may be seen in some animals (CFSPH, 2008).

Diagnosis Sheep and goat Pox can be diagnosed based on observable clinical sign like, fever, dyspnea and pox lesion in different parts of the skin. Epidemiology, clinical pathology and species of affected host are also important in the diagnosis of this disease. As the virus of sheep and goat pox are very closely related it's indistinguishable serologically (Pandey, 1972). Sheep and goat antigen can be detected using the polymerase chain reaction (PCR) method in combination with a clinical history consistent with generalized capripox infection. Other laboratory diagnoses include observation of the virus using electron microscope, virus isolation, indirect fluorescent antibody test, detection of antibody by virus neutralization test (Davies and Otema, 1978), and characteristic histopathologic lesions (Davies, 1981).

Goat pox has no effective treatment so treatment should be directed to control secondary bacterial infection. So parentral administration of broad spectrum antibiotic may be important to control secondary bacterial infection (CFSPH, 2008).

The disease is endemic in most parts of the world such as Africa North of Equator (including Ethiopia), Middle East, Turkey and Nepal (Worldcatorg, 2018). Vaccination with commercially available live attenuated vaccines has been applied as the main control measure for SPP/GTP in endemic regions. Annual vaccinations using live attenuated SPP vaccines provide good protection and are able to control the outbreaks when the minimal coverage of 75 % is reached and maintained (Davies, 1981).

Case History

An owner presented four goats to the Veterinary Teaching Hospital of Addis Ababa University, College of Veterinary Medicine and Agriculture in January, 2018. The owner mentioned that one of the goats, the old one, was newly bought, 1 week before, from Adama. All the goats were kept in extensive type of farming. The history of the case was generalized skin lesions, lacrimation, nasal discharge, complete loss of appetite and depression, coughing (the old goat only) and also

swollen limbs. The lesions first appeared on the newly bought goat, old goat, and on the next days the other goats were also having the same type of lesion.

Clinical Examination

On clinical examination, all the goats were febrile with body temperature of 39.9^{o}c, 39.9^{o}c, 41.2^{o}c and 42.6^{o}c with respiratory rate of 20 breaths/min, 24 breaths/min, 20 breaths/min and 28 breaths/min respectively. The nasal air flow was also having a slightly wheezy sound. There were bilateral serous ocular and mucopurulent nasal discharges. At the tip of the nostrils there was dried nasal discharge that could be responsible for the wheezy sound on the nasal air flow. Palpation of the retropharyngeal and prescapular lymph nodes revealed swollen lymphonodes. The skin lesions, papules, were found all over the skin but abundantly found in face, leg and tail areas.

Fig. 69 The initial four goats (Day one)

Fig. 70 The newly bought; old goat (Day one)

Fig. 71 Rapid respiration (Day one)

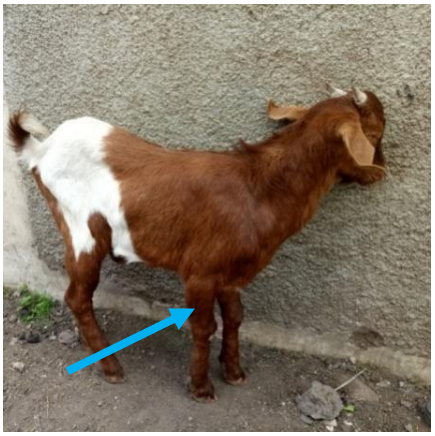

Fig. 72 Depression and swollen limbs; young goat (Day one)

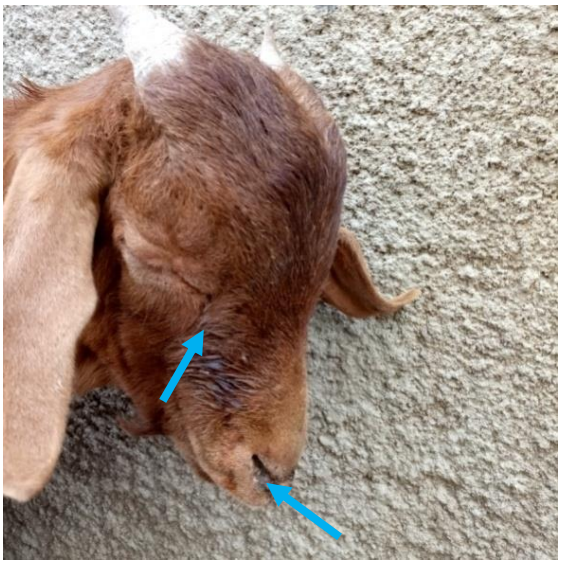

Fig. 73 Serous occular and mucopurulent nasal discharge (Day one)

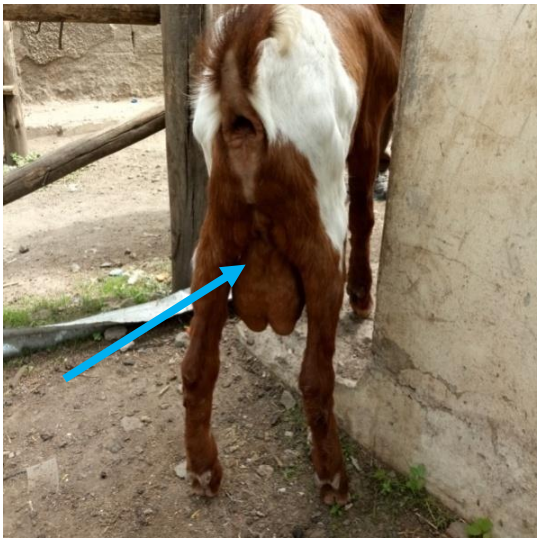

Fig. 74 Papules clearly seen around the scrotum (Day one)

Differential Diagnosis

Goat pox, Dermatophilosis, Mange mite

Sample Collection

The goat with typical lesion was selected for taking tissue sample. A papule on the left hind limb was selected and the hair over the papule was clipped off. With waterproof marker the area of the papule was marked and with 25 gauge needle 5 ml of 2% lidocaine was infiltrated around the papule. Using scalped blade No. 24 and thumb forceps, the marked papule was surgically removed and the tissue sample was kept in sterile universal bottle that contain Phosphate-buffered saline (PBS). On the goat the area where the sample was taken, was cleaned with 1% iodine and covered with gauze for three days. The sample was then transported with cold chain to NVI for molecular identification of antigen.

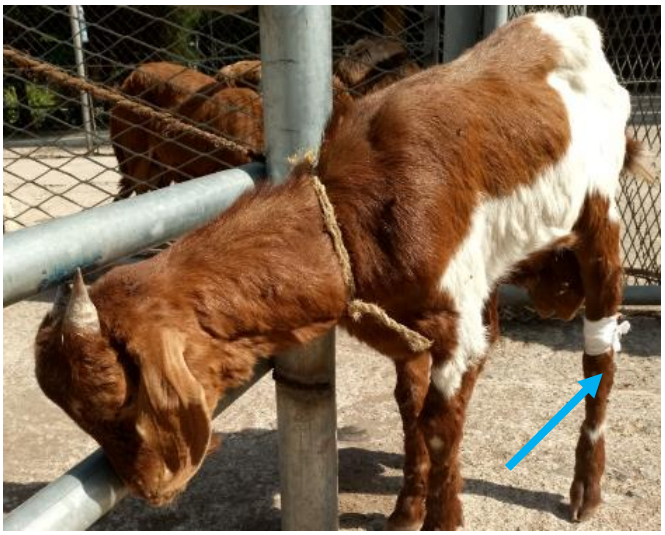

Fig. 75 Site of Biopsy

Laboratory Diagnosis and Result

National Veterinary Institute had done Polymerase chain reaction (PCR) test and confirmed the presence of Goat Pox Virus in the tissue sample.

Treatment

In two of the goats, lesions around the eye and nose were super infected so it was cleaned with water and 2% povidion iodine.

All patients were treated with Flunixin meglumine (1.1 mg/kg/day IV) for 3 days as antipyretic and antipain. And Oxytetracycline (10 mg/kg/day IM) was given for 5 days to avoid secondary bacterial complication.

Outcome

The case was reported to the Ada'a woreda Livestock and Fishery department and vaccination was given to the goats that were living in the same area.

On the fifth day of follow up, one of the goats, the old one, died. And on the other three the papules that were observed in some body parts, (on the mouth, fore limb, and hind limb)

progressed and covered almost all parts of the body of the animals. At the 10th day of follow up, one additional goat, the young one, died.

Fig. 76 The young goat just before death

And the other two were getting better where the papules were gradually changed in to scabs. On the 60th day of visit the two goats were totally free from any visible pox lesion.

Fig. 77 The two recovered goats (Day sixty)

Discussion

Goat pox is serious, fatal viral systemic diseases resulted from infection caused by sheep pox virus (SPV) or goat pox virus (GPV), of family poxoviaride. The clinical sign and lesion observed in this case, which include generalized skin lesions, lacrimation, nasal discharge, complete loss of appetite and depression, are in agreement with clinical findings on literatures (Nasroallah et al, 2012; Kamran *et al.*, 2015).

Coughing that was observed on the newly bought, old goat could be due to the further progression of the pox lesion to the respiratory tracts and lungs.

Goat pox is malignant in kids and benign in adults (Animal Health Australia, 2018). The death of the kid in the study is in consistent with the malignancy of the disease in kids. But the death of the adult goat, though it should be benign, could probably be due the advancement of the disease to the respiratory system (evidenced with calf) or it could be due to the weak body defense mechanism as the goat had poor body condition.

Quarantine of newly purchased animal atleast for three weeks, immediate isolation of diseased animal and vaccination of the healthy sheep and goats should be advised to the farmers.

Acknowledgment

My thankful appreciation goes to Mr Shelemew, for giving me all the follow up information even through phone calls.

4.3.2. Coccidiosis

Summary

Coccidiosis is a gastrointestinal disease of farm animals that is caused by Eimeria spp. This case report summarizes the case study of Coccidiosis in a sheep presented to Veterinary Teaching Hospitalof CVMA Bishoftu, Ethiopia. The sheep was newly bought from market three days before and it was mixed with other sheep that were kept for fattening. The sheep was directly introduced to the feed that was given to the sheep kept for fattening. On Clinical examination there was a sign of diarrhea on the hind quarter of the sheep. The sheep looked depressed. But body parameters were in normal range. The level of dehydration was very minimal. Faecal sample was taken and wet smear was prepared and Coccidia eggs were detected on microscopic examination. The sheep was treated with Sulfadimethoxine for 3 days. Stressful conditions like immediate feed change could expose animals to coccidiosis so sudden changes should be avoided.

Keywords: Coccidiosis, stress, diarrhea

Literature Review

Coccidiosis is a gastrointestinal disease of farm animals. It is caused by Eimeria spp. Eimeria are protozoa, a unicellular microorganism naturally found in the soil. Coccidia are host-specific (Levine and Ivans, 1970; Lima, 1981).

Coccidia are obligate intracellular parasites whose development is within the cytoplasm of epithelial cells result in the hyperplasia or death of each cell that is parasitized. Mechanisms and degree of tissue damage depend on the species of Eimeria involved, the size of the infective dose of oocysts, stress, and various host-related factors including age, physical condition, genetic susceptibility, and degree of immunity that has developed from previous low levels of infection. Because of diminished epithelial turnover in young animals, they are most susceptible to disease (Jolley and Bardsley, 2006).

The life cycle of Eimeria species includes an exogenous phase of maturation of the oocyst (sporogony), and a parasitic endogenous phase within the host with an asexual followed by a sexual multiplication. The unsporulated oocysts are passed in the faeces and develop into the

infective stage after 2–7 days according to the species of Eimeria and the environmental conditions, moisture, oxygen and temperature. The original single cell divides, forming four sporoblasts, each of which develops into one sporocyst, and within each sporocyst two sporozoites develop. The pathogenesis begins with infection of a cell in the intestinal mucosa, by a sporozoite released from a sporocyst in the lumen of the gut. The life cycle of coccidia are similar and comprises three phases including one or more generations of asexual multiplication by merogony, sexual reproduction by gamogony, and asexual reproduction by sporogony. Gamogony is characterized by the independent development of macrogametes (female) and microgametes (male), with the latter being motile and often produced in large numbers. In addition, the sporozoites are typically enclosed in sporocysts that form within oocysts which are passed into the environment as a resistant stage (Hausmann,1996; Lee *et al.*, 2001).

Specific immunity to each coccidial species develops after infection, so that young animals exposed for the first time are often more susceptible to a severe infection and clinical disease than other animals. The immunity induced by the first infection seems to protect most lambs from reinfections later in the grazing season (Catchpole *et al.*, 1993).Age, genetic susceptibility, physical condition, the degree of immunity and stress factors, such as inclement weather, weaning, dietary changes, traveling, and regrouping have important roles in clinical coccidiosis (Dai, *et al.*, 2006; Gul., 2007). The stress factors results in immunocompromised, so these animals could be more susceptible to Eimeria infections.

The main symptom is diarrhea. The change in the feces appearance is coincided with the first appearance of oocystswhich varies according to theprepatent period of each species. In many cases, the faeces are watery with clumps of mucus and color changes from brown to yellow or dark tarry (Constable et al., 2012; Koudela and Bokova, 1998). During the period of diarrhea, the affected animals rapidly show marked dehydration, paleness of conjunctiva, listlessness, abdominal pain, tenesmus, and weight loss. The general condition of the animal is worsened because of decreased appetite. In certain conditions, coccidiosis can be characterized by sudden mortality without preceding digestive signs, in particular amongst young animals between 2 and 4 months old (Uzal *et al.*, 2016).

Treatment must be done as early as possible and concern the whole group of animals (age, paddock) as animals showing no obvious signs may contaminate the environment. Treatment has

to be associated with a move of the animals to a cleaner environment (Chartier and Paraud, 2012).

Case History

An owner presented a sheep to the veterinary teaching hospital of CVMA Bishoftu, Ethiopia. The owner mentioned he has bought the sheep from market three days before and mixed it with the sheep at his home that were kept for fattening. The new sheep was directly introduced to the feed that was given to the sheep kept for fattening.

Clinical Examinations and Findings

There was a sign of diarrhea on the hind quarter of the sheep. The sheep looked depressed. Body parameters were; temperature 37.6^{o}C, respiratory rate 20 breaths/ minute and pulse 36 beats/ minute. The level of dehydration was very minimal.

Fig. 78 Depressed sheep

Fig. 79 Evidence of diarrhea on the hind quarter of the sheep

Differential Diagnosis

Coccidiosis, Carbohydrate engorgement,

Sample Collection

Faecal sample was taken and wet smear was prepared.

Laboratory Diagnosis and Result

A drop of fecal sample was placed on a microscopic slide and covered with cover slip and observed under 10X magnification power of a binocular microscope.

The result showed coccidia eggs.

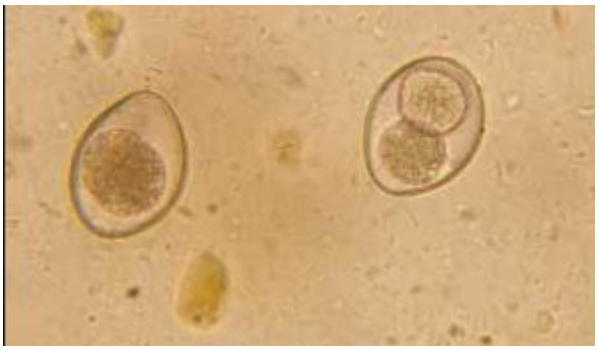

Fig. 80 Coccidia egg in the faecal sample

Treatment

Sulfadimethoxine 30mg/kg IV

Outcome

After the end of treatment the diarrhea stopped and the sheep was bright. After 2 days of treatment when the sheep was visited at home it was bright.

Fig. 81 Bright sheep after two days of treatment

Discussion

Coccidiosis is a gastrointestinal disease of farm animals that is caused by Eimeria spp. Stressful conditions could expose animals to coccidiosis (Dai, *et al.*, 2006; Gul., 2007). In this case study the diet and living environment of the sheep may have been changed which could cause stress to the sheep and the coccidia would take that opportunity to multiply in higher number and cause disease.

Acknowledgment

I thank Mr Dereje Gudeta (Vet technician at CVMA) for assisting in the clinical procedures with this case.

4.4. Case Reports on Poultry

4.4.1. Collibacillosis

Summary

Collibacillosis is a disease caused by pathogenic *Escherichia coli (E. coli)*. This case report summarizes the case management of Collibacillosis in one of the chicken farm, Bishoftu, Ethiopia. There was sudden death of chickens without clinical signs. A dead chicken, which died shortly before, was taken for observation of gross pathological. Heart blood sample was also taken with a sterile syringe for isolation of the possible bacteria. The gross pathological lesions were accumulation of fibrin in the abdominal organs especially on the liver and around peritoneum. The blood sample taken was cultured on blood agar and incubated for 24 hours. Big, circular and hemolytic colonies were observed. For further identification the suspect a colony, from the blood agar, was sub-cultured on MacConkey agar plate and incubated for 24 hours. Circular, smooth, pink and lactose fermenting bacteria were observed. The results on biochemical tests were; on catalase test, the bacteria were Catalase positive and on motility test, the bacteria were motile. Gram staining of the colony revealed Gram negative rod bacteria. All the characteristics of colonies on cultures, biochemical tests, Gram staining done on the sample and post mortem lesions found confirmed the disease; Collibacillosis. Chlortetracycline **(**Aureomycin) 500g/1000 liter of water was given for 5 consecutive days. The mortality of chicken continued until the fourth day of treatment but stopped after the fifth day. Cleaning the environment and avoiding stressful condition was recommended as unhygienic environment of the farm could cause suffocation and stress that could give favorable condition for normal flora of E. coli to be changed to pathogenic flora.

Key words: E. coli, chicken, culture, biochemical test

Literature Review

Collibacillosis is a disease caused by pathogenic *Escherichia coli (E. coli)*. The causative agent, *E. coli*, is a gram-negative, non-acid-fast, uniform staining, non-spore-forming bacillus that

grows both aerobically and anaerobically and may be variable in size and shape. Many strains are motile and have peritrichous flagella (Barnes *et al.,* 1997).

Collibacillosis is a common disease in poultry flocks worldwide especially in the intensive farming system (Chansiripornchai, 2009). It affects birds of all ages but especially has high prevalence rate in adult layer birds (Rahman *et al.,* 2004).

Intensification of poultry production will increase the incidence of colibacillosis through greater exposure of birds to pathogens and stress (Hossain *et al.,* 2004). This disease has an important economic impact on poultry production worldwide (Schouler *et al.,* 2012). It is one of the most common causes of mortality in commercial layer and breeder chickens (Nolan, 2013).

E. coli is one of member of the normal microflora of the poultry intestine. Although most strains of E. coli are not regarded as pathogens, they can be opportunistic pathogens, in case of stress and unfavorable conditions, spread into various internal organs and cause Collibacillosis characterized by systemic fatal disease (Barnes and Gross, 1997; De Carli *et al.,* 2015).

The mechanisms by which avian pathogenic *E. coli* cause infection are largely unknown. But the disease is characterized by fibrinous lesions around visceral organs (Kemmett *et al.,* 2014), septicaemia, enteritis, granulomas, omphalitis, sinusitis, airsacculitis, arthritis/synovitis, peritonitis, pericarditis, perihepatitis, cellulitis, and swollen head syndrome (Kunert *et al.,* 2015). APEC infections also lead to reduced yield, quality, and hatching of eggs. The signs in birds may also include coughing, sudden death of birds and birds being off-color with their necks pulled into their bodies (Johnston, 2007).

Faeco-oral route is the main route of infection followed by ingestion of contaminated feed and water. Intestinal tract of animals including poultry is the most important reservoir site of *E.coli.* Transmission of avian pathogenic *E. coli* (APEC) through the egg, penetrating the shell from fecal contamination, is a common route and may cause high mortality in chicks. Pathogenic coliforms are more frequent in the gut of newly hatched chicks than in the eggs from where they hatched, suggesting rapid spread after hatching (Otaki, 1995).

Colibacillos is suspected based on the clinical features and the typical macroscopic lesions. The diagnosis is obtained by *E. coli* isolation from cardiac blood and affected tissues, like liver,

spleen, pericard or bone marrow (Gomis *et al.*, 1997). Selective media like McConkey, eosin-methylene blue or drigalki agar are used for isolation. Further identification of the isolated colonies is based on biochemical reactions (indol production, fermentation of glucose with gas production, presence of ß-galactosidase, absence of hydrogen sulphite production and urease, and the inability to use citrate as a carbon source) (Dho-Moulin, 1999).

A first step is the prevention of egg contamination by fumigating them within two hours after lay, and by removing cracked eggs or eggs soiled with faecal material. It is recommended to vent the incubators and hatchers to the outside and to have as few breeder flocks as possible per breeding unit (Barness and Gross, 1997). In chicks, contamination with APEC from the environment must be controlled by reduction and control of intestinal infection. This can be achieved using competitive exclusion i.e., inoculating day-old chicks with normal bacterial flora of healthy adult chickens (Hofacre *et al.*, 2002).

Treatment strategies include attempts to control predisposing infections or environmental factors and early use of antibacterials indicated by susceptibility tests (Kabir *et al.*, 2005).

Case History

There was a phone call, for veterinary service, from one of the poultry farm in Bishoftu. The farm owner mentioned that he had more than 2000 chickens. The owner mentioned that there was sudden death of chicken (a total of 10 chickens died in two days' time). Some of the dead chickens were having twisted neck.

Clinical Examinations and Findings

On the day of visit of the farm, there was one dead chicken with little swollen head. The personnel confirmed that there was no dead chicken before an hour so the whole dead chicken was taken as a sample for disease confirmation. Randomly selected chickens were examined for any clinical sign but no clinical sign observed.

Fig. 82 Dead chicken (Day one)

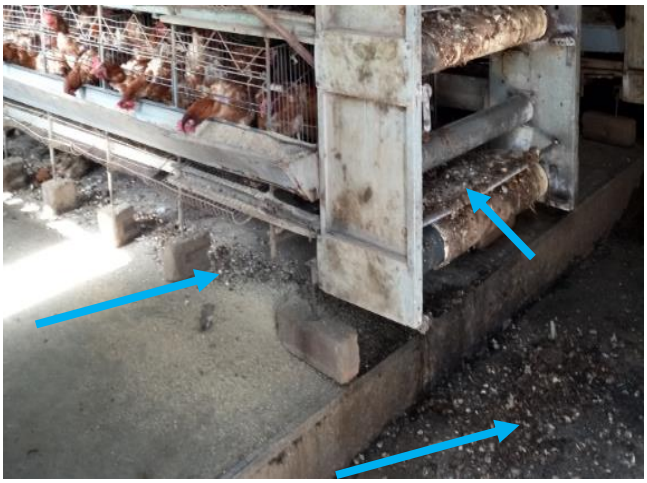

Fig. 83 Unhygienic environment (Day one)

Fig. 84 Random Clinical examination (Day one)

Tentative Diagnosis

Collibacillosis

Sample Collection

At a separate room of the farm, on cleaned and disinfected table, the carcass of the dead chicken was opened with No. 24 surgical blade, for taking blood sample directly from the heart and for observing gross pathological lesions. 2 ml of blood was taken directly from the heart with 21gauge, 5ml syringe. The sample was kept in a cold chain and transported to laboratory for microbial isolation.

Gross Post Mortem Lesion

The carcass was opened with scalpel and the gross pathological lesions found were fibrinous lesions around most internal organs especially on liver and around peritoneum.

Fig. 85 Fibrinous lesion on liver

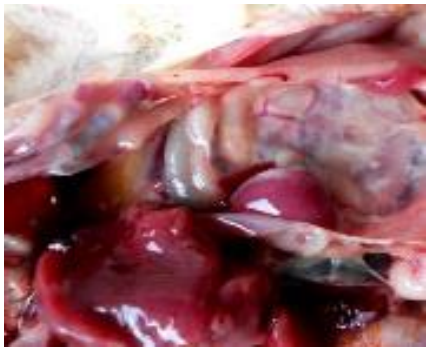

Fig. 86 Fibrinous lesion around peritoneum

Laboratory Diagnosis and Result

A loop (that was sterilized) of blood sample was streaked on Blood agar and incubated for 24 hours. Big, circular and non-hemolytic colonies were observed. With sterilized and cooled loop, a small colony was taken from the blood agar and sub-cultured on MacConkey agar plate and incubated for 24 hours. Circular, smooth, pink and lactose fermenting bacteria were observed. On biochemical tests done; on catalase test (APPENDIX 3), the bacteria were Catalase positive.

For Gram staining; with a sterile cooled loop, a drop of sterile water was placed on a clean labelled microscopic slide. The loop was again sterilized over Bunsen burner flame and allowed to cool. With the cooled 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 (APPENDIX 1).

A drop of oil immersion was added on the stained slide and observed under 100X objective lens of binocular microscope. Gram negative rod bacteria were observed on the slide.

All the characteristics on culture, biochemical tests and Gram staining done on the sample confirmed the presence of *E. coli* in the heart blood sample taken.

Treatment

Chlortetracycline **(**Aureomycin) 500g/1000litre water was given to all chicken in the farm continuously for 7 days. And the shed thoroughly cleaned two times a day during the five days of treatment.

Outcome

Staring from the first day treatment until the fourth day of treatment 10 chickens died. But from the 5^{th} day to the 30^{th} day of follow up there was no report of death of chicken.

Discussion

Collibacillosis is a disease caused by pathogenic *Escherichia coli*. It affects birds of all ages but especially has high prevalence rate in adult layer birds (Rahman *et al.,* 2004). The history of sudden death with no clinical signs reported in this case report is in consistent with other findings (Kemmett *et al.,* 2015; Johnston, 2007).

The twisted neck, which was no reported in literatures, but in the history of this case report, could be due to incorrect observation of the farm personnel or any uninvestigated underlying condition.

The deposition of fibrin around the liver and peritoneum is a similar finding with others (Kemmett *et al.,* 2014; Kunert *et al.,* 2015; Johnston, 2007).

Escherichia coli are one of the normal floras of the gastro-intestinal tract of Chicken. Stressful conditions like suffocated environment (Barnes *et al.,* 1997; De Carli *et al.,* 2015) could predispose poultry to Collibacillosis. So the personnel of the farm were advised to keep floorings clean.

Acknowledgment

My thankful appreciation goes to Mr Misgana Tefera (Lab technologist in CVMA), for his help in Sample Collection and laboratory confirmation of the case.

4.4.2. Newcastle disease

Summary

Newcastle disease is one of the most contagious viral diseases of poultry worldwide. This case report summarizes case management of Newcastle Disease in one of the small scale poultry farm found in Modjo, Ethiopia. Initially the farm had 30 cross bred chicken. They were kept under semi-intensive farming. Additional five new chickens, bought from the market, were introduced to the farm. After four days six chickens, from the old stock, died. In addition some of the chickens from the old stock started to show signs of depression, paralysis of wings and legs, greenish diarrhea and air gasping. Two live chicken samples were sent to National Veterinary Institute (NVI) for confirmatory diagnosis. Reverse-transcriptase polymerase chain reaction (RT-PCR) test confirmed the cause of the disease is Newcastle Disease Virus (NDV). In the remaining old stock, even though Chlortetracycline treatment was started to hinder secondary bacterial complication, it was unable to save any from the old stock. But the newly bought five chickens did not show any clinical sign of Newcastle disease. Newly bought chicken, with history not known, should not be introduced in to old stock before quarantined atleast for 21 days is recommended.

Key words: Newcastle, Poultry, RT-PCR

Literature Review

Newcastle disease (NCD) is a contagious viral disease of birds and considered one of the most important poultry diseases worldwide. NCD is caused by avian Paramyxovirus serotype 1 (APMV-1) viruses (Lamb *et al.,* 2000). The virus is mostly known by the name Newcastle disease virus (NDV).

NDV is infective for almost all avian species, both domestic and wild. Chickens are highly susceptible to infection with Newcastle disease virus, including the pigeon variant of APMV-1. Chickens are considered to be the most susceptible of domestic poultry species. Newcastle disease virus is heat stable when compared with most of paramyxovirus. It remain infectious in bone marrow and muscle of slaughtered chicken at least six month at -20°C and for up to four

month in refrigerator temperature and also infectious virus may survive for months at room temperature in eggs laid by infected hens (Merck, 1995).

Higher prevalence of NCD is during dry season than wet season. Human activity and increased turnover in the chicken markets during dry season could leads to outbreaks of the disease that have been attributed to high prevalence during dry season (Nega *et al.,* 2012).

Studies on NCD indicated high significant difference in the prevalence of the disease between local and cross breeds of chickens. Highest prevalence's are recorded in cross breeds of chickens than local breed. Mortality may be very high, often reaching 50 to 100% (Aschelew *et al.,* 2005).

The transmission of NDV occurs through respiratory aerosols, exposure to fecal and other excretions from infected birds, through newly introduced birds, selling and giving away sick birds and contacts with contaminated feed, water, equipment and clothing. The usual source of virus is an infected chicken, and spread is usually attributed to the movement of chickens through chicken markets and traders (Desalegn, 2015).

The infection takes place by inhalation or ingestion of the virus or by contact with mucous membranes, especially the conjunctiva. Incubation period is 2–15 days with an average of 5–6 days; some species may be over 20 days (Serkalem *et al., 2005)*

The clinical signs in birds infected with NDV vary greatly from very high morbidity and mortality to asymptomatic carriers. The severity of an infection is dependent on factors like the virulence and tropism of the virus, host species, age of host, immune status, other diseases and environmental conditions (Kahn, 2005). Symptoms from the respiratory tract are gasping, coughing, sneezing and rales. Signs from the nervous system include tremors, paralyzed wings and legs, twisted necks, circling, spasms and sometimes complete paralysis. Other general symptoms that can be seen are greenish diarrhea, depression and inappetance, partial or complete drop in egg production and an increased production of deformed eggs (Kahn, 2005).

Clinical diagnosis based on history, signs and lesions may establish a strong index of suspicion but the laboratory confirmation must be done. Hemagglutination and hemagglutination inhibition test, virus neutralization test, Enzyme linked immune-sorbent assay, plaque neutralization test and reverse-transcriptase polymerase chain reaction (RT-PCR) can be used for confirmation of

the ND virus (Alexander and Allan, 1974). Now RT-PCR is the most exclusively used method to detect NDVs (Haque *et al.*, 2010).

The general approaches to the control of Newcastle disease are hygiene and vaccination, this is always important, especially in the control of NCD in semi-intensive systems where birds are confined within a fenced yard or house. Hygiene includes measures such as cleaning, disinfection, limiting access to wild birds, and personal hygiene of the farm staff. Vaccination in combination with appropriate hygiene measures, this remains the most effective way of controlling NCD (Moerad, 1987).

NDV may cause conjunctivitis in humans, when a person has been exposed to large quantities of the virus (Alexander, 2000). Mostly, Laboratory workers and vaccinators are affected. The use of personnel protective equipment and biological safety cabinet has reduced the exposure of laboratory workers. Infection is rarely seen in the workers of a farm; moreover, persons handling or consuming poultry products do not appear to be at risk (Nolen, 2003).

Case History

A phone call was received from small poultry farm in Modjo, Ethiopia. The owner mentioned that he had 30 cross bred chicken. He said the chickens were bought from one of his friend's farm before one month. They were kept under semi-intensive farming. The owner bought five new chickens, with history not known, from the market five days ago and mixed them with the others in the farm. A day before, 6 chickens died with twisted neck. He mentioned also there were chickens with twisted neck and leg paralysis at the time of his call. He was advised to bring two chickens, with a marked signs, to National Veterinary Institute (NVI) for confirmatory diagnosis.

Clinical Examination

The two chickens were depressed and had sneezing and paralyzed wings and legs. There was evidence of green diarrhea around the cloaca.

Fig. 87 Depressed & paralyzed chickens (Day one)

Fig. 88 Examination of chickens

Fig. 89 Green diarrhea around the cloaca and Gasping air

Differential Diagnosis

Newcastle disease, Mareks disease

Sample Collection

Two live chickens were given to NVI for confirmatory diagnosis.

Laboratory Diagnosis and Result

NVI had done RT-PCR, and confirmed the presence of paramyxovirus from the tissue sample taken from spleen.

Treatment

To protect the secondary bacterial complication, chlortetracycline 10g/20kg feed and given continuously for 5 days was prescribed.

Outcome

After two days of the start of therapy, the owner reported that all the chickens died out except the five that were newly bought.

Discussion

Newcastle disease (NCD) is a contagious viral disease of birds. Chickens are highly susceptible to infection with Newcastle disease virus.

The clinical signs found in this case report; depression and reluctance to move, paralyzed wing and leg, greenish diarrhea and gasping of air are all in consistent with the literatures (Merck, 1995, Kahn, 2005; Alexander, 2000; Nolen, 2003).

The newly introduced five chickens, which did not die, could probably be vaccinated against Newcastle disease. But ND vaccination usually protects the chickens from the more serious consequences of disease, but virus replication and shedding may still occur (Brugh *et al.,* 1998; Guittet *et al.,* 1993). So the virus that could be shed from the five chickens could be the source of infection for the old cross bred stock. And cross bred chicken are very susceptible to NCD, where the mortality rate could reach as high as 100% (Aschalew *et al.,* 2005).

Post mortem findings could further confirm the case. It was assumed that post mortem examination could be done after the confirmation of the disease by NVI but by then all the chicken were dead.

4.4.3. Salmonellosis

Summary

Salmonella infection caused by a variety of *Salmonella* species is one of the most important bacterial diseases in poultry. This case report summarizes case management and follows up of Avian Salmonellosis in one of the poultry farm found in Bishoftu, Ethiopia. The farm had 2,600 layers. Some chickens showed reduced feed intake, droopiness, decreased egg production and poor quality of the shell of the eggs, ruffled feathers and diarrhea. A representative chicken, with marked clinical signs, was selected and sent to National Veterinary Institute (NVI) for confirmation of the case. NVI has done Polymerase chain reaction (PCR), the disease was confirmed to be Salmonellosis. Antibiotic, Sulphadimidine, and oral rehydration therapies were given. After the end of the treatment most of the clinical signs resolved; but the poor quality of the shell of the egg continued which could be due to less amount of calcium in the feed or due to any underlying uninvestigated cause.

Key words: Salmonellosis, Poultry, PCR

Literature Review

Salmonella infection caused by a variety of *Salmonella* species is one of the most important bacterial diseases in poultry causing heavy economic losses through mortality and reduced production (Haider *et al.*, 2004). Salmonella are Gram negative, short plump shaped rods, non-spore forming, non- capsulated, aerobic and facultative anaerobic organisms and classified under the family Enterobacteriaceae (OIE, 2006). Avian salmonella infection may occur in poultry either acute or chronic form by one or more member of genus Salmonella (Hofstad *et al.*, 1992). *Salmonellapullorum* is the causative agent of Pillorum disease whereas *Salmonella enterica biovars Gallinarum* is of fowl typhoid (OIE, 2013).

Salmonella is capable of producing peracute infection and hemolytic anemia in both young and adults (Christense *et al.*, 1996). Age wise prevalence of avian salmonellosis showed highest infection rate in adult layers (Lowry *et al.*, 1999). Most stains of salmonella does not possess

zoonotic potential unless like *S. typhimurium or S. enteritidis*, but they cause severe mortality among poultry birds which results in massive economic loss (Shivaprasad, 2000).

The pathogenesis of Salmonella depends on the invasive properties and the ability of the bacteria to survive and multiply within the cells, particularly macrophages (Humbert, 1997). After multiplying the bacteria in the digestive tract the organisms are engulfed by macrophages and spread to the reticloendothelial tissues rich organ like liver and spleen through blood stream which are the main sites of multiplication (Barrow *et al.*, 1994).

Various routes of infection have been described. Oral route of infection represents the normal route of infection (Britto *et al.,* 1995). Infections in newly hatched chicks by nasal and cloacal routes are also considered as the important route of transmission. Chicks may be infected early by vertical transmission either from an infected ovary, oviduct or from the infected eggs during the passage through the cloacal faeces from infected or carrier hens (Berchieri *et al.,* 2001).

In mature chickens Salmonellosis causes either acute enteritis with greenish diarrhea or a chronic disease of the genital tract that reduces egg production. Other chronic signs include ruffled feathers, inappetance, thirst, yellow diarrhea, dejection and reluctant to move (Proux et al., 2002).

The organs which show pathological changes mainly include lungs, liver, heart, kidney, intestine, pancreas, bursa of fabricius. In lungs red hepatization, hemorrhages, congestion, pneumonic lesions, serofibrinous exudates in alveoli has been reported (Shivaprasad, 2000; Kumari *et al.,* 2013). They also reported splenomegaly along with multiple necrotic foci, reticulo endothelial cell hypoplasia and depletion of lymphoid cells in spleen. Liver shows bronze discoloration, congestion, hepatitis and degenerative changes (Sujatha, 2003).

Detection of Salmonella by bacteriologic method is known to be time consuming (Wallace et al., 1999). Rapid detection of Salmonella from poultry by Real-Time Polymerase Chain Reaction with Fluorescent Hybridization Probes has the potential for use in routine monitoring and detection of Salmonella in infected flocks and carcasses (Eyigor and Carli, 2003)

Treatment of infected birds is required to decrease the rate of mortality and its spread in a flock. To combat the dehydration loss due to diarrhea, fluid and electrolyte therapy is the first line of

treatment for the affected birds. Antibiotics therapy either in intravenous or oral route are recommended for reducing the infection after doing antimicrobial sensitivity test (Quinn *et al.*, 2011).

Case History

A phone call was received from one poultry farm in Bishoftu. The owner of the farm mentioned that she had 2,600 chickens. But they reduced their feed intake and showed droopiness, decreased production (form 85% egg production/day to 60% egg production/day) and poor quality of the shell of egg, ruffled feathers, diarrhea (yellowish in color) of some of the chicken. The personnel in the farm managed to isolate thus diseased chicken in to isolation pen.

Fig. 90 Overview of the farm (Day one)

Fig. 91 Isolation pen

Clinical Examinations and Findings

Chickens in the main shed of the farm did not show any clinical sign but chickens in the isolation pen, were showing clinical signs; depression, yellow diarrhea and refused to move. The body temperatures of selected five chickens were measured and it ranges between 40.1^{o}c to 41.0 ^{o}c, which was in a normal range. The feed in the store house was and no moldy growth.

Fig. 92 Depressed chicken (Day one)

Fig. 93 Ruffled feather (Day one)

Fig. 94 Poor egg shell quality (strength)

Differential Diagnosis

Salmonellosis, Collibacillosis, Coccidiosis

Sample Collection

One chicken with the marked clinical signs was selected and sent to National Veterinary Institute (NVI) for confirmatory diagnosis.

Fig. 95 Representative sample sent to NVI

LaboratoryDiagnosisandResult

After doing r-PCR, NVI reported the disease as salmonellosis.

Treatment

Sulphadimidine sodium 1 kg per 2000 litres of drinking water according to the 3-2-3 scheme: (3 days on, 2 days off, 3 days on) was given to all chicken in the farm (including the apparently healthy group).

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.

Outcome

After the end of the treatment the diarrhea stopped. After a month, the chickens in the isolation pen were bright with good feed intake. The egg production of the farm increased to 80%/day. But the egg shell quality was still poor.

Fig. 96 Recovered chicken in the isolation pen (Day thirty)

Discussion

Commercial poultry farming is one of the fastest growing sectors in the country. One of the challenges of the growing poultry industry is salmonellosis, causing heavy economic loss through mortality and reduced productivity. Salmonellae may cause varieties of clinical signs ranging from acute systemic disease and gastrointestinal symptoms in poultry flocks to embryonic problem in hatchery (Gast, 1997).

The clinical signs observed; reduces egg production, ruffled feathers, inappetance, diarrhea, reluctant to move are in consistent with chronic form of salmonellosis in mature chicken (Proux., 2002).

Due to unavailability of antibiotic impregnated disks and urgency of the need of treatment antibiotic sensitivity test was not performed.

The qualities of the shells of the eggs remain poor even after the end of treatment of salmonellosis. This could be due to lack of green fodder or due to less amount of calcium in the food that needed further investigation.

Acknowledgment

I thank Mrs Aster Alemayehu, the farm owner, for covering the costs of diagnosis at NVI and giving me all the information every three to four days.

4.5. Case Reports on Canine

4.5.1. Canine parvovirus disease

Summary

Canine parvovirus infection (CPV) is acute disease of dogs caused by Canine parvovirus 2. This case report summarizes the case management of Canine parvovirus infection in eight months old puppy presented to veterinary teaching hospital at the College of Veterinary Medicine, Bishoftu, Ethiopia. The puppy was vomiting and had watery diarrhea since two days ago. The owner lost one other puppy with similar clinical sign two months ago. On clinical examination the puppy had fever (42.2^{o}C), weak pulse and around 10% degree of dehydration. Whole blood sample was used for packed cell volume determination and polymerase chain reaction. The PCV result was 48% that could justify fluid loss. Metoclopramide HCl and sulphadimidine were used for five days to stop vomit and secondary bacterial complication, respectively. Additionally, 1120ml of 5% dextrose solution per day was administered intravenously for 5 days, to compensate both the deficit and maintenance fluid volumes. After the five days of treatmentvomit and diarrhea stopped and the puppy started to take up food and water by itself. At its 30th day ofcheckup the puppy was happy, grown up and healthy. Early detection of the disease and appropriate fluid and symptomatic therapy were recommended to save the lives of infected puppies.

Key words: puppy, canine parvovirus, vomit

Literature Review

Canine parvovirus infection (CPV) is acute disease of dogs caused by Canine parvovirus 2. 'Parvo' means small (Latin), canine parvovirus 2 belongs to genus *Parvovirus* and family *Parvoviridae*. The genome is a single stranded DNA (Murphy *et al.,* 1999).

Canine parvovirus can affect dogs at any age. Severe infection is most common in puppies between 6 weeks and 4 months old. All breeds of dogs are susceptible (Houston *et al.,* 1996). If a dog survives the first 4 days, they will usually recover rapidly and become immune to the virus for life. Most puppies die without medical treatment (Jacob *et al.,* 1980).

Canine parvovirus spreads through oral contact with infected faeces or contaminated surfaces. The source of CPV infection is faecal waste from infected dogs. Dogs that are confined to a house or yard and are not in contact with other dogs have much less chance of exposure to CPV. It's easily transmitted via the hair or feet of infected dogs and also by contaminated objects such as cages. CPV is hardy and can remain in faeces-contaminated ground for 5 months or more if conditions are favorable (Jacob *et al.,* 1980).

The virus enters the body through the mouth as the puppy cleans itself or eats food off the ground or floor. There is a 3–7 day incubation period before the puppy seems obviously ill. Upon entering into the body, it replicates to large numbers in the lymph nodes (Stann *et al.,* 1984). After a couple of days, significant amounts of virus have been released free into the bloodstream. Over the next 3–4 days, the viruses go to new organs containing the rapidly dividing cells like the bone marrow and the delicate intestinal cells and form large eosinophilic intranuclear inclusion bodies. Within the bone marrow, the virus is responsible for destruction of young cells of the immune system and then knocking out the body's best defense mechanism, characterized by a drop in white blood cell count. The virus causes most devastating effects in the gastro-intestinal tract, destroying the intestinal villi, resulted in less absorption of nutrients from intestine (McCandlish *et al.,* 1981).

Diarrhea occurs in dogs of any age but appears in serious proportions in pups. Dogs with enteritis act like they are in extreme pain. Early symptoms are depression, loss of appetite, vomiting, high fever and severe diarrhea. There is slight rise of temperature in the initial stage of the disease but gradually turn to subnormal level with advancement of vomiting and diarrhea (Kramer *et al.,* 1980). There is no consistent character of the stool, it may be watery, yellow in color or tinged with frank blood in severe cases. Rapid dehydration is a danger, and dogs may continue to vomit and have diarrhoea until they die. The course of illness is highly variable depending on the infectious dose of the virus and clinical signs usually develop from 3 to 5 days following infection and typically persist for 5–7 days (Fletcher *et al.,* 1979). The morbidity and mortality vary according to the age of the animals, the severity of challenge and the presence of intercurrent disease problems. Puppies can die suddenly of shock as early as 2 days into the illness (Stann, 1984).

A presumptive diagnosis of CPV enteritis can be made based on the clinical signs such as depression, vomiting, diarrhea, anorexia and fever. The tests should be performed on any dog with diarrhea that is also exhibiting signs of systemic disease: vomiting, lethargy, fever, loss of appetite, dehydration or dogs with unusually copious, smelly/bloody diarrhea, or any dog with known exposure to parvovirus within the preceding 14 days of developing diarrhea. Definitive diagnosis could be HA (Haemagglutination) (Carmichael *et al.*, 1980), Electron Microscopy (EM) (Burtonboy *et al.*, 1979), Virus neutralization test, Polymerase chain reaction (PCR), nucleic acid sequencing (Cho *et al.*, 2004).

The restoration of the electrolyte and fluid balance is the most important goal of therapy (Woods *et al.*, 1980). The affected dog should be put under broad spectrum antibiotic umbrella (ampicillin, chloramphenicol, erythromycin, gentamycin, etc). Norfloxacin and nalidixic acid have been proved to be effective against canine hemorrhagic gastroenteritis (Kelly and Atwell, 1979). Symptomatic treatment with steroid, broad spectrum antibiotic, fluid and electrolyte may save the life of the animal (Kelly and Atwell, 1979).

Case History

A young boy presented an 8 months old puppy, about 8kg in weight, to veterinary teaching hospital at the College of Veterinary Medicine, Bishoftu, Ethiopia on December 2017. The owner mentioned that he lost one puppy with a sign of vomit and diarrhea 2 months ago. And he adopted this new puppy 15 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 vomit. Then after the puppy became very weak and reluctant to eat and drink.

Fig. 97 Weak puppy (Day one)

Clinical Examination

The puppy was having a body temperature of 42.2^{o}c, respiratory rate of 28 breaths/minute and pulse rate of 52 beats/minute. The pulse quality was weak. Based up on physical examination the estimated percent of dehydration was 10% (moderate degree of skin turgor, dry oral mucous membrane, slight tachycardia, and decreased pulse pressure).

Differential Diagnosis

Parvovirus infection, lead poisoning

Sample Collection

The area below and just above the elbow, on top of right forelimb of the dog was shaved. The cephalic vein was identified by applying thumb pressure along the vein line so that the vein filled up, engorged with blood. A 22 gauge needle size was twisted over 5ml syringe. Alcohol was applied over the area that was going to be punctured. Again thumb pressure was applied over the cephalic vein and puncture was made with the needle over the engorged area of the vein. After it was checked that the needle was inside the vein, the thumb, that was used to create pressure, was released and 5 ml of blood was withdrawn with the syringe. The needle was slowly taken out and the area was disinfected with alcohol and the blood in the syringe was immediately transferred to an EDTA vacutainer tube. The sample was taken to lab and placed on +4^{o}c until processed.

Laboratory Diagnosis and Result

For determination of packed cell volume two plain capillary tubes were used (as the blood was collected in EDTA vacutainer tube). From the EDTA vacutainer tube blood was filled up to its ¾ level of capillary tubes by the capillary action. The ends of the capillary tubes, in contact with the blood, were sealed with clay up to 2 mm depth. The two capillary tubes were placed in to two opposite grooves of hematocrit centrifuge. They were centrifuged at 15,000 rpm (resolution per minute) for one minute. The capillary tube was removed from the centrifuge and it was read using hematocrit reader. The reading of the packed cell volume was 48%, higher than the normal range.

Treatment

The deficit volume was calculated as 800 ml (Fluid deficit calculation: Body weight (kg) * % dehydration= volume (L) to correct) (Muir et al., 1983). And the maintenance volume deficit was calculated as 320 ml (Maintenance fluid calculation=40ml/kg/day) (Wanamaker *et al.*,2008). So 1120ml of 5% dextrose was given per day intravenously (ten drips in one second) for consecutive 5 days.

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.

Fig. 98 Intravenous fluid administration (Day one)

Outcome

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 so there was no need to further continue the fluid therapy. After one month, when the puppy was visited, it was grown up and healthy.

Fig. 99 Recovering puppy (Day three)

Fig. 100 Bright puppy (Day five)

Fig. 101 Well recovered and grown up puppy (Day thirty)

Discussion

Canine parvovirus infection (CPV) is acute disease of dogs. CPV can affect dogs at any age. The Tentative Diagnosis in this case report is in agreement with the report of (Hoskins, 1997; Prittie, 2004) that CPV can occur in dogs of any age though puppies are mostly affected.

CPV is hardy and can remain in faeces-contaminated ground for 5 months or more if conditions are favorable (Jacob *et al.*, 1980) so the current puppy may probably acquire the virus from the contaminated materials that were being used by the puppy died 2 months ago.

Clinical signs like vomiting, fever and diarrhea are in agreement other studies on CPV (Hoskins, 1997; Prittie, 2004)

Fluid therapy, aimed at restoring fluid and electrolyte balance and antibacterial therapy, aimed at reducing the secondary bacterial complication were similar treatment protocols with other studies (Hoskins, 1997; Prittie, 2004).

In consistent with the present case study, in other findings also ((Hoskins, 1997; Prittie, 2004), about 80 to 85 percent of affected dogs will survive and live normal lives if disease could be detected early and proper treatment could be administered.

Due to lack of automated blood counting machines, the number of white blood cells (that was assumed to decrease in number) was not determined. The final confirmation of the disease was better to be confirmed by polymerase chain reaction, but due to lack of primer this was not done. But the epidemiology, history and clinical signs and response to treatment were confirmatory to Parvovirus infection.

Acknowledgment

I would like to thank the child of the owner for his enthusiastic approach for his puppy. He brought the puppy on each of his appointment and happy to follow every advice given to him.

4.5.2. Flea allergy dermatitis

Summary

Flea allergy dermatitis (FAD) in dogs is a hypersensitive condition that is caused due immune system reaction to the saliva of fleas on dogs. This case study summarizes the case management of Flea allergy dermatitis in a dog presented to Veterinary Teaching Hospital, Bishoftu, Ethiopia. The dog was always kept in-door and its shade was clean. The dog would take bath at least two times a week. And it was vaccinated against rabies before two months (the vaccine had one year protection time). A day before its presentation to the clinic, the owner took the dog to outside just for recreation. After about 20 minutes stay outside, the dog was brought back in to its compound. Just after few minutes the dog started to itch itself badly. The dog couldn't get rest; itching itself badly the whole night. It started to bit around its tail. The owner washed and combed the dog and there were fleas. But the dog could not stop itching and biting itself and even after combing some fleas were still on the dog. On clinical examination the body parameters (temperature, respiratory rate and pulse rate) were in normal range but the dog was itching and biting itself. When the dog was inspected around its inguinal area, there were fleas. For two consecutive days, Hydrocortisone (IV) injection was used as anti-allergic and Fibronil spray was used to kill the fleas. After few minutes of administration of the Hydrocortisone on the first day, the dog stopped itching and the dog was when it was presented the next day. 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. And if fleas are detected anti-allergic treatments are necessary, in addition to drugs used to control fleas, to give symptomatic relief to the dog.

Keywords: Flea Allergy Dermatitis, itching, hydrocortisone

Literature Review

Flea allergy dermatitis (FAD) is the leading cause of allergic, hypersensitive conditions in dogs. In an allergic reaction, the immune system overreacts and produces antibodies to a substance that

it would normally tolerate or in an attempt to fight infection (Scott *et al.,* 2011; Cole, 2008; Dryden, 2008; Wilkerson *et al.,* 2004).

The life cycle of the flea ranges from as few as 12 to as many as 190 days, with an average of 21 days. The time needed for development depends heavily on environmental conditions, particularly temperature and humidity. The optimal environment is a low-altitude geographic location, a temperature of 23.8°C, and a relative humidity of 78% (PetMD, 2018).

An adult flea takes its first blood meal from a host within minutes of contact. Female fleas lay their first egg 24 to 36 hours after this blood meal. A single female flea can produce up to 50 eggs per day and about 2,000 in her lifetime. Flea eggs are smooth and slick. Only 30% of eggs remain on the haircoat; the remainders fall off the host into the environment. Hatching takes place within 1 to 10 days, again depending on humidity and temperature (Soulsby, 1982;Bitam *et al,* 2010).

Although eggs can hatch anywhere in the environment, development of the larvae that emerge from the eggs must take place off the host because mammalian body temperatures are too high for survival. Larvae are highly sensitive to heat and desiccation and therefore tend to move downward and away from direct light sources. The larvae feed on adult flea feces (partially digested blood) in the environment. Within 5 to 11 days, a larva undergoes two separate molting stages before forming a pupa (Veterinary Manual, 2018).

The pupal stage is the most resilient of all stages because the cocoon is highly resistant to desiccation. It also has a sticky surface that helps to prevent premature removal from the environment andaccumulates dust and other household particulates to provide protection. On average, the pupal stage lasts 8 to 9 days; however, fleas can pupate for up to 6 months if the environmental conditions are not ideal for emergence. Only with proper environmental stimuli, such as an increase in carbon dioxide, warmth, physical pressure, and vibration, will an adult flea emerge from its cocoon (Dobler and Pfeffer 2011).

After emerging from the cocoon, adult fleas search for an appropriate host. Adult fleas are attracted to light and tend to migrate upward toward surfaces where contact with an appropriate host is more likely. Once a host is found, feeding and mating take place within 8 to 24 hours.

Female fleas can consume 15 times their body weight in blood per day. Adult fleas act as obligate, permanent ectoparasites, preferring to remain on a host rather than in the environment (Veterinary Manual, 2018c).

Flea saliva contains histamine-like compounds, proteolytic enzymes, and anticoagulants. These proteins are released into the host during feeding and can act as inflammatory or antigenic stimuli in sensitive animals. Various immunologic responses are provoked, including immediate & delayed hypersensitivity reactions (Gross and Halliwell, 1985).

Clinical signs associated with FAD are variable and depend on frequency of flea exposure, duration of disease, presence of secondary or other concurrent skin disease, degree of hypersensitivity, and effects of previous or current treatment. Non-allergic animals may have few clinical signs other than occasional scratching due to annoyance of flea bites. Those that are allergic will typically have a dermatitis characterized by pruritus (Veterinary Manual, 2018c).

History and physical examination findings are the keys to making an appropriate diagnosis of flea allergy dermatitis. There is no breed or sex predilection, and flea allergy dermatitiscan develop in animals of any age (Veterinary Manual, 2018c).

The goals of flea control are to eliminate the adult fleas on all the animals in the house as well as immature fleas in the environment. The best approach incorporates mechanical, physical, and chemical measures. Source points should be identified and treated aggressively.

Case History

An owner presented a dog with severe pruritus to the Veterinary Teaching Hospital, Bishoftu, Ethiopia on January 2018. The owner mentioned that the dog was always kept in-door and its shade was clean. The dog would take bath at least two times a week. And it was vaccinated against rabies before two months (the vaccine had one year protection time). A day before its presentation to the clinic, the owner took the dog to outside just for recreation. After about 20 minutes stay outside, the dog was brought back in to its compound. The owner saw some fleas on his trouser and he put this trouser in hot water. Just after few minutes the dog started to itch itself badly. The dog couldn’t get rest; itching itself badly the whole night. It started to bit around

its tail. The owner washed and combed the dog and there were fleas. But the dog could not stop itching and biting itself and even after combing some fleas were still on the dog.

Clinical Examinations and Findings

The body parameters of the dog were: body temperature 38.1^{o}C, respiratory rate 28 breaths/ minute and pulse rate 80 beats/minute. All parameters were in normal range. The dog was itching and biting itself.

When the dog was inspected around its inguinal area, there were fleas.

Fig. 102 Salivation due to severe licking and biting itself

Fig. 103 Clinical examination of the dog

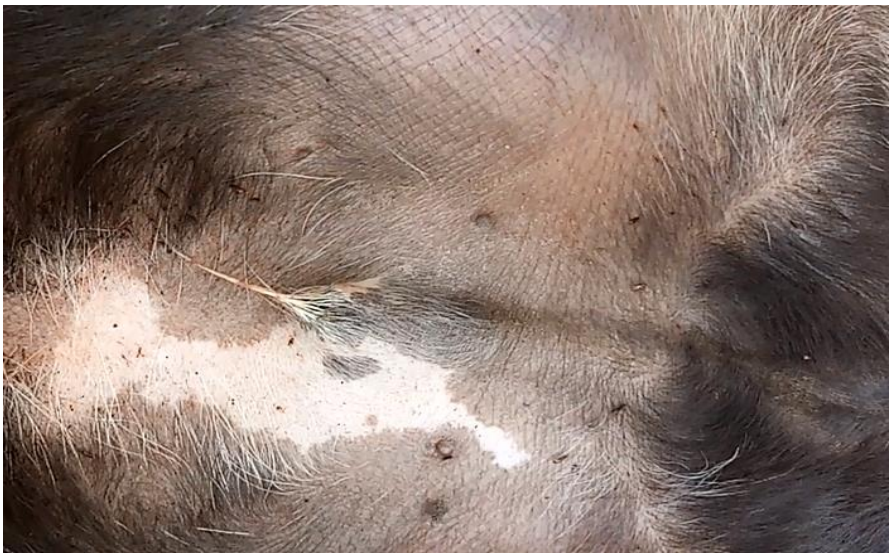

Fig. 104 Fleas in the inguinal area of the dog and red spots indicating dermatitis

Differential Diagnosis

Flea Allergy Dermititis, rabies,

Treatment

Hydrocortisone 3.0mg/kg, SID, IV was given; a 100mg Hydrocortisone powder was suspended in 5 ml sterile water and 1.5ml (because the dog was 10kg its needed 30mg which is calculated to be 1.5ml) was withdrawn with 21 gauge needle. And after disinfection of the area over the

cephalic vein with 75% alcohol, the Hydrocortisone solution was given through the cephalic vein.

Fipronil spray was applied all over the body except the face of the dog and Ivermectin 0.2 ml (1ml/ 50kg) SID, SC was given. And the owner was informed about the life cycle of fleas so that he should apply the spray everywhere the dog had contact during the last two days.

Outcome

After few minutes of administration of the Hydrocortisone, the dog stopped itching. When the dog was presented the next day, it was bright and not itching itself.

After three days of end of treatment, when the owner brought the dog for checkup, the dog was bright and the owner confirmed that the dog stopped itching after the treatment.

Fig. 105 Bright dog brought for checkup (Day five)

Discussion

Flea allergy dermatitis (FAD) in dogs is a hypersensitive condition that is caused due immune system reaction to the saliva of fleas in dogs.Flea saliva contains histamine-like compounds, proteolytic enzymes, and anticoagulants. These proteins are released into the host during feeding and can act as inflammatory or antigenic stimuli in sensitive animals(Gross and Halliwell, 1985).

Clinical signs of FAD are variable among different dogs including itching, biting itself, diarrhea and vomiting (Veterinary Manual, 2018c). The clinical sign that was observed in this case study was pruritus and presence of fleas on the dog's body. This finding is similar with different researches and literatures (Veterinary Manual, 2018c; Cole, 2008; PetMD, 2018).

History, clinical findings and response to treatment (Specially the fast response to Hydrocortisone treatment) were used to confirm this case.

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. And if fleas are detected anti-allergic treatments are necessary, in addition to drugs used to control fleas, to give symptomatic relief to the dog

Acknowledgment

I want to thank Mr Dereje Gudeta for his help in the management of this case.

5. CONCLUSION AND RECOMMENDATION

Agriculture is the basis of Ethiopia's economy and is the most important economic sector in terms of generation of foreign currency. The sector is the primary source of livelihood for more than 85 % of Ethiopian rural households who practice subsistence crop and livestock production. But the country couldn't earn much from the sector due to different problems among which is animal disease.

In addition to the existence of many different types of diseases in the country, diagnosis of diseases based on only clinical signs worsen the health condition of the livestock. But investigation of any animal disease problem should be done on careful and thorough clinical examination with the objective of recognizing the nature of the infection, so that effective treatment and, where practicable, control measures are adopted.

So based on the above conclusion the following points were recommended:

- Confirmatory diagnosis by taking appropriate sample is recommended to keep the welfare of animals, reduce the risk of transmission of diseases to other animals and human being
- Further and repeated case studies should be done to prepare treatment guideline at a national level

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7. APPENDICES

APPENDIX – I 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.

APPENDIX – II Wright's staining procedure

1. Make a blood smear and allow it to air dry
2. Flood the smear with Wright's stain and keep it submerged for 15-30 seconds
3. Flood with distilled water and blow gently to mix well for 40 seconds
4. Rinse the slide in distilled water
5. Allow to dry before examination
6. Examine under oil immersion on the microscope using high power to identify blood cells and blood parasites

APPENDIX – III Slide-Catalase test procedure

1. Use a loop or sterile wooden stick to transfer a small amount of colony growth in the surface of a clean, dry glass slide.
2. Place a drop of 3% H_2O_2 in the glass slide.
3. Observe for the evolution of oxygen bubbles.

APPENDIX – IV Normal body parameters ranges

Species	Temperature °C	Heart Rate (Beats/Minute)	Respiratory Rate (Breaths/Minute)	Capillary refill time
Cattle	37.5-38.5	60-70	30	Less than 2 sc
Goat	39.1	70-80	12-20	Less than 2 sc
Horse	37.5-38.5	32-36	10-14	Less than 2 sc
Sheep	39.1	60-70	19	Less than 2 sc
Dog	38.9	80-90	22	Less than 2 sc
Donkey	36.7 -38.3	28 - 44	8 - 16	Less than 2 sc
Poultry	40.5 -42	350- 450	15 -30	Less than 2 sc

APPENDIX – V Case recording sheet

Case number_____ Reference photo Numbers_____

Date Examined_____ Weather condition of the month_____

Species_____ Breed_____ Age_____ Sex_____ Color_____

Estimated body weight_____

Case history _____

_____

_____

_____

_____

_____

Animal husbandry system_____

Observation of the animal from distance_____

_____

_____

Clinical findings

Body temperature_____ Respiratory rate_____ Respiratory rhythm_____

Heart rate_____ Pulse rhythm_____

Ruminal Motility_____ Gut sound_____ Color of VMM_____

Capillary refill time_____

Abnormality head and neck region _____

_____

_____

Abnormality in thorax and abdominal region_____

_____

_____

_____

Abnormal in the extremities_____

_____

_____

Aabnormality in regional lymphnodes_____

Tentative diagnosis_____

Samples taken_____

Sample code_____

Sample transporting medium_____

Laboratory procedure_____

Name of the laboratory_____

Laboratory technichian_____

Laboratory result_____

_____

_____

_____

Treatment given_____

_____

_____

_____

_____

_____

Advice given to the owner_____

_____

_____

_____

_____

Follow up appointment_____

Final outcome of the disease_____

_____

_____

_____

_____

_____

APPENDIX – VI Goat pox PCR result

	NATIONAL VETERINARY INSTITUTE	Document No. NVI -QMS - QF - 158		
Title: - Laboratory Result Report Form		Effective Date 19/09/2014	Issue No. 4	Page No. 1of 1

Client Name and Address: Hana Zewde. Tel_____

Sample History	Animal species	Sample type	Number of Samples	Origin of animals	Lab. Ref. No.
-	Goat	Nodule suspension	01 (One)	-	MB 48/18

Date of Submission: - 21/03/2018

Samples Collected By: - Customer

Date of Examination: - 04/04/2018

Date of Report: - 09/04/2018

Report No: - MO-16/18

Disease to Diagnosed: - GTP antigen detection.

Test Method/s Recommended: -

Classical PCR

Purpose: -GTP antigen detection.

Type of test under taken: Classical PCR.

Criteria for Positive Result: - Positive sample had around 172 bp.

Number of positive samples	Number of negative samples	Total number tested
01 (One)	-	01 (One)

ID of positive sample: - 01.

Tested By:	Checked By:	Approved By:
Name: - Alebachew Belay	Dr. Esayas Gelaye (Lab. Sup.)	Dr. Esayas Gelaye (TM)
Signatures: -		

Tel. +251-11-433-8411/12 Fax: +251-11-433-9300 E-mail: nvi-rt@ethionet.et
Website: https://www.nvi.com.et P.O.Box:19, Debre-Zeit, Ethiopia

APPENDIX – VII Lumpy skin disease PCR result

NATIONAL VETERINARY INSTITUTE	Document No. NVI -QMS - QF - 158		
Title: - Laboratory Result Report Form	Effective Date 19/09/2014	Issue No. 4	Page No. 1of 1

Client Name and Address: Hana Zewde Tel_____

Sample History	Animal species	Sample type	Number of Samples	Origin of animals	Lab. Ref. No.
-	bovine	Nodule suspension	01 (One)	-	MB 48/18

Date of Submission: - 21/03/2018

Samples Collected By: - Customer

Date of Examination: - 04/04/2018

Date of Report: - 09/04/2018

Report No: - MO-17/18

Disease to Diagnosed: - LSD antigen detection.

Test Method/s Recommended: -

Classical PCR

Purpose: -LSD antigen detection.

Type of test under taken: Classical PCR.

Criteria for Positive Result: - Positive sample had around 172 bp.

Number of positive samples	Number of negative samples	Total number tested
01 (One)	-	01 (One)

ID of positive sample: - 01.

Tested By:	Checked By:	Approved By:
Name: - Alebachew Belay	Dr. Esayas Gelaye (Lab. Sup.)	Dr. Esayas Gelaye (TM)
Signatures: -		

Tel. +251-11-433-8411/15 Fax: +251-11-433-9300 E-mail: nvi-rt@ethionet.et
Website: https://www.nvi.com.et P.O.Box:19, Debre-Zeit, Ethiopia

APPENDIX – VIII Blackleg PCR result

NATIONAL VETERINARY INSTITUTE	Document No. NVI -QMS - QF - 158		
Title: - Laboratory Result Report Form	Effective Date 19/09/2014	Issue No. 4	Page No. 1of 1

Client Name and Address: Hana Zewde Tel_____

Sample History	Animal species	Sample type	Number of Samples	Origin of animals	Lab. Ref. No.
	-	Bacterial culture	1 (One)	-	MB 122/18

Date of Submission: - 11/06/2018

Samples Collected By: - Customer

Date of Examination: - 12/06/2018

Date of Report: - 12/06/2018

Report No: - MO-32 /18

Disease Diagnosed: Cl.chauvoei antigen detection.

Test Method/s Recommended: -

Classical PCR

Purpose: -Cl.chauvoei antigen detection.

Type of test under taken: Classical PCR.

Criteria for Positive Result: - Positive sample had around 601 bp.

Number of positive samples	Number of negative samples	Total number tested
01 (one)	-	01 (one)

ID of positive sample: - Code 01.

Tested By:	Checked By:	Approved By:
Name: - Alebachew Belay	Dr. Esayas Gelaye (Lab. Sup.)	Dr. Esayas Gelaye (TM)
Signatures: -		

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