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ARTICLES

Atresia ani associated with recto-vaginal fistula in two months old Sudanese crossbreed lamb

Bovine mastitis: Prevalence, Isolation and identification of major bacterial pathogens in selected areas of Bench Maji Zone, Southwest Ethiopia
Teshome Gemechu, Hasen Awel Yunus, Morga Soma and Amare Beyene

Common health and welfare problems of working donkeys in Addis Ababa and its surrounding area: Retrospective and questionnaire survey
Samson Terefe, Asnakech Ashine and Getachew Mulugeta

Distribution and molecular characterization of avian hepatitis E virus (aHEV) in domestic and wild birds in Burkina Faso
Jean B. Ouoba, Kuan A. Traore, Alphonsine K. M’Bengue, Solange Ngazoa, Hortense Rouamba, Moussa Doumbia, Alfred S. Traore, Pierre Roques and Nicolas Barro

Comparative cost analysis of three injectable ivermectin preparations in the control of gastrointestinal nematodes of sheep in Makurdi, Benue State Nigeria
Mathew Adamu, Paul Amuta, Anthony Ameh and Samuel Ode
Case Report

Atresia ani associated with recto-vaginal fistula in two months old Sudanese crossbreed lamb

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A case of atresia ani with recto-vaginal fistula was observed in a two-month old Sudanese crossbreed lamb. On physical examination, the abdomen was distended, and there was bulging around the perineal region and severe straining. Upon palpation, the anal ring was felt at the anal area, voiding of faeces and urine from the vaginal opening was seen, while the fistula was felt upon vaginal palpation. Reconstructive surgery by incising the bulged, palpable region was done. The rectum was located and sutured to the perineal wall. The patency of the newly constructed anal opening was however maintained with the use of fabricated syringe barrel. Full recovery was attained within 21 days. The animal was relieved of pressure strain, pain through the surgical intervention with resultant increase in body condition score.

Key words: Atresia ani, fistula, anal, palpation.

INTRODUCTION

Congenital anomalies of gastrointestinal tract occurs among different species of animals with an incidence of about 4.3% (Leipold et al., 1971).

Atresia ani is a developmental anomaly of the newborn which occurs as a result of an autosomal recessive gene (Bademkiran et al., 2009). This condition is characterized by absence of anal opening and may be associated with recto-vaginal fistula, recto-cystic fistula, vagino urethral agenesis, taillessness, hypospadias (Singh et al., 1993) and diphallus (Loynachan et al., 2006). Recto-vaginal fistula or anus vaginalis is an inherited lethal abnormality in which there is an abnormal passage between rectum and vagina; also, faeces are passed through the vagina as a result of the imperforate anus (Oehme and Prier, 1974). Atresia ani associated with recto-vaginal fistula has been reported in many species. These include calves (Shakoor et al., 2012; Mahesh et al., 2014), lambs (Kamalakar et al., 2014, 2015), dogs (Rahal et al., 2007) and pigs (Monsang et al., 2014).

These anomalies are usually noticed at birth whereas in some cases, usually diagnosed at a later age. Early diagnosis of non-lethal anomalies aids in efficient
Table 1. Haematology (complete blood count).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Test results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV (%)</td>
<td>32</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>10.6</td>
</tr>
<tr>
<td>RBC (× 10^{12}/L)</td>
<td>8.3</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>52.1</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33.3</td>
</tr>
<tr>
<td>HCT</td>
<td></td>
</tr>
<tr>
<td>MCH(pg)</td>
<td>16.8</td>
</tr>
<tr>
<td>Platelets(×10^3/L)</td>
<td></td>
</tr>
<tr>
<td>WBC (10^9/L)</td>
<td>6.9</td>
</tr>
<tr>
<td>Neutrophil-Band</td>
<td></td>
</tr>
<tr>
<td>Neutrophil-Mature</td>
<td>31</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>63</td>
</tr>
<tr>
<td>Monocytes</td>
<td>2</td>
</tr>
<tr>
<td>Eosinophil</td>
<td>4</td>
</tr>
<tr>
<td>Basophil</td>
<td>0</td>
</tr>
</tbody>
</table>

All hematological values are within the normal range.

management of the condition.

There are four major types of atresia ani which consists of type I-IV. In type I, a mucosal barrier obstructs the lumen of the intestine. Animals with type II have an intestine with two segments without any communication usually with a fibrous cord joining them together. In type III, two segments of intestine separated completely which may be coiled at the distant end in some animals. Type IV atresia involves multiple site of atresia (Bademkiran et al., 2009; Rahal et al., 2007). Congenital rectovaginal fistula usually associated with type II atresia ani in which the rectum ends as a blind pouch immediately cranial to the imperforated anus (Rahal et al., 2007).

**CASE HISTORY AND CLINICAL EXAMINATION**

A 2-month old female Sudanese crossbred lamb was presented to the Veterinary Teaching Hospital, University of Maiduguri, Nigeria with the complaint of inability to void feaces normally. An examination of perineal region, revealed absence of anal opening, tenesmus, bulging at the anal region and communication between rectal floor and vaginal roof, through which the feaces was voiding out. Based on the prevailing clinical signs, the condition was however diagnosed as congenital atresia ani associated with recto-vaginal fistula.

**Treatment plan**

Complete blood count was conducted so as to ascertain whether the animal is anemic or not; auscultation of the heart and lungs was done to know the status of the cardiopulmonary system, whether or not the animal can withstand both anaesthesia and surgery. Unfortunately, ultrasound scan was not done to know the type of atresia ani we were dealing with and lastly, reconstructive surgery was performed to correct the condition.

**Vital parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory rate</td>
<td>65 breaths/min (60-90)</td>
</tr>
<tr>
<td>Heart rate</td>
<td>120 beats/min (70 - 100)</td>
</tr>
<tr>
<td>Temperature</td>
<td>38.5 (38 - 40) (Table 1)</td>
</tr>
</tbody>
</table>

**Surgical treatment**

The rectum and vagina were evacuated of feaces, the perineum was shaved and prepared for aseptic surgery (Figure 1). Fluid therapy using 5% normal saline was instituted. Anesthesia was effected with xylazine at 0.084 mg i.m. and local infiltration of the perineum using 20% lignocaine hydrochloride. The animal was placed on lateral recumbency and a cruciate skin incision was made on the skin at the bulging area. The rectum was opened and the contents were evacuated. The fistulous orifice, which was about 3 cm in diameter and 2” away from anus, was reached through anal route and was closed using simple interrupted suture pattern with chromic catgut size 0/2 (Figure 2). The area was irrigated with normal saline and rectal mucosa was sutured to the skin.
Figure 1. Black arrow shows the fistula opening between rectum and vagina.

Figure 2. Arrow shows suturing of fistula No. 2 (3.5 metric) using simple interrupted pattern.

Figure 3. Arrow shows syringe barrel.

in simple interrupted pattern using black braided silk. A sterile 20-mL syringe barrel (Figure 3) was cut at non-winged end and two holes were made at the centre of each wing. The non-winged end was lubricated with liquid paraffin and inserted into rectum in other to hold the rectum tightly to the skin, to avoid closure through healing. The wings of barrel were secured to the perineal skin by passing nylon suture material from skin through hole in that side wing and tied to outside using simple interrupted sutures.

Outcome

The animal fully recovered 90 min after the surgery and stood on its entire limbs (Figure 4). After another 90 min, a rising appetite was noticed, hence small quantity of feed (wheat bran) was provided. After 24 h, normal defecation even though softer than normal was noticed, which may be due to the wheat bran.

Post-operative care

Post operatively, 5% dextrose for rehydration, procine penicillin with streptomycin was given at 9 mg/kg × 3/7. Diclofenac sodium was given at 3 mg/kg × 3/7 as an analgesic. The animal was fed wheat bran continuously for 7 days at the Veterinary Hospital so that it could pass out soft feaces and for monitoring, should there be any complication.
DISCUSSION

Atresia usually arises during the embryonic period which results from autosomal recessive gene (Loynachan et al., 2006). Though environmental teratogens, plant toxins and some viruses (Loynachan et al., 2006) are recognised complicating factors in calves. In the present case, the reason could not be ascertained and unspecific as reported by Johnson et al. (1980). The increased faecal pressure may have caused an abnormal opening between rectal wall and vagina forming recto-vaginal fistula and thus causing defeation via vulva (Norrish and Rennie, 1968). Atresia ani is frequently associated with recto-vaginal fistula between dorsal wall of vagina and ventral wall of terminal rectum. A clinical sign is mainly the absence of anal opening; however, while Amith et al. (2017) reported tenesmus, abdominal discomfort in a 5-day old lamb, Prasad et al. (2016) did not report such clinical signs in a 3-day old lamb. Tenesmus, abdominal distension, abdominal discomfort was equally not observed in this present study, which means there are variations in clinical signs regardless of age. Furthermore, radiographs are considered important to determine the position of the fistula and to differentiate the four types of congenital atresia ani (Rahal et al., 2007). However, in our present case, the defects were rectified individually as reported by Rahal et al. (2007).

Conclusion

Surgical intervention (anal reconstruction) is the only possible solution to cope with these congenital anomalies in animals and to make affected animals economically profitable for the keepers. However, it is worthy of note that female animals, especially the Sudaneese breed with these conditions may live up to 2 months or more and still have a good body condition score until corrected.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

A cross-sectional study was conducted to determine the prevalence of mastitis in bovines and to, isolate and identify the major bacterial pathogens of lactating cows in six selected woredas of Bench Maji Zone of Southwestern Ethiopia from June 2017-October 2017. Three hundred eighty four lactating cows were examined for mastitis by combination of udder physical examination, California Mastitis Test and subsequent bacteriological isolation. During the study period, 116 (30.21%) cows had mastitis, of which 35 (30.17%) and 81 (69.83%) showed clinical and subclinical mastitis, respectively. The prevalence rates of mastitis in cross breed and local breed cattle were found to be 71.43 and 28.65%, respectively. Based on parity, higher prevalence (45.45%) was recorded in cows which had greater than 5 parturitions and the lower prevalence (13.04%) was recorded in cows with 1-2 parturitions. Based on lactation stage, the prevalence was (45%) in late stage, followed by middle stage (36.60%) and early stage of lactation (13.85%). The prevalence rates of mastitis based on different age groups of lactating cows were found to be 48.78, 30.54 and 18.52% in cows of greater than 8 years old, 4-8 years old and in cows less than 4 years old, respectively. Except parity, the other associated risk factors (breed, lactation stage and age group) had significant association (P<0.05) with the prevalence of mastitis in the study animals. Upon subsequent bacterial culturing, the quarter milk samples yielded three types of bacteria. *Staphylococcus aureus* (59.26%), *Streptococcus agalactiae* (38.27%) and *Escherichia coli* (2.47%) were the major isolates. In conclusion, the overall prevalence of mastitis in lactating cow of the study area was high and this suggests the need of improved hygienic practices and applies different methods for prevention and strategic control of the disease.

**Key words:** Bacteria, cows, Ethiopia, mastitis, prevalence.

**INTRODUCTION**

Ethiopia is believed to have the largest livestock population in Africa. This livestock sector has been contributing considerable portion to the economy of the country and still promising to rally round the economic...
development of the country. Among livestock, cattle play a significant socio-economic role in the livelihoods of the Ethiopian people. Livestock products (meat, milk, cheese and butter) and by-products supply animal protein that contributes to the improvement of the nutritional status of the people (CSA, 2015). Conversely, low annual per capita consumption of milk in Ethiopia (19 kg) revealed that current milk production in Ethiopia is insufficient to fulfill the requirements due to a multitude of factors (FAO, 2017).

Mastitis can reduces milk yield, increase culling rate, incur treatment cost and occasionally result in death from severe infection. Moreover, mastitis had been known to cause a great deal of loss or reduction of productivity, to influence the quality and quantity of milk yield and to cause culling of animals at an unacceptable age (Radostits et al., 2007). To increase milk production cross breeding of indigenous zebu cattle with exotic breeds particularly Holstein Friesian is widely practiced. This resulted in a larger portion of the dairy cattle population especially in urban areas to be with a high level of exotic blood. However, this market oriented dairy production in many African countries, is subjected to diseases of intensification including mastitis and reproductive disorders (Lemma et al., 2001).

Oviedo-Boyso et al. (2007) and Suriyasathaporn et al. (2000) revealed that mastitis is a multifactorial disease. As such, its incidence depends on exposure to pathogens, effectiveness of udder defense mechanisms and presence of environmental risk factors, as well as interactions between these factors. Seegers et al. (2003) indicated that mastitis has been described as the most common and costly disease in dairy production causing over 38% economic losses due to health problems. Many infectious agents have been identified as cause of mastitis in cattle. The most common organisms being Streptococcus agalactiae and Staphylococcus aureus whereas, environmental mastitis is associated with Coliforms and environmental Streptococci that are frequently found in the cow's environment (Radostits et al., 2000; Quinn et al., 2002; Endale et al., 2016; Jafer et al., 2016; Belay and Tadele, 2017).

It is a serious problem in the dairy industry of Ethiopia (Mekonnen et al., 2005). Bovine mastitis is among the major health problem hindering dairy productivity in Ethiopia, which requires the development of methodologies of control program under the prevailing husbandry system (Fufa et al., 2013). However, the information about prevalence of the disease is inadequate. Such information is important when designing appropriate strategies that would help to reduce its prevalence and effects. From the economic point of view, mastitis especially the-subclinical form causes extensive economic losses that include reduction of milk yield, changes in the milk composition and reduction in milk as well as shortens the productive life span of the affected animals (Radostits et al., 2007).

Mastitis is one of the most important destructive infectious diseases of dairy cattle industry and it is considered of quite vital importance to the public health as it is associated with many zoonotic diseases in which milk acts as a vehicle for the infectious agents. Mastitis not only brings huge economic losses of dairy cow production, but it also cause public health and food safety. The safety of milk with respect to food born disease is a great concern around world this is especially true in developing countries like Ethiopia where the production of milk often take place under unsanitary conditions and consumption of raw milk which is typically produced in small dairy farm under unsatisfactory hygienic conditions is a common practice (FHR, 2006; Teshome and Tesfaye, 2016). Most of the studies in Ethiopia were carried out in Addis Ababa and its surrounding, which may not representative of other regions of the country (Almaw et al., 2009). In Bench Maji Zone, mastitis is commonly observed in dairy cattle. However, scientific data and literature is not available on the current status of mastitis in the targeted area. Therefore, the objectives of this study were to determine the prevalence of mastitis and to isolate and identify the most common bacteria associated with the subclinical and clinical mastitis of cows in study area.

MATERIALS AND METHODS

Description of the study area

This study was conducted in Bench Maji zone (BMZ) of Southwestern part of Ethiopia. The zone is found at a distance of about 561 km from Addis Ababa and 830 km from the regional capital Hawassa. Agro-ecologically, BMZ consists of 52% lowland (<1500 m above sea level (masl)), 43% mid altitude (1500-2300 masl) and 5% highland (>2300 masl). The zone is found at 34°45'–36°10’ east and 5°40’–7°40’ north. The annual average temperature ranges from 15.1 to 27.5°C, while the annual rainfall ranges from 400 to 2,000 mm. The total cattle, sheep and goats population in the zone is about 334,502, 181, 203 and 93,952, respectively (CSA, 2016/2017). The study was conducted in six woredas of the zone namely Sheko, Gurara, Debib Bench, Shey Bench, Semen Bench, Menitgoldia and Maji.

Study animals

The study was conducted on lactating local (indigenous zebu, Sheko) and cross breed cows that were managed under extensive, semi intensive and intensive farming system.

Study design

A cross-sectional study was carried out in June 2017 - October 2017 to investigate the prevalence of mastitis and to isolate and identify the most common bacteria associated with the subclinical and clinical mastitis of cows in study area.

Sampling method and sample size

Out of 10 woredas of the BMZ six woredas were selected purposively based on accessibility for transportation of milk samples.
and population of cattle. From each woreda, two kebeles were selected purposively and the household with at least one lactating cow was involved in the study. From each selected kebele, 32 lactating cows were selected by using simple random sampling method for CMT and bacteriological examinations. The sample size for the study is calculated based on the formula developed by Thrustfield (2007) for random sampling method. A 5% absolute precision and 95% confidence interval was used for determining sample size. Since there is no previous study on the prevalence of mastitis in the study areas, an expected prevalence of 50% was used to determine the maximum sample size.

\[
\frac{(Z_{0.025}^2 \cdot p(1-p))}{S_d^2} \leq \frac{\chi^2}{n} = \frac{(196)^2 \cdot 0.5(1-0.5)}{(0.05)^2} = 384.16
\]

Where, \( P = \) is the expected prevalence, \( S_d = \) is standard deviation (desired absolute precision). \( n = \) the total sample size

Accordingly, the calculated sample sizes was 384 samples.

Clinical examination

Physical examination for evidence of clinical mastitis was conducted in all lactating cows that were sampled in the study area. The udders of the selected cows were examined visually and by palpation for any presence of clinical mastitis. During examination, attention was paid to cardinal signs of inflammation, blindness, injuries, milk clots, symmetry, size, consistency of udder quarters and swelling (Radostits et al., 2007). A cow was considered to have clinical mastitis if it fulfilled at least two of the clinical findings, (1) pain reaction upon palpation, (2) changes in colour and consistency of milk (blood tinged milk, watery secretions, clots, pus) and (3) change in consistency of the udder (Lakew et al., 2009). Cows that did not have clinical mastitis were tested further for sub-clinical mastitis based laboratory investigation.

Milk sample collection and Laboratory investigation

According to Quinn et al. (2002) procedures of mastitis testing, the lactating cows' milk samples were directly collected using universal sample collection bottles. The first 3-4 streams of milk were discarded. The collecting bottle was held as near horizontal as possible and by turning the teat to a near horizontal position and approximately 10 ml of milk were collected into the container. After collection, the sample was labeled and placed in ice box and transported to the Mian Regional Veterinary Diagnostic Laboratory. The analysis was performed within two to three hours after sampling.

California mastitis test (CMT)

The CMT was conducted to diagnose the presence of subclinical mastitis (Quinn et al., 1999). Collected milk samples were poured in to four shallow cups in the CMT paddle and equal amount of CMT reagent was added to each cup and gentle circular motion was applied to the mixture on the horizontal plane. Based on the thickness of the gel formed by CMT reagent and milk mixture, test results were scored as 0 (negative), 1 (weak positive), 2 (distinct positive) and 3 (strong positive). Milk samples with test result of CMT 1 to 3, was classified as evidence of subclinical mastitis (Quinn et al., 1999; Radostits et al., 2007).

Bacterial isolation and identification

Bacteriological study was performed on milk samples from the sepositive CMT reactive and mastitis milk for culture. Identification of mastitis pathogens was carried out following microbiological procedures for diagnosis of bovine udder infection described in Quinn et al. (1999). One standard loop (0.01 ml) of milk was streaked on 7% blood agar. The inoculated plate was incubated aerobically at 37°C. The plates were checked for growth after 24, 48 and up to 72 h to rule out slow growing bacteria species. A milk sample was considered positive for mastitis pathogens if at least single colonies of a potential pathogen were detected and the positives were identified by biochemical tests. For primary identification, size, shape, color, hemolytic characteristics, Gram's reaction and catalase production was used. For confirmation, biochemical tests were used after sub culturing isolated distinct colony on selective media. MacConkey agar (Oxoid) and Edward's agar (Oxoid) were used to detect the most aerobic pathogens, enteric bacteria and Streptococci, respectively. Primary identification of Staphylococci was based on colony morphology, catalase test. Gram-staining morphology and differentiated from micrococi on the basis of the oxidative fermentative (OF) test carried out on semi-solid OF medium(Difco, Becton, Dickinson and Company, Franklin Lakes, NJ,USA). The Staphylococci were also tested for production of coagulase enzyme by the tube method as described by Quinn et al. (1994).

Isolates that produced Gram-positive cocci in clusters, and were catalase positive, glucose-fermentative, resistant to bacitracin and did not produce coagulase were identified as coagulase-negative staphylococci (CNS). S. aureus isolates were differentiated from other coagulase-positive staphylococci on the basis of mannitol fermentation on mannitol salt agar (Oxoid). The enteric bacteria were identified using colony morphology, oxidase test. Lactose fermentation on MacConkey agar (Oxoid), indole production test, citrate utilization Quinn et al. (1999). Interpretation was made according to NMC (1990). The culture was considered negative if no growth occurs after 72 h of incubation and plates showing mixed and confluent growths, with no evidence of single discernible colonies, were not investigated further.

Data analysis

Data collected from the laboratory test and the questionnaire survey was recorded and coded in Microsoft excel spread sheets 2010 and analyzed using statistical data analysis of SAS version 9.10. The prevalence of mastitis was calculated as the number in study population testing positive divided by the total study units tested. The Chi-square (\( \chi^2 \)) test was applied to determine existence of any association between the laboratory test positivity and the associated risk factors (such as breed type, parity, lactation stage and age of milking cows). For all analysis, a P-value of less than 0.05 was taken as significant.

RESULTS AND DISCUSSION

Prevalence of mastitis

The overall prevalence of mastitis of cow level in the study areas were tested by using CMT and clinical inspection of the udder (Table 1). From the total 384 lactating cows examined during the study period, 116 (30.21%) cows had mastitis, of which 35 (30.17%) and 81 (69.83%) showed clinical and subclinical mastitis, respectively. In the current study, the clinical and
Table 1. Clinical and subclinical mastitis in lactating cows in study area (n=384).

<table>
<thead>
<tr>
<th>Type of mastitis</th>
<th>No. of cow examined</th>
<th>Positive</th>
<th>%</th>
<th>Culture positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical mastitis</td>
<td>384</td>
<td>35</td>
<td>30.17</td>
<td>35</td>
<td>100</td>
</tr>
<tr>
<td>Sub-clinical mastitis</td>
<td>384</td>
<td>81</td>
<td>69.83</td>
<td>79</td>
<td>97.53</td>
</tr>
<tr>
<td>Total</td>
<td>384</td>
<td>116</td>
<td>30.21</td>
<td>114</td>
<td>98.28</td>
</tr>
</tbody>
</table>

n= number of lactating cows.

Table 2. The prevalence of mastitis in association with potential risk factors in the study areas.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total number of examined cows</th>
<th>CMT positive</th>
<th>Prevalence (%)</th>
<th>X²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breed type</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Cross breed</td>
<td>14 (3.65%)</td>
<td>10</td>
<td>71.43</td>
<td>21.9429</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>(ii) Local breed</td>
<td>370 (96.35%)</td>
<td>106</td>
<td>28.65</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) 1-2</td>
<td>161 (41.93%)</td>
<td>21</td>
<td>13.04</td>
<td>44.82</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(ii) 3-4</td>
<td>146 (38.02%)</td>
<td>60</td>
<td>41.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(iii) ≥5</td>
<td>77 (20.05%)</td>
<td>35</td>
<td>45.45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactation stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) Early Lactation</td>
<td>130 (33.85%)</td>
<td>18</td>
<td>13.85</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(ii) Mid Lactation</td>
<td>194 (50.52%)</td>
<td>71</td>
<td>36.60</td>
<td>38.99</td>
<td></td>
</tr>
<tr>
<td>(iii) Late Lactation</td>
<td>60 (15.63%)</td>
<td>27</td>
<td>45.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(i) &lt;4 years</td>
<td>135 (35.16%)</td>
<td>25</td>
<td>18.52</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(ii) 4-8 years</td>
<td>167 (43.49%)</td>
<td>51</td>
<td>30.54</td>
<td>73.20</td>
<td></td>
</tr>
<tr>
<td>(iii) &gt;8 years</td>
<td>82 (21.35%)</td>
<td>40</td>
<td>48.78</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The potential associated risk factors

Breed, parity, lactation stages and age had significant influence on the prevalence of bovine mastitis (P<0.05). The result showed that the prevalence of mastitis was significantly higher in cross breed (71.43%) than local breed of cows (28.65%) (Table 2). The effect of cross breed on the current prevalence of mastitis was relatively comparable with the reports of Jafer et al. (2016) in Dire Dawa city (71.1%). Compared to present study results, Belay and Tadele (2017) reported the lower prevalence in cross breeds (58.46%) and higher prevalence (38.2%) in local breeds in HoroGuduru Wollega Zone. In Ethiopia, many studies showed statistically significant difference in mastitis between local and cross breeds. Furthermore, cows with high milk yield is more susceptible to mastitis whereas low-yielding cows tend to be more resistant (Biffa et al., 2005; Mekibib et al., 2010; Megersa et al., 2012; Moges et al., 2012). This may be due to genetic improvement for milk yield is accompanied by gradual decline in genetic resistance to mastitis (Radostits et al., 2008). Parity also showed an effect on the occurrence of mastitis. Higher prevalence (45.45%) was recorded in cows multiparous (greater than 5 parturition) and the lower prevalence (13.04%) was recorded in cows with first and second parity. Similarly, Alebachew and Alemu (2015) reported the higher prevalence (90.8%) in cows with subclinical mastitis examined was higher than that reported by Alebachew and Alemu (2015) who found 21.2% clinical and 46.8% subclinical mastitis in selected commercial dairy farms in Addis Ababa. Jafer et al. (2016) also reported the minimum clinical mastitis (15.27%) and maximum subclinical mastitis (84.73%) in dairy farm of Dire Dawa City.

The present study showed an overall prevalence of 30.21% lower than that of Biffa et al. (2005) in and around Addis Ababa and Getahun (2006) in Haramaya who reported 38.9 and 36.9%, respectively. The difference in results could be due to variations in the distribution of mastitis risk, laboratory techniques, study design, climate, the level of management and animals studied. As indicated in the Table 1, all CMT positive samples were cultured for etiological agent identification. From 81 samples cultured, 79 were positive for known subclinical mastitis pathogens while all of the samples cultured from clinical mastitis were positive for mastitis.
with 4-7 parurition and the lower prevalence (61.6%) in cows with 1-3 parurition in Addis Ababa.

Lactation stage had association with the occurrence of mastitis where the prevalence was higher (45%) in late stage, followed by middle (36.60%) and early stages of lactation (13.85%). Belay and Tadele (2017) reported that the similar results based on the stage of lactation which was 34.21, 38.24 and 56.1% in the 1st, 2nd and 3rd trimester of lactation, respectively. The high prevalence of mastitis at late lactation might be due to an increased period of exposure of the udder during previous stages of early and mid-lactations.

There was a significant difference in prevalence between animals of different age of lactating cows (P<0.05). The highest prevalence (48.78%) was found in lactating cows of ages greater than 8 years, followed by cows of ages 4-8 years (30.54%) and the lowest prevalence (18.52%) was recorded in cows of ages less than 4 years. Correspondingly, Belay and Tadele (2017) reported that the highest prevalence (61.16%) of older cows (>9.5 years), followed by cows age 6-9.5 years (36.96%) and the lowest prevalence (34.15%) by cows age of 2.5-6 years. The high prevalence of the mastitis revealed in older animals might be due to the physiology of exhausted canal which is more dilated and remains partially open due to years of repeated milking. This facilitates the entrance of environmental and skin-associated microorganisms leading clinical or sub clinical mastitis. Blowey and Edmondson (2010) also reported the high occurrence of mastitis in older aged cows compared to young and adult cattle. This could be due to damage of teat canals in old animals facilitates access of bacteria into the mammary gland.

Identified and isolated major pathogens

As shown in Table 3, milk samples collected from 116 mastitis positive cows (35 clinical cows and 81 CMT positive subclinical cows) were cultured on blood agar. The dominant bacteria isolated were *Staphylococcus aureus* species followed by *Streptococcus* species and other Gram negative enteric bacteria, of which *Escherichia coli*. By using further biochemical tests and selective media, three major strains of pathogenic bacteria namely *S. aureus*, *S. Agalactiae* and *E. coli* were found. Among three major pathogenic bacteria, *S. aureus* was the highest prevalent organism (59.26%); followed by *S. Agalactiae* (38.27%) and *E. coli* (24.7%).

Table 3. The identified and isolated major pathogenic bacteria species in the areas.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Isolated bacteria</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Escherichia coli</em></td>
<td>2</td>
<td>2.47</td>
</tr>
<tr>
<td>2</td>
<td><em>Staphylococcus aureus</em></td>
<td>48</td>
<td>59.26</td>
</tr>
<tr>
<td>3</td>
<td><em>Streptococcus agalactiae</em></td>
<td>31</td>
<td>38.27</td>
</tr>
</tbody>
</table>

The prevalence of *S. aureus* in the present study was higher (59.26%) than early findings of Milne et al. (2002); Fufa et al. (2013) and Jafer et al. (2016) who reported 44.4% in Sebeta, 21.13% in Addis Ababa city and 48.4% in Dire Dawa, respectively. Likewise, this finding was disagreeing with the report of Bitew et al. (2010), Biruке and Shimeles (2015) who reported 20.3% in Bahir Dar and 45.1% in Addis Ababa, respectively. Present findings are comparable with the results of Endale et al. (2016) who reported 57.14% *Staphylococcus* species and 28.57% *Streptococcus* species in and around Sodo Town, Wolaita Zone, Ethiopia. The relative high prevalence of *S. aureus* in this study could be associated with the absence of post milking teat dipping, poor udder and teat washing before milking, poor hand milking practice and wide distribution of the organism inside the mammary gland and on the skin of teat and udder.

Available literature also showed that *Staphylococcus* species causing mastitis is the common and economically the greatest concern wherever dairy farming is practiced (Workineh et al., 2002; Fufa et al., 2013; Jafer et al., 2016). *S. aureus* has adopted to survive in the udder and established chronic and subclinical infection (Radostits et al., 1994). The isolation of *S. aureus* is of public health significance since it is a commonly recovered pathogen in outbreaks of food poising due to milk and milk product. This could be due to *S. aureus* is environmentally robust, surviving wide extremes of temperature and moisture. *S. agalactiae* (38.27%) was the second major pathogenic isolated in the study areas. This result was higher than the early findings of Kerro and Tareke (2003); Almaw (2004) and Bitew et al. (2010) who reported isolation rates of 13.1, 8.15 and 13.9%, respectively.

The justification given for *S. aureus* could also be a factor for *S. agalactiae* relative high isolation rate since both of them are contagious pathogens. The isolation of *streptococcus* species is of public health significance as it causes various gastrointestinal upset ranging from abdominal pain to diarrhea. Generally, the present study showed that contagious mastitis pathogens were the predominant isolated bacteria. This might be due to lack of effective udder and teat washing and drying, inter-cow hand washing and poor cleaning of milking area. Contamination of milkers’ hands, cloths and milking utensils leads to high spread of mastitis disease.

The present result also indicated that *E. coli* was the third predominant pathogens (24.7%) isolated in the study areas. This finding was much lower than the early findings of Iqbal et al. (2004) and Biruke and Shimeles (2015) who reported 18.6 and 40.7%, respectively.

However, it is comparable with the previous reports of Mekhibib et al. (2010) at Holeta (4.6%) and Sori et al. (2005) in and around Sebeta (0.75%). The prevalence of *E. coli* is probably due to the fact that *E. coli* is the commonest environmental contaminants which are
closely associated with hygiene. It becomes pathogenic whenever the hygienic conditions of the animal or environment become poor. In addition, the existence of high concentration of \textit{E. coli} in milk also indicates the relatively poor quality of milk, related with substandard hygiene of the farm management.

CONCLUSION AND RECOMMENDATIONS

The overall 30.21\% prevalence of mastitis at cow level was tested by using California Mastitis Test (CMT) and clinical inspection of the udder. Breed, parity, lactation stages and age have a significant influence on the prevalence of bovine mastitis (P<0.05). Increasing age, lactation stage, parity and poor management increased the risk of mastitis. The major pathogenic strains isolated were: \textit{S. aureus} (59.26\%), \textit{S. agalactiae} (38.27\%) and \textit{E. coli} (2.47\%). This indicates that mastitis caused by \textit{S. aureus} is one of the major problems of dairy cows in milk production followed by \textit{S. agalactiae}. The distribution of these bacterial pathogens in the herd indicates the economic impact of the disease. Beside the disease has economic importance it also harm the health and well-being of human being. The professionals should apply different methods for prevention and strategic control of the disease and should be informed to the public about the relevance of pasteurization of milk before consumption to avoid food born infection and intoxication. There is a need of further study on drugs to which the bacterial are sensitive to use it as primary choice to treat the disease in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES


Full Length Research Paper

Common health and welfare problems of working donkeys in Addis Ababa and its surrounding area: Retrospective and questionnaire survey

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A retrospective study was conducted to assess the health and welfare problems of working donkeys at the Merkato Donkey Sanctuary clinic in Addis Ababa, Ethiopia. Data on 12,991 working donkeys from 2008 to 2010 were analyzed from the data record sheet kept by the clinic. Results on the age distribution of donkeys showed that the average age of the donkeys was around nine years old. Only 7.37% of the donkeys were with good or ideal body condition (≤2.5), while the majority, 90.56% were with poor body condition (≤2). Multiple health problems were a common feature. Among the common health problems encountered parasitosis, hoof problems, wounds, musculoskeletal disorders, colic, ophthalmic cases and traffic accidents accounted for 55.68, 16.71, 14.9, 3.28, 2.59, 2.37, and 1.71%, respectively. Wounds due to ill fitted harnesses and inappropriate harnessing materials accounted for majority of the wounds observed. Back sore due to the absence of paddings, improper padding, inadequate and inappropriate padding materials was a common feature, and accounted for 62.6% of the overall different wounds. Hoof overgrowth and solar puncture and/or abscess due to sharp objects were the common hoof problems recorded, while hoof overgrowth accounts for 13.56%. Traffic accidents leading to death and injuries were common problems. Apart from the health related welfare problems, the questionnaire survey made also revealed that abuses and negligence by the owners, management constraints like overloading and overworking, beating, and shortage of feed, housing problems, wounds due to harnessing and physical injuries mainly due to traffic accidents were the major welfare problems of urban working donkeys. The retrospective study, the questionnaire survey and observations made provided the significant health and welfare problems of working donkeys that need to be addressed in order to improve their health and working efficiency.

Key words: Retrospective study; working donkey; health and welfare problems.

INTRODUCTION

World domesticated equines (horses, donkeys, and mules) population is 115.2 million consisting of 44.3 million donkeys. Global distribution indicated that 98% of all donkeys are found in developing countries (Jannke, 1983). Ethiopia having 5.2 million donkeys is the second in donkey population in the world and first in Africa...
possessing nearly 40% of African donkey population. According to the present regional classification of Ethiopia 97% of donkey population is found in three regions: 44% in Oromia, 34% in Amahara and 19% in Tigray regional state (Feseha, 1998).

In Ethiopia they have been used as beasts of burden for a long time and still render their valuable services mostly as pack animals throughout the country, particularly in areas where modern means of transportation are absent, unaffordable or inaccessible (Getachew et al., 2002). Donkeys have been completely neglected and omitted from the national livestock development programs. This is because the contribution of donkey's power in the agricultural systems and their role in production are not yet well recognized and magnified (Fielding, 1987). The treatment services provided to these species of animals have been far below that is given to other species of animals; this can be due to age old erroneous concept that when donkeys do get sick they are quick to die and probably because they are not provider of meat and milk (Yoseph et al., 2001).

Due to minimum management attention is given to donkeys, particularly in countries like Ethiopia; they are exposed to some diseases. Donkeys in Ethiopia are subjected to numerous health and welfare problems. These include: polyparasitism, back sores, and other harness inflicted wounds, hoof problems, ophthalmic problems, colic, car accident, overloading and overworking, and various infectious diseases such as strangles and Tetanus. Donkey Sanctuary-Donkey Health and Welfare project (DS-DHWP) in Ethiopia has been providing veterinary, education and extension services since its inception in 1994. It opened its second stationary clinic in December 2007 in Addis Ababa, Merkato grain market, where more than 5000 donkeys are giving their pack transport services to the urban communities. The clinic provides veterinary services to many health and management problems of working donkeys. The health and welfare problems of these working donkeys are not well documented and readily available. Therefore, the primary objective of the present study was to identify the common health, welfare, and management problems of working donkeys in Addis Ababa-Ethiopia, Merkato grain market.

MATERIALS AND METHODS

Study area

The study was conducted in Addis Ababa, Merkato Donkey Sanctuary (DS) clinic. Donkeys are coming to get treatments from within Addis Ababa and the surrounding areas of Sululta and Gefeersa. Merkato is known for its high donkey population used by limited resource communities for their livelihood. Addis Ababa located at 9°22’N, 38°04’E with an altitude of about 2400 m above sea level, Addis Ababa city possesses a complex mix of highland climates zones with temperature differences of up to 10°C depending of elevation and prevailing wind patterns, high elevation moderate temperature year round, and the city’s position near the equator mean that temperatures are constant from month to month. Addis Ababa receives a mean annual rainfall of 1800 mm in bimodal pattern. The long rainy season extends from June to September followed by a dry season ranging from October to February; the short rainy season lasts from March to May. The average minimum and maximum temperature of Addis Ababa is 10.7 and 23.6°C, respectively (NMSA, 2005).

Study animals

The study animals were working donkeys in Addis Ababa, and the surrounding areas of Sululta and Gefeersa coming to the Merkato DS clinic for treatment against different health problems. Most of the donkeys were from the Merkato grain market and some from the different sub-cities of Addis Ababa.

Study design

Retrospective studies

Data on the different health, management and welfare problems of working donkeys that came to the Merkato DS clinic for treatment were stored either electronically or on a data record sheet as a hard copy since 2008. Data from daily clinical record formats/sheet were entered into Microsoft Excel spread sheet. The data were coded into categories of clinical findings for each donkey. The categories were defined according to the systems affected, types of the problem and the cause of the problems. A total of 12,991 records of donkeys from 2008 to 2010 were extracted and organized for further analysis.

Questionnaire survey

A structured questionnaire was designed and validated to cover a wide range of socio-economic aspects including the number of donkeys owned, size at house hold levels, uses, family income through the use of donkeys, frequency and magnitude of work, nutrition and management of donkeys, health and welfare constraints and significant causes of abandoning working donkeys. The questionnaire was randomly administered to donkey owners, drivers coming to the Merkato clinic to collect relevant information about working donkey's welfare issues in the study area. For the purpose, a total of 71 donkey owners were interviewed.

Data analysis

Descriptive statistics for the common health problems of working donkeys were calculated using Minitab statistical software. Graphs and tables were produced using Microsoft Excel program.

RESULTS

Retrospective study

The data record format showed that some donkeys were coming to the clinic to get treatments against different health problems (Figure 1). The study made showed that 96.3% of the donkeys in and around Addis Ababa used for work were adults greater than four years old. However, more than 3% of the donkeys were less than three years of age. The average age of the donkeys was
nine years old. Age distribution of the donkeys is shown in Figure 2. The frequency distribution of the body condition score of the donkeys is shown in Figure 3. More than 90% of the donkeys were with poor body condition score (≤2), while it is only 7% of the donkeys had well to ideal (≥2.5) body condition. Further analysis of the data revealed that donkeys were suffering from multiple health and welfare problems. The common health problems of working donkeys are summarized in Table 1.

Parasitosis, Wounds, hoof problems, musculoskeletal disorders, colic, ophthalmic problems and traffic accidents were the major problems encountered in working donkeys during the year 2008-2010 (Table 1). Wounds due to the use of inappropriate harnessing materials, absences of padding for the back and ill fitted harnessing materials were the primary cause of wounds and sores. The most common wound was back sore (Figure 4). Among hoof problems, hoof overgrowth and
Figure 3. Frequency distribution of body condition of working donkeys treated at the Merkato DS clinic in Addis Ababa during the year 2008-2010, Ethiopia.

Table 1. Common health problems of working donkeys diagnosed and treated at Merkato DS clinic in Addis Ababa (n=12,991).

<table>
<thead>
<tr>
<th>Cases attended</th>
<th>Number of cases</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back sores</td>
<td>1195</td>
<td>9.2</td>
</tr>
<tr>
<td>Other wounds</td>
<td>734</td>
<td>5.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>1929</strong></td>
<td><strong>14.9</strong></td>
</tr>
<tr>
<td>Colic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Due to Foreign bodies</td>
<td>154</td>
<td>1.2</td>
</tr>
<tr>
<td>Parasitic</td>
<td>38</td>
<td>0.29</td>
</tr>
<tr>
<td>Impaction</td>
<td>10</td>
<td>0.08</td>
</tr>
<tr>
<td>Unknown causes</td>
<td>132</td>
<td>1.02</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>338</strong></td>
<td><strong>2.59</strong></td>
</tr>
<tr>
<td>Hoof problems</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hoof overgrowth</td>
<td>1762</td>
<td>13.56</td>
</tr>
<tr>
<td>Solar puncture/abscess</td>
<td>268</td>
<td>2.08</td>
</tr>
<tr>
<td>Hoof thrush</td>
<td>141</td>
<td>1.09</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2171</strong></td>
<td><strong>16.71</strong></td>
</tr>
<tr>
<td>Musculoskeletal disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanical injury</td>
<td>41</td>
<td>0.32</td>
</tr>
<tr>
<td>Muscular strain</td>
<td>385</td>
<td>2.96</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>426</strong></td>
<td><strong>3.28</strong></td>
</tr>
<tr>
<td>Ophthalmic cases</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mechanical injury</td>
<td>222</td>
<td>1.71</td>
</tr>
</tbody>
</table>
Table 1. Contd.

<table>
<thead>
<tr>
<th>Cause</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unknown cause</td>
<td>86</td>
<td>0.66</td>
</tr>
<tr>
<td>Total</td>
<td>308</td>
<td>2.37</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respiratory problems</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Drenching pneumonia</td>
<td>18</td>
<td>0.14</td>
</tr>
<tr>
<td>Suspected strangles</td>
<td>9</td>
<td>0.07</td>
</tr>
<tr>
<td>GIT Parasites</td>
<td>59</td>
<td>0.45</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>0.66</td>
</tr>
<tr>
<td>Parasitosis</td>
<td>7234</td>
<td>55.68</td>
</tr>
<tr>
<td>Traffic accidents</td>
<td>183</td>
<td>1.41</td>
</tr>
<tr>
<td>Dental problems</td>
<td>89</td>
<td>0.69</td>
</tr>
<tr>
<td>Infectious cases</td>
<td>49</td>
<td>0.38</td>
</tr>
<tr>
<td>Tumours</td>
<td>55</td>
<td>0.42</td>
</tr>
<tr>
<td>Rectal prolapse</td>
<td>23</td>
<td>0.18</td>
</tr>
<tr>
<td>Suspected anthrax</td>
<td>30</td>
<td>0.23</td>
</tr>
<tr>
<td>Suspected rabies</td>
<td>11</td>
<td>0.08</td>
</tr>
<tr>
<td>Tetanus</td>
<td>10</td>
<td>0.08</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>35</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Figure 4. Common wound problems of working donkeys treated at the Merkato DS clinic during the years 2008-2010 (n=1945).

puncture wounds leading to solar abscess and lameness were common (Figure 5).

Questionnaire survey

Ownership, owners’ education back ground and working condition of donkeys:

Questionnaire survey showed that 83% of the owners get 60-100% of their income from the work of their donkeys. Over 90% of the donkey owners were more than 25 years old ranging from 15-85 years. Education background of the donkey owners showed 56% were illiterate and only 31 and 14% attended elementary and secondary school level, respectively. Ownership of the donkeys showed that more than 83% had 2 to 3 donkeys, and the average number of donkeys per owner was 3. Eighty seven percent of the owners reported that the average weight they load their donkeys was 85 kg (50-130 kg), travelling 7 h per day covering an average distance of 20 km. Seventy six percent of the owners use
their donkeys 2-5 days per week covering a distance of 2.5-35 km.

**Water, feed, feeding and housing practices**

Almost all donkeys in urban areas depend on hand feeding since grazing is quite scarce. Over 83% of the owners reported that they supplement their animals with ‘kortebe’, the local name for a mixture of different cereals/grain leftovers while other provides grains and straws/grass. Over 66% of the owners reported that they feed their donkey only once a day, while 33% feed twice a day. Majority of the owners provide water twice a day. Most of the donkeys spend day time working, and have a limited time roaming around and/or grazing, if available. Over 91% of the owners reported that donkeys are housed at night separately in purposely built enclosures.

**Health and management problems**

Ninety five percent of the owners reported parasitosis as a significant disease problem incriminated in reducing the working performance of their donkeys. Hoof problems and wound/traumatic injuries were ranked second and third major essential health problems, respectively. The owners do not practice castration of male donkeys because they believe that castration reduces working capacity and life span of their donkeys. From the interviews and observation made, health care given to the animals is negligible and more than 70% of the owners practice traditional methods of treatment when their donkeys get sick. Drenching herbal plants mixed with some spices and alcohol (Areke), the use of engine oil for wound treatment, and branding for lameness cases related to musculoskeletal problems are common practices.

**DISCUSSION**

The present retrospective study has revealed multiple health, management and welfare problems of working donkeys in and around Addis Ababa. Although the majority of the donkeys were adults, over four years of age, the 3% of donkeys below three years of age indicate that donkey owners begin to use donkeys for work before they are mature enough. Age at maturity of donkeys is estimated at four years and it is recommended not to work with them until this age as this predisposes them to structural deformities such as sagged (lordosis) back and early demise. Although good to ideal body condition was observed in 7% of the population, the majority of the donkeys were with poor body condition (bcs<2). Donkeys studied were, on the whole, in poor body condition with a mean/median body condition of 2. This clearly shows that apart from health and management related problems, shortage of feed may be a major contributory factor for poor body condition of the donkeys the result obtained by a questionnaire survey, they have low priority regarding access to good quality feed. Pearson et al. (2001), similar finding. Such poor body condition might be one of the contributory factors for the high incidence of back sore, due to less muscle cover.
A large proportion of donkeys were seen with various degrees of wounds and abrasions. These results were consistent with the finding of similar works done by Mohammed (1991), Pearson et al. (2001) Demelash and Moges (2006) in Ethiopia, Rodriguez-Maldonado (1990) in Mexico, Harris (1971) in UK. The major causes of wounds and injuries were harness related problems due to inappropriate harnessing material, ill-fitted harnesses and the absence of padding on the back of the animals. Abnormalities in locomotion incident to joint sprains and unknown causes suggested that donkeys were overloaded, leading to tendon damage.

The observed high incidence of hoof problems could have resulted from lack of proper hoof trimming and care due to the lack of veterinary services (Soliman, 1989). Similar hoof problems were reported by Getachew et al. (2002a) and Yilma et al. (1991). The finding of questionnaire survey also reflects this problem, the high incidence of hoof overgrowth and lameness in the present study is not consistent with the findings of Getachew et al. (2002) in rural donkeys. The travelling of donkeys in a narrow lane in the city might have predisposed them to hoof puncture with sharp objects such as nails and broken glasses, which are quite common in such urban areas. Traffic accidents also played a significant role in causing hoof problems leading to lameness. According to the study made by Morgan (2006), lameness related to foot problems due to traffic trauma was a common problem in urban donkeys that is consistent with current finding.

Wounds from donkey bites are results of fighting between males donkey, as the practice of castrating donkey is not known in the areas, and this may lead to aggressive behavior to each other. The low incidence of hobble wounds in this study indicates that hobbling donkeys is not a common practice in urban donkeys as compared to rural donkeys, which is one of the common causes of injury and abrasion (Feseha, 1997; Getachew et al., 2002b). Both the retrospective study and questionnaire survey showed that traffic accidents are quite common in urban working donkeys. The present 1.4% traffic accident cases were those lucky ones with treatable injuries and able to recover. However, according to the clinician and our observation, the majority of traffic accidents result in death and serious structural damage, which necessitate euthanasia. Study made by Getachew et al. (2002) reported traffic accident as one of the major problems in working donkeys living in the urban setups and by the road sides, which frequently cross roads on their way to graze or home. The fact that donkeys carrying heavy loads and sharing the same road in the urban setup with vehicles predisposes them to the accident.

Colic cases due to the ingestion of a foreign body, particularly plastic materials were found to be one of the significant gastrointestinal problems, which are consistent with the finding of Getachew et al. (2002). Apart from ingestion of foreign bodies, impaction due to ingestion of excessive coarse, dry and high fiber feeds such as the fine residue of teff (Eragosteris abyssinica) is a frequently encounter cases of colic in rural donkeys during the harvesting and threshing seasons (Getachew, et al., 2002).

The present finding of respiratory problems is consistent with the reports by Feseha (1997), Pearson et al. (2001) and Getachew et al. (2002). Majority of the respiratory problems were due to drenching of herbal remedies to treat other problems, particularly colic cases. The common use of traditional herbal remedies from the reflection of those. The finding of cases of rabies, anthrax, tetanus, strangles, rectal prolapse and tumour in this study was consistent with the results of the study made by Getachew (1999), Pearson et al. (2002), Getachew et al. (2002) and Ayele et al. (2006).

CONCLUSION AND RECOMMENDATIONS

The present retrospective study and questionnaire survey revealed that there are a significant health, management and welfare problems of working donkeys in the urban and per-urban setup of Addis Ababa. The study has shown that majority of the problems were due to mismanagement, neglect and cruelty which could easily be preventable, however, deliberate maltreatment is rare and health problems of donkeys are more likely due to ignorance. The ubiquitous nature of the problems may result in donkey owners becoming indifferent or being unaware that anything is wrong. The main reasons for the mismanagement and ill-treatment of donkeys could be many folds:

(i) The weak economy of the owners
(ii) Lack of education and training
(iii) Lack of material essential drugs
(iv) Lack of professional advice
(v) The perception by the people that donkeys do not get ill or can tolerate problems may also play a significant role.
(vi) Fundamental lack of understanding of the potential productivity of their animals giving the correct care by the owners.

The health, management and welfare problems of donkeys can be improved and controlled, and the increased use of donkeys can be enhanced:

(vii) Proper veterinary care and advice
(viii) Through the development and dissemination of proper harnesses to control wounds and sores.
(ix) Through better education and training of both professionals and donkey owners as to the primary health care, management and welfare problems of donkeys.
(x) When the donkey is seen by the whole community as an animal for collective benefit, and not just for the
CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Distribution and molecular characterization of avian hepatitis E virus (aHEV) in domestic and wild birds in Burkina Faso

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Avian hepatitis E virus (aHEV), clinically important in poultry industry, can cause death and reduce egg production of chickens, resulting in significant economic losses in the poultry industry. However, little is known about this aHEV infection in Burkina Faso. This study presents the results of distribution and characterization of aHEV in domestic and wild birds without clinical disease. In total 173 birds liver samples were collected from four Burkina Faso provinces, between February 2015 and June 2016. Reverse transcription polymerase chain reaction (RT-PCR) with aHEV specific degenerate primers was used to screen the presence of aHEV. RNA of aHEV was detected in 29 (16.8%) liver samples. Of these, the prevalence was diverse in different species of birds; the most frequent level was 35.3% in Numida meleagris, respectively followed by 23.5% in Gallus gallus domesticus, 13.3% in Streptopelia turtur, 13.3% in Columba livia, 6.7% in Anas platyrhynchos and 3.3% in Pternistis natalensis. The present study firstly revealed the prevalence of HEV infection in six species of birds in Burkina. It is therefore important to conduct further research on the impact on poultry mortality and egg production in our country.

Key words: Avian hepatitis E virus, zoonosis, birds, prevalence, Burkina Faso.

INTRODUCTION

Hepatitis E virus (HEV), known to have zoonotic potential (Pavio et al., 2010), is transmitted enterically, mainly

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through the consumption of contaminated food or water (Yugo and Meng, 2013). HEV is the causative agent of a self-limiting acute hepatitis, ranges from an asymptomatic to a severe course, as described in immune-compromised patients and pregnant women (Purdy and Sue, 2017; Zuin et al., 2017). The severity in pregnant women is reflected in a mortality rate reaching up to 10 to 30% compared with 0.5 to 4.0% in young adults (Ward et al., 2011). HEV is divided into two genera: Orthohepevirus with four species (A–D) and Fischepevirus with one species (Spahr et al., 2018). Orthohepevirus A has at least 8 recognized genotypes of mammalian HEV. Orthohepevirus B consists of avian viruses and is divided into four proposed subtypes (I–IV) associated with geographical distribution (Sridhar et al., 2017).

Avian Hepatitis E virus (aHEV) was first isolated from chickens with big liver and spleen disease (BLSD) or hepatitis-splenomegaly (HS) syndrome. Phylogenetic analysis of the full or nearly complete genome of aHEV strains identified four different genotypes and showed a distant relationship to mammalian and swine HEVs (50 to 60% nucleotide sequence identity) (Smith et al., 2015). The aHEV genotype 1 has been described in Australia and Korea, genotype 2 in USA, genotype 3 in Europe and China, and more recently, genotype 4 in Hungary and Taiwan (Payne et al., 1999; Park et al., 2015; Wang et al., 2015; Moon et al., 2016; Zhang et al., 2016).

HEV infections are completely asymptomatic in many animal species, but it seems to have some pathogenic importance for chickens (Yugo et al., 2016). Besides the enlargement of spleen and liver, both ovarian regression and presence of serosanguinous abdominal fluid or clotted blood in the abdomen are commonly associated with the HS syndrome (Ritchie and Riddell, 1991; Payne et al., 1999; Haqshenas et al., 2001; Thiry et al., 2017). The disease mainly causes a decrease in egg production and an increase in mortality in birds (Sun et al., 2004; Peralta et al., 2009). However, aHEV can be detected in birds without symptoms as well (Yugo et al., 2016; Zhang et al., 2016; Zhang et al., 2017). The virus appears to spread easily within and between flocks via the fecal-oral route transmission (Yugo et al., 2016). Other routes of transmission, including aerosol, vertical, vector-borne, or mechanical carrier, have not been demonstrated in natural or experimental avian models (Meng, 2011).

Based on serological evidence, it appears that avian HEV is widespread in chicken flocks with seropositive rates of approximately 71% in the United States, 90% in Spain, 20% in Brazil and 57% in Korea (Kwon et al., 2012). The overall detection rate of avian HEV RNA in fecal samples was 62.9% in the United States (Gerber et al., 2015).

Human infection with aHEV has not been observed up to now as it was for swine HEV (Meng, 2010). However, aHEV exposure of human population have largely increase in relationship to the consumption of contaminated poultry eggs and meat, the use of poultry viscera as a culinary delicacy, and the handling of poultry (Hsu and Tsai, 2014). In addition to the already described capacity of the virus to recognize human hepatocyte (Hsu and Tsai, 2014), the existence of a yet unknown aHEV variant able to enter and infect human liver may have a critical public health implication in the future.

In West Africa, the status of avian HEV infection in chickens is largely unknown. Considering that aHEV infection is most prevalent and dangerous among birds, it is imperative to access the contribution of aHEV to poultry and wildlife in Burkina Faso. The aim of the present study were to determine the possible circulation of avian HEV both in domestic and wild birds without clinical symptoms in Burkina Faso.

MATERIALS AND METHODS

Sample collection

In total, 173 samples of different symptomless bird flocks (4 domestic bird species) or hunted animals (2 wild bird species) currently in food chain in Burkina, were collected between February 2015 and June 2016 from four Burkina Faso district (Figure 1): 34 Guinea fowls (Numida meleagris), 34 chicken (Gallus gallus domesticus), 30 mallards (Anas platyrhynchos), and 15 doves (Columba livia) for domestic flocks and 30 turtle dove (Streptopelia turtur), 30 natal francolins (Pternistis natalis) hunted in the hunting areas of Burkina Faso.

0.5 g of liver samples from each animal were collected and stored at -20°C in the RNA later Buffer, until further use as source of HEV genomic RNA. Wild animals were samples in the provinces of Houet and Gourma where there are hunting areas. Kadio is in the center and does not have a hunting area, so no wild birds were taken in this area.

Samples RNA extraction and aHEV detection

RNA extractions on the liver samples were performed using the SV total RNA isolation system kit (Promega, France). Extracts were subsequently used for detection of the partial capsid gene of aHEV using primers described previously (Bilic et al., 2009) in a reverse transcriptase polymerase chain reaction (RT-PCR). Briefly, external primers set Forw1_C-BLSv (5'-GGATGGTTGTTCGACATGTGG-3') and Rev1_C-BLSv (5'-GCTGNCNGAAGTCTGTCGA-3') were used. The reverse transcriptase reaction and polymerase chain reaction were performed with the OneStep RT-PCR kit (Promega, France), according to manufacturer's instructions under the following conditions: 50°C for 30 min; 95°C for 5 min; 45 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, followed by a final elongation step of 72°C for 10 min. The negative control was water treated in the same way as the liver samples. Polymerase chain reaction (PCR) products with the expected size (280 bp) were revealed on a 1% agarose gel containing SybrGreen (Figure 2).

Statistical analysis

We performed the statistical analysis using R software version 2.13.0, through the package ‘Rcmdr’ version 2.5-1 (Fox et al., 2018). The differences in avian HEV RNA positivity between different variables (Locality and Species) were evaluated using
logistic regression binomial. The best model was judged by Fisher's scoring algorithm. All tests were two-sided, and values of p < 0.05 were considered statistically significant. Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were estimated to explore the strength of the association between aHEV positivity and the conditions investigated.

RESULTS

Avian HEV RNA were detected in 29 (16.8 and 95% CI [11.2 – 22.3]; p=2.2.10^{-16}) of the 173 examined bird liver samples by RT-PCR (Tables 1 and 2). Of these, the prevalence was diverse in different birds species; the most frequent level was 35.3% (12/34, 95% CI [19.2 – 51.4]) in N. meleagris, followed by 23.5% (8/34, 95% CI [9.3 – 37.8]), in G. gallus domesticus; 13.3% (4/30, 95% CI [1.2 – 25.5]), in S. turtur; 13.3% (2/15, 95% CI [0 – 30.5]), in C. livia; 6.7% (2/30, 95% CI [0 – 15.6]), in A. platyrhynchos and 3.3% (1/30, 95% CI [0 – 9.8]) in P. natalensis. The highest proportions of positive samples were found in the domestic species 21.2% (24/113 95% CI [13.7 – 28.8], p=9.7.10^{-10}) against 8.3% in wild birds (5/60, 95% CI, [1.3 – 15.3], p=1.1.10^{-10}); Domestic birds had 4.8-fold higher risk than wild birds ([OR], 4.8; 95% CI, [1.8 – 15.9]; p=4.0. 10^{-3}) (Table 2).

Comparison between domestic and wild birds within the area where both species were tested in sufficient numbers, show that the prevalence of N. meleagris (3/3
**DISCUSSION**

Evidence of aHEV infection of poultry has been well documented from the United States, Canada, China, Australia, Israel, and several countries in Europe (Haqshenas et al., 2001; Swwayne, 2003; Agunos et al., 2006; Guo et al., 2006; Peralta et al., 2009; Xiao et al., 2013; Zhang et al., 2017). This study represents the first report on the distribution and molecular characterization of avian Hepatitis E Virus in domestic and wild birds without clinical symptoms in Burkina Faso. The overall aHEV RNA prevalence was 16.8% (29/173) in six birds species sampled from four districts of Burkina Faso, which was lower than that in chickens in the United States (29.9%; Gerber et al., 2014), Brazil (20.0%; Billam et al., 2005), and Korea (28%; Kwon et al., 2012), by ELISA. The low prevalence recorded in detecting avian HEV genome could be attributed to sampling of apparently healthy birds and alternatively to the primers used (Gerber et al., 2015), as the avian HEV genome shows a high variability (Sprygin et al., 2012). Besides, detection rate of aHEV RNA in the pooled fecal samples was 62.9% (39/62) (Gerber et al., 2015), hence fecal samples could be another samples source, suitable for the molecular detection of avian HEV.

This prevalence of aHEV RNA was diverse in different birds species; the most frequent level was 35.3% in *N. meleagris*, 23.5%, in *G. gallus domesticus*, 13.3%, in *S. turtur*, 13.3%, in *C. livia*, 6.7% in *A. platyrhynchos* and 3.3% in *P. natalensis*. The differences could be related to differences in ecological and geographical factors (Cong et al., 2014). Thus, the high rate of aHEV RNA showed in the domestic species (*G. gallus domesticus, N. meleagris, A. platyrhynchos* and *C. livia*), could be due the poultry were highly congested in livestock areas, feces likely serve as the main source for virus spread within the flock (Haqshenas et al., 2001; Saif et al., 2008; Ahmad et al., 2010; Meng, 2011; Yugo et al., 2016). Domestic birds could be most often subject to a re-infection, because proper sanitation conditions in the henhouse are lacking and bird drinking water can contain feces (Crespo et al., 2015). This results suggests the possibility of aHEV transmission from asymptomatic
cases or repeated introduction through an unknown common source (Hsu and Tsai, 2014). Some studies have also shown that the high density of poultry increases the risk of disease transmission (Ricard and Marche, 1988). The low prevalence of aHEV RNA observed in wild birds (P. natalensis and S. turtur) also reflects that the congesting increases the likelihood of positive. Indeed, these birds live in liberty and are less congested compared to domestic birds. As for, A. platyrhynchos living in semi-liberty, the rate of positive sample (6.7%) was higher than in wild birds (Crespo et al., 2015). Burkina Faso is a developing country with low health and educational standards. Utilization of untreated bird feces for agriculture could increase the risk of virus dissemination, which in turn can infect wild birds. The high frequency of aHEV occurrence in bird livers in our country must be monitored to avoid an eventual outbreak. We have not investigated the source of aHEV infection in this study, but the role of wildlife in spreading the disease cannot be ignored (Crespo et al., 2015). The present study demonstrates the circulation of avian HEV in the domestic and wild birds without clinical symptoms, in Burkina Faso. This asymptomatic circulation of the virus in birds is of great interest and should be better monitored to avoid large epidemics. Thus we have to undertake studies on public health issues related to aHEV and the genetic diversity of aHEV inside the country.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Comparative cost analysis of three injectable ivermectin preparations in the control of gastrointestinal nematodes of sheep in Makurdi, Benue State Nigeria

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The efficacy and comparative cost analysis of three injectable ivermectin preparations was evaluated in West African Dwarf (WAD) sheep naturally infected with gastrointestinal nematodes. Three anthelmintics: ivomec classic® (IVC), ivomec super® (IVS) and ivomec gold® (IVG) were administered at a dose rate of 200µg/kg to control gastrointestinal nematodes in three treatment groups comprising five animals each. The faecal egg counts (FEC) for each animal pretreatment, and thereafter for a period of 16 weeks post-treatment was carried out using the modified McMaster technique. The results are a pretreatment mean FEC for groups A, B and C of 970±550.36, 880±279.55 and 1640±893.78 eggs per gram (epg), respectively and a mean FEC of zero  for all treatment groups one week post treatment. The mean FEC of zero was maintained for 28, 35 and 56 days, respectively. A mean FEC threshold for re-treatment of 500 epg was exceeded at days 42, 49 and 84 for groups A (615±167.26), B (830±287.49) and C (737.5±448.10), respectively. The results were subjected into a deterministic model to estimate the costs of using IVC, IVS or IVG in an annual control program. The costs of a one-time treatment were $20.6, $20.8 and $21.0, respectively. The average annual costs were $82.39, $83.22 and $41.99 for groups A, B and C, respectively. Thus, veterinary service and labour are two variables that contributed more to cost of treatment when compared with the price of drugs and average weight of the animals treated.

Key words: Ivermectin, gastrointestinal nematodes, West African Dwarf sheep, efficacy, cost analysis.

INTRODUCTION

Gastrointestinal (GI) parasite infection is considered as the most important limiting factor to sheep productivity in

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most parts of the world especially in developing countries (Waller, 1997; Roeb et al., 2013; Blackie, 2014; Singh et al., 2017a, b). The most common GI parasitic diseases in sheep in Nigeria are Haemonchosis, Strongyloides, Oesophagostomosis, Bunostomosis and Trichostrongylosis.

*Haemonchus contortus* has been singled out as the most important nematode of small ruminants in the tropics (Adamu et al., 2013; Singh et al., 2013; Zvinorova et al., 2016). Gastrointestinal nematode infections poses serious economic consequences to small ruminant production due to the associated morbidity, mortality, veterinary service and cost of treatment, as well as costs of other control measures (Singla, 1995; Zinsstag et al., 1998; Nwosu et al., 2007).

In ruminant production systems, parasite control has consistently shown a very high correlation with increased production (Kumar et al., 2013; Kenyon et al., 2013). Gastrointestinal nematode control strategies are almost entirely on the use of anthelmintic. The frequent use and mismanagement of these drugs has led to development of wide-spread resistance to the major groups of anthelmintic except for monepantel (Pomroy, 2006; Molento, 2009; Adamu et al., 2013; Melaku et al., 2013).

Ivermectin (IVM) is a macrocyclic lactone with activity against GI and lung nematodes (Nolan, 2012; Campbell, 2012), as well as against ectoparasites of clinical relevance in domestic animals (Campbell et al., 1984; Shoop et al., 1995; Merola and Eubig, 2012). Ivermectin has extensive tissue distribution, low biotransformation and high plasma-GI recycling that guarantees its persistent activity. The broad spectrum of activity and wide margin of safety has made it a drug of choice for nematode and arthropod parasitism in cattle, sheep, goat, swine, dog and horses (Campbell et al., 1983). Consequently, IVM is the most widely used anthelmintic and this extensive use has led to the emergence of IVM-resistant nematode populations in several countries (Jackson and Coop, 2000; Waller, 2006; Pomroy, 2006; Molento, 2009). The efficacy of IVM against gastrointestinal parasites under different control strategies has been demonstrated (Kenyon et al., 2013).

Farmers and veterinarians should be interested in information concerning the cost analysis of using different drugs and strategies to help with decision making for better control options. The decision making process is dependent on the costs of the anthelmintic, veterinary service and labour. These three variables may change depending on cost of anthelmintic, efficacy and duration of action against GI nematodes.

Currently, there are three injectable IVM preparations in the market produced by Merial. These IVM preparations are ivomec classic®, ivomec super® and ivomec gold®. The preparations differ in composition, duration of action and price. The aim of this study was to determine the efficacy, duration of action and cost implications of these IVM preparations as demonstrated by faecal egg counts pre and post-treatment for a period of 16 weeks in a flock of sheep.

**MATERIALS AND METHODS**

Experimental animals and ivermectin preparations

A total of fifteen West African Dwarf sheep kept at the University Teaching and Research Farm, University of Agriculture, Makurdi were randomly selected for this experiment. The sheep included 7 rams and 8 ewes out of a herd of 35 sheep. The sheep were kept under the semi-intensive system of management. Pregnant ewes and lambs were excluded from this experiment.

The compositions of the three injectable IVM preparations used in this experiment were: Ivomec classic® (IVC) contains 1% m/v of ivermectin; Ivomec super® (IVS) contains 1% m/v of ivermectin and 10% clorsulon and Ivomec gold® (IVG) contains 3.15% m/v of ivermectin. All three formulations used were manufactured by Merial South Africa (Pty) Limited. The anthelmintic was administered at a dose rate of 200 µg of IVM per kilogram body weight according to manufacturer’s instructions.

Experimental design

The fifteen sheep were randomly assigned to 3 treatment groups (A, B and C), with each group comprising 5 sheep. Sheep in group A were treated with IVC, while sheep in groups B and C were treated with IVS and IVG, respectively.

Prior to the administration of the IVM preparations, a baseline faecal examination was carried out to determine the faecal egg counts of individual sheep pre-treatment. The sheep were weighed individually using a Camry® weighing scale.

The months of August to October represent the second half of the rainy season including its peak. During this period, it is expected that *Haemonchus* L3 are well established in grazing pasture to pose a sufficient challenge to the experimental animals. The IVM formulations were administered to the sheep in the different groups at the same dose rate of 200 µg/kg subcutaneously, as recommended by the manufacturer.

Sampling and determination of faecal egg counts

Following treatments, faecal samples were collected per rectum from all the sheep in each group once weekly for 16 weeks. Samples were placed in polythene bags, labeled and transported on ice packs to the laboratory for further processing and examination.

The faecal samples were examined for helminth eggs and the faecal egg counts (FEC) for each sample was determined using the Modified McMaster technique using saturated sodium chloride solution as the floatation medium (Hansen and Perry, 1990).

Data collection and analysis

Prior to treatment with the respective injectable IVM preparations, the FEC for each of the 5 sheep per group was examined and recorded. The mean and standard error of the mean (SEM) were calculated and recorded for each group. The FEC of each sheep in the three groups was determined once weekly for a duration of 16 weeks. The mean FEC for each group and the SEM was similarly calculated for each group weekly. The FEC of 500 epg was used as the cut-off value in this study for a repeat of treatment (the week in which the mean FEC exceeded 500 epg was referred to as “re-treatment week”). The time interval between the week of first treatment and the re-treatment week was regarded as the "duration
Economic analysis

A deterministic economic model was developed using Microsoft Excel to demonstrate the cost implications of using each of the IVM formulations with representing the treatment groups (IVC, IVS and IVG). Table 2 shows the input values for variables used to develop the model. The calculated variables include: drug price per ml (dp), average weight of sheep in each group (AW), average dosage administered per group (DA), average cost of drug per treatment per group (DC), service cost per group (VSc), labour cost per treatment per group (Lc), duration (weeks) till next treatment for each IVM preparation (DT) and number of treatments required per year (NT). The outcome of these calculations is the average annual cost of GI nematode control per group (ATC). VVc represents veterinary visit cost.

Economic model calculations

\[ DC_i = DA_i \times dp_i \]  

[1]

The average cost of a one-time treatment per group (Tc) is the sum of veterinary visit cost (VVc); and DC, VSc, and Lc for five sheep as follows:

\[ TC_i = VVc + \sum_{i=1}^{5} DC_i + VSc_i + Lc_i \]  

[2]

The NT, was determined based on the fact that the rainy season provides an environment conducive for the proliferation of GI nematodes on pasture. The rainy season usually lasts about 6 months (24 weeks) during which several life-cycles of the parasites are expected to occur. Therefore, NT, differed between groups based on their respective DA, as follows:

\[ NT_i = \frac{24 \text{ weeks}}{DA_i} \]  

[3]

The average annual cost of GI parasite control for one group (ATC) is the product of the Tc and the NT, of the respective groups.

\[ ATC_i = TC_i \times NT_i \]  

[4]

Sensitivity analysis

A sensitivity analysis was conducted in order to determine the level of impact of some variables on the annual cost of GI parasite control for each drug. This was done by adjusting the input values of certain variables by ±20% one at a time, while all other variables remained constant at their default values. The resulting average annual cost of GI parasite control (ATC) from each simulated scenario was compared with the default ATC calculated from the values from the field experiment. Variables for which sensitivity analysis was done include: veterinary visit cost (VVc), veterinary service cost (VSc), labour cost (Lc), the price of the drugs (DP) and the average weight of the animals (AW).

RESULTS

The mean faecal egg count for the three groups of 5 sheep treated with the three different ivermectin preparations are illustrated in Figure 1. The pre-treatment mean FEC across the groups is as follows: 970 epg for group A, 880 epg for group B and 1640 epg for group C (Table 1). One week after treatment, all the animals in the three groups presented 0 epg on examination of faecal samples. This is indicative of the efficacy of all three IVM preparations 7 days post treatment as 100%.

The three IVM formulations delayed re-infection for different durations. Sheep in group A treated with IVC maintained 0 epg status until day 28 post treatment, sheep in group B treated with IVS maintained 0 epg status until day 35 post treatment and sheep in group C treated with IVG maintained 0 epg status until day 56 post treatment. This implied that following clearance of infection, IVC, IVS and IVG prevented patent infection for a period of 28, 35 and 56 days, respectively.

Garg et al. (2007) reported a mean FEC of 500 epg in sheep requires anthelmintic treatment. The results in Table 1 therefore indicate the need for re-treatment on day 42 following treatment using IVC, day 49 following treatment with IVS and day 84 following treatment with IVG. The period of importance for the control of H. contortus is the rainy season which lasts about 6 months (180 days) on average in the study area. This implies the use of IVC and IVS will require 4 treatments per year.
Table 1. Mean faecal egg count ± standard error of the mean for groups of 5 sheep treated with IVC (Group A), IVS (Group B) and IVG (Group C).

<table>
<thead>
<tr>
<th>Day</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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<tbody>
<tr>
<td>0</td>
<td>970±550.36</td>
<td>880±279.55</td>
<td>1640±893.78</td>
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<tr>
<td>7</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
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<td>14</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
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<tr>
<td>21</td>
<td>0±0</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>28</td>
<td>*430±175.78</td>
<td>0±0</td>
<td>0±0</td>
</tr>
<tr>
<td>35</td>
<td>365±171.32</td>
<td>*200±187.75</td>
<td>0±0</td>
</tr>
<tr>
<td>42</td>
<td>**615±167.26</td>
<td>155±64.42</td>
<td>0±0</td>
</tr>
<tr>
<td>49</td>
<td>1170±436.92</td>
<td>**830±287.49</td>
<td>0±0</td>
</tr>
<tr>
<td>56</td>
<td>1945±924.37</td>
<td>880±279.55</td>
<td>*50±25.82</td>
</tr>
<tr>
<td>63</td>
<td>**2720±1533.59</td>
<td>880±279.55</td>
<td>150±85.63</td>
</tr>
<tr>
<td>70</td>
<td>1630±768.21</td>
<td>4765±2879.71</td>
<td>400±242.90</td>
</tr>
<tr>
<td>77</td>
<td>2020±1018.90</td>
<td>*6005±4205.36</td>
<td>425±335.91</td>
</tr>
<tr>
<td>84</td>
<td>1700±711.34</td>
<td>1800±725.95</td>
<td>**737.5±448.10</td>
</tr>
<tr>
<td>91</td>
<td>1205±440.77</td>
<td>3760±2244.35</td>
<td>256.25±130.50</td>
</tr>
<tr>
<td>98</td>
<td>960±678.49</td>
<td>1030±615.14</td>
<td>312.5±124.33</td>
</tr>
<tr>
<td>105</td>
<td>1970±905.62</td>
<td>1755±874.66</td>
<td>475±121.79</td>
</tr>
<tr>
<td>112</td>
<td>230±93.01</td>
<td>410±137.30</td>
<td>512.5±250.58</td>
</tr>
</tbody>
</table>

*Reinfection

while farmers that use IVG will require only 2 treatments per year (Table 2). Hence, on the basis of efficacy and duration of action, IVM appears to be the more preferable choice among these injectable IVM formulations for the control of GI nematodes in a flock of sheep.

The outcome of the economic analysis shows that the average cost of a one-time treatment (Tc) for GI nematode infection using IVC, IVS and IVG for a group of 5 sheep are $20.6, $20.8 and $21.0, respectively. While farmers that use IVC and IVS will require 3 treatments per year (Table 2). Hence, on the basis of efficacy and possible resistance development by GI parasites is nevertheless important since AR has developed in sheep to all known anthelmintic groups including IVM in South Africa by Van Wyk et al. (1999). The difference may be due to a large scale sheep farming in South Africa and other southern hemisphere countries like Australia and New Zealand (Pomroy, 2006; Leathwick and Besier, 2014). These large-scale farms use IVM more frequently in GI nematode control as compared to the predominant small holder sheep farming structure in Nigeria. Periodic evaluation of the efficacy of common anthelmintics and possible resistance development by GI parasites is nevertheless important since AR has developed in sheep to all known anthelmintics except for monepantel (Kaminsky et al., 2011).

The different formulations of IVM (IVC, IVS and IVG) following clearance of infection prevented re-infection for a period of 28, 35 and 56 days, respectively. These differences in duration for re-infection to occur between treatment groups can be attributable to the different concentrations of IVM in the preparations used. The concentration of IVM in IVG may be responsible for the prolonged anthelmintic effect of IVG resulting from an extended half-life of the drug in plasma of treated sheep.

DISCUSSION

This study evaluated the efficacy of three different IVM preparations in the treatment of GI nematodes in sheep as well as the onset of parasite re-infection after treatment. The efficacy of all three IVM preparations 7 days post treatment was 100% further strengthens claims about the susceptibility of GI nematodes to IVM in this region of Africa by Idika et al. (2012). Similarly, Peña-Espinoza et al. (2014) reported a 100% efficacy of IVM against *H. contortus* in small ruminants in Denmark. This finding is contrary to the report of anthelmintic resistance (AR) to all known anthelmintic groups including IVM in South Africa by Van Wyk et al. (1999). The difference may be due to a large scale sheep farming in South Africa and other southern hemisphere countries like Australia and New Zealand (Pomroy, 2006; Leathwick and Besier, 2014). These large-scale farms use IVM more frequently in GI nematode control as compared to the predominant small holder sheep farming structure in Nigeria. Periodic evaluation of the efficacy of common anthelmintics and possible resistance development by GI parasites is nevertheless important since AR has developed in sheep to all known anthelmintics except for monepantel (Kaminsky et al., 2011).
Table 2. Default input values of costs and prices used in the economic analysis of the costs of GI nematode control using three different IVM preparations in WAD sheep.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Abbreviation</th>
<th>IVC</th>
<th>IVS</th>
<th>IVG</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug Price/500 ml</td>
<td>DP</td>
<td>29.18</td>
<td>91.23</td>
<td>148.28</td>
<td>^OVAH S/Africa</td>
</tr>
<tr>
<td>Drug Price/ml</td>
<td>Dp</td>
<td>0.06</td>
<td>0.18</td>
<td>0.3</td>
<td>Calculated</td>
</tr>
<tr>
<td>Dosage (ml/kg)</td>
<td>D</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>*Merial®</td>
</tr>
<tr>
<td>Average weight (kg)</td>
<td>AW</td>
<td>16.73</td>
<td>16.73</td>
<td>16.73</td>
<td>Calculated</td>
</tr>
<tr>
<td>Dose Administered (ml)</td>
<td>DA</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>Calculated</td>
</tr>
<tr>
<td>Drug cost/treatment/sheep</td>
<td>DCT</td>
<td>0.02</td>
<td>0.06</td>
<td>0.1</td>
<td>Calculated</td>
</tr>
<tr>
<td>Drug cost/treatment/5 sheep</td>
<td>Dc</td>
<td>0.1</td>
<td>0.3</td>
<td>0.5</td>
<td>Calculated</td>
</tr>
<tr>
<td>Vet visit cost</td>
<td>VVc</td>
<td>8</td>
<td>8</td>
<td>8</td>
<td>Authors</td>
</tr>
<tr>
<td>Veterinary service cost/sheep</td>
<td>VSc</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Authors</td>
</tr>
<tr>
<td>Veterinary service cost/5 sheep</td>
<td></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>Calculated</td>
</tr>
<tr>
<td>Labour cost/5 sheep</td>
<td>Lc</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>Authors</td>
</tr>
<tr>
<td><strong>Cost of one-time treatment</strong></td>
<td>Tc</td>
<td>20.6</td>
<td>20.8</td>
<td>21.0</td>
<td>Calculated</td>
</tr>
<tr>
<td>Duration till re-Rx of flock (wk)</td>
<td>DT</td>
<td>6</td>
<td>7</td>
<td>12</td>
<td>As implied from Garg et al. (2007)</td>
</tr>
<tr>
<td>No. of Rx req./year</td>
<td>NT</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>Calculated</td>
</tr>
<tr>
<td><strong>Average annual treatment costs</strong></td>
<td>ATc</td>
<td>82.39</td>
<td>83.22</td>
<td>41.99</td>
<td>Calculated</td>
</tr>
</tbody>
</table>

^OVAH S/Africa: Onderstepoort Veterinary Academic Hospital, Pretoria South Africa. *Merial®: The direction on the leaflets for each of the drugs was followed. R-treatment, wk:week, yr:year; IVC Ivomec classic, IVS Ivomec super, IVG Ivomec gold

Figure 2. Tornado graph showing sensitivity analysis for Group A treated with Ivomec Classic®.

Tornado graphs showing sensitivity analysis of cost variables. The values displayed are the differences between the average annual costs of GI nematode control in the normal scenario (with default prices for all cost variables) and the average annual costs of GI nematode control in a group of 5 WAD sheep under scenarios in which the value of one cost variable is altered. The negative values represent reduced marginal costs compared to the normal scenario, while the positive values represent additional marginal costs compared to the normal scenario in US Dollars.

(McKellar and Marriner, 1987; Garg et al., 2007).

The economic model used to evaluate the comparative cost analysis of the three IVM preparations shows that in spite of the relatively large difference in price between the drugs (that is, $119.10 between the cheapest option, IVC and the most expensive option, IVG), the consequential difference in the average costs of a one-time treatment for a group of 5 sheep was relatively small, that is, $1.40 between the cheapest option (IVC) and the most expensive option (IVG). This may be due to the proportion of treatment costs attributable to the cost of the drug used was only about 0.5, 1.5 and 2.4% for IVC, IVS and IVG, respectively. Whereas, other complementary costs that make up the treatment costs contributed much more. The proportion of treatment costs attributable to veterinary service cost is almost 50% for all three treatment options. A scenario manipulation of the model showed that the cost of veterinary services would account for as much as 75% of treatment costs in a flock of 50 sheep kept under the same circumstances as those in the current study. The relatively high cost associated with veterinary services make farmers to by-pass professionals there
by administering the drugs or employing the services of unqualified individuals (quacks). This abuse of the veterinary profession may increase the risk of anthelmintic resistance as a result of the use of incorrect dosages and routes of administration, as well as the practice of sub-optimal control strategies. Most farmers do not consider the cost of their labour (time and energy spent on catching and restraining the sheep during treatment) into account. This is taken for granted, but this study considered this an important input as persons may be employed to perform the duty. The farmer may perform the duty himself, the time spent should be valued based on the value of other profitable activities he/she could have been engaged in during the period. In the current study, labour costs accounted for about 12% of treatment costs, which is much more than the cost of the drugs, indicating that they should not be overlooked. The sensitivity analysis buttresses the fact that veterinary services and labour costs are the highest contributors to the overall costs and should be considered more importantly in the choice of control strategy, rather than the price of the drug which is usually the major consideration by farmers.

The major reason for which IVG emerged as a cheaper option, this is less than half the cost of either IVC or IVS is when used for an annual control programme. The
sheep were protected for 12 weeks, indicating that the treatment procedure will only need to be carried out twice a year as against four times when compared with the other two options used. This reduction in the frequency of treatment cuts down the cost of the procedure such as veterinary services and labour by 50%. This will reduce the exposure of the anthelmintic and possibly delaying the onset of anthelmintic resistance. Farmers may be more willing to employ the services of a vet when the costs of veterinary services are lowered. To optimize the cost of anthelmintic control, the choice of anthelmintic should be based on the efficacy and duration of action of the drug.

**Conclusion**

The GI nematode parasites in circulation among West African Dwarf (WAD) sheep in the study area may not have developed resistance to ivermectin. The IVG confers a longer duration of protection when compared with IVC and IVS formulations. While IVG is costly than both IVC and IVS in a one-time treatment of GI nematodes in sheep, it is much cheaper to use in an annual control programme of GI nematodes of sheep in the study area. Veterinary service and labour costs contributed more to treatment costs than the price of the anthelmintic in GI nematode control. Thus, the decision for choice of anthelmintic for optimization of the cost analysis of GI nematode control should be based on the efficacy and duration of action of the drug rather than its price.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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