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Volume 10 (2); March 25, 2020



Research Paper

***In vitro* efficacy of Tylosin and Enrofloxacin in treatment of bovine mastitis causing bacteria in Omdurman locality.**

Almobarak ME, Mohammed Salih RR and Gibreel HH.

Online J. Anim. Feed Res., 10(2): 53-58, 2020; pii: S222877012000007-10
DOI: <https://dx.doi.org/10.36380/scil.2020.ojaf7>



Abstract

Dairy industry has recently grown as a very important economic national source of income. In Sudan, many dairy owners introduced foreign blood. This might result in a progeny of mixed blood cows with lowered resistance to endogenous and locally prevailing diseases such as mastitis. In this study 60 milk samples were obtained from Frisian cows in Elrudan and Elmouileh Convention in Omdurman, Khartoum State, Sudan. Samples positive for bacterial growth were identified using the gram stain and various conventional biochemical tests. Hundred species of bacteria were isolated from 60 samples of milk. A total of 70 (70%) were gram positive, and 30 (30%) were gram negative bacteria. Among the total of the gram positive isolates, 40 (57.1%) were *Staphylococcus* spp., 18 (25.7%) were *Bacillus* spp., 6 (8.6%) *Streptococcus* spp., 4 (5.7%) *Corynebacterium* spp., and 2 (2.9%) were *Actinomyces* spp. and from gram negative isolates, 26 (86.7%) were *Enterobacter* spp. and 4 (13.3%) were *E. coli*. Antibiotic susceptibility tests to Tylosin and Enrofloxacin were performed for the isolated bacteria (*Staphylococcus aureus*, *Staph. epidermidis*, *Enterobacter aerogenes* and *Enterobacter faecalis*). The isolated bacteria were found to be highly sensitive to Tylosin and Enrofloxacin.

Keywords: Tylosin, Enrofloxacin, Bovine, Mastitis, *In vitro*

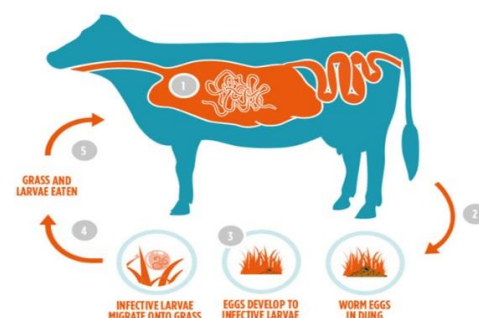
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Research Paper

Prevalence of bovine gastro intestinal parasitic infection in and around Kombolcha town, Ethiopia.

Ayele A, Abay M, Birhan M, Yayeh M, Erara M, Gessese T, Mohammed A and Demoze G.

Online J. Anim. Feed Res., 10(2): 59-65, 2020; pii: S222877012000008-10
DOI: <https://dx.doi.org/10.36380/scil.2020.ojaf8>



Abstract

A cross-sectional study was conducted in and around Kombolcha from October 2017 to April 2018 to determine the prevalence of gastro-intestinal helminthes parasites in cattle. A total of 384 randomly selected cattle were sampled and examined using standard coprological procedure. The overall prevalence was 39.8% of gastrointestinal (GI) helminthes and the prevalent helminthes eggs identified were 15.6% *Paramphistomum* species (spp), 10.4% strongly type eggs, 8.6% *Fasciola* spp., 3.1% *Trichuris* species and 2.1% *Toxocara* species. This result indicated the highest prevalence of *Paramphistomum* spp. eggs than other helminthes egg and the lowest prevalence of *Toxocara* species egg. There was statistically significant difference among the age groups in paramphistomum and strongly infection ($\chi^2=24.960$, $p \leq 0.001$) and ($\chi^2=17.047$, $p \leq 0.001$) respectively. Higher prevalence rate was shown in 2-5 years age of cattle. Between body conditions there was also significant ($p \leq 0.000$ and $p \leq 0.013$) difference in paramphistomum and strongly and which was higher in moderate animals and lower in animals with good body condition. Sex had no significant effect on the prevalence of helminthes parasite, except for strongly type of egg. The present study revealed that there is high prevalence of GI helminthes infection in cattle in the study area. Therefore, strategic prevention should be advocated to prevent the problem in and around Kombolcha.

Keywords: Cattle, Gastrointestinal, Prevalence, Helminthes parasites, Kombolcha

[Full text-[PDF](#)]

Research Paper

Comparative advantages of cervical insemination over natural mating on production of crossbred lambs for export market.

Besufkad Sh, Bisrat A, Demis Ch, Abebe A, Abebe A, Goshime Sh, Zewude T.

Online J. Anim. Feed Res., 10(2): 66-70, 2020; pii: S222877012000009-10
DOI: <https://dx.doi.org/10.36380/scil.2020.ojafr9>



Abstract

A study was conducted at Debre Birhan agricultural research center to evaluate the success of artificial insemination (AI) following oestrous synchronization in Washera ewes. A total of 32 ewes were selected from this center. All experimental animals were synchronized using single injection of 1 ml Enzaprost® intramuscularly at unknown stage of estrous cycle. Then ewes were divided into Dorper and Awassi fresh semen cervical insemination. Fixed time cervical insemination was performed in estrous ewes 48-51 hrs following 1 ml Enzaprost® injection with 0.25 ml fresh diluted Dorper and Awassi semen. Out of 32 ewes synchronized, 22 (68.8%) of ewes exhibited overt sign of oestrous within 48 hrs of hormone administration. Ewe's body condition score (BSC) wasn't found to be a significant factor determining oestrous response to hormone treatment. The overall pregnancy, lambing and fecundity rates for cervical insemination and natural mating were 63.64, 77.27, 121.43% and 57.46, 61.57, 107.14%, respectively. There was no significant difference in pregnancy, lambing and fecundity rates among ewes mated with fresh semen cervical insemination and controlled ram mating ($P < 0.05$). The pregnancy, lambing, and fecundity rates for Awassi and Dorper semen were 58.3, 75.0, 128.57% and 70.0, 80.0, 114.29%, respectively. No significance differences were observed in pregnancy, lambing and fecundity rates among the genotypes ($P < 0.05$). The current work strongly indicated that artificial insemination following oestrous synchronization has a tremendous impact on terminal crossing, that allows us to import fewer exotic genotypes and producing large number of crossbreed lambs from imported breed with in few years.

Keywords: Artificial insemination, Commercial lamb production, Washera, Enzaprost

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Research Paper

Hemato-biochemical profiles of sheep infected with fasciolosis in comparison with health controls.

Yesuf M, Erara M, Kenubih A, Belay A and Ahmedin N.

Online J. Anim. Feed Res., 10(2): 71-75, 2020; pii: S222877012000010-10
DOI: <https://dx.doi.org/10.36380/scil.2020.ojafr10>



Yesuf M, Erara M, Kenubih A, Belay A and Ahmedin N (2020). Hemato-biochemical profiles of sheep infected with fasciolosis in comparison with health controls. *Online J. Anim. Feed Res.*, 10(2): 71-75. DOI: <https://dx.doi.org/10.36380/scil.2020.ojafr10>

Abstract

Fasciolosis can cause considerable change in hematological parameters and liver driven serum enzymes. Hence, the aim of this research is to assess the hematological and biochemical changes in sheep infected with Fasciolosis in comparison with healthy controls. A total of 52 local breed (26 from naturally infected and 26 from healthy control group) matched with sex and age were enrolled in the study. Five ml blood using EDTA vacutainer tube for hematology and another 5 ml blood by serum separating tubes for serum biochemical profiles were taken and analyzed using Sysmex automated hematological analyzer and Vegasys chemistry analyzer respectively. The hematological analysis indicated there were significant mean reductions ($P < 0.001$) in tRBC, hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC) in case group (5.62 ± 1.15 , 8.92 ± 1.66 , 20.12 ± 5.78 , 26.05 ± 4.16 , 9.444 ± 2.00 and 29.72 ± 2.55) than control group (10.27 ± 1.36 , 12.68 ± 1.66 , 38.9 ± 6.05 , 40.50 ± 3.35 , 13.100 ± 1.35 and 34.327 ± 3.06) respectively. On the contrary the mean value of tWBC and eosinophil were significantly higher in the infected group (11.27 ± 2.7 and 7.19 ± 2.81) than the flock free of Fasciolosis (7.12 ± 2.61 and 2.82 ± 1.07) respectively. Regarding biochemical parameters, the significant mean elevation ($P < 0.001$) in serum ALT (125.92 ± 20.71) and AST (34.73 ± 11.97) in the infected sheep were observed than ALT (77.04 ± 13.96) and AST (23.69 ± 7.37) of the control groups. Whereas, the serum total protein and glucose level (5.17 ± 1.05 and 21.15 ± 11.08) in Fasciola infected sheep were significantly lower ($P < 0.001$) than healthy control (7.33 ± 1.06 and 32.62 ± 9.48 , respectively). The alteration in hemato-biochemical profile suggested a great impact of Fasciolosis on liver physiology leading to loss of production and productivity in sheep industry.

Keywords: Biochemical Profile, Fasciola, Hematology, Sheep.

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IN VITRO EFFICACY OF TYLOSIN AND ENROFLOXACIN IN TREATMENT OF BOVINE MASTITIS CAUSING BACTERIA IN OMDURMAN LOCALITY

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✉ Supporting Information

ABSTRACT: Dairy industry has recently grown as a very important economic national source of income. In Sudan, many dairy owners introduced foreign blood. This might result in a progeny of mixed blood cows with lowered resistance to endogenous and locally prevailing diseases such as mastitis. In this study 60 milk samples were obtained from Frisian cows in Elrudoan and Elmouileh Convention in Omdurman, Khartoum State, Sudan. Samples positive for bacterial growth were identified using the gram stain and various conventional biochemical tests. Hundred species of bacteria were isolated from 60 samples of milk. A total of 70 (70%) were gram positive, and 30 (30%) were gram negative bacteria. Among the total of the gram positive isolates, 40 (57.1%) were *Staphylococcus* spp., 18 (25.7%) were *Bacillus* spp., 6 (8.6%) *Streptococcus* spp., 4 (5.7%) *Corynebacterium* spp., and 2 (2.9%) were *Actinomyces* spp. and from gram negative isolates, 26 (86.7%) were *Enterobacter* spp. and 4 (13.3%) were *E. coli*. Antibiotic susceptibility tests to Tylosin and Enrofloxacin were performed for the isolated bacteria (*Staphylococcus aureus*, *Staph. epidermidis*, *Enterobacter aerogenes* and *Enterobacter faecalis*). The isolated bacteria were found to be highly sensitive to Tylosin and Enrofloxacin.

Keywords: Tylosin, Enrofloxacin, Bovine, Mastitis, *In vitro*

INTRODUCTION

Bovine Mastitis is a multi-etiological and complex disease, which is defined as inflammation of parenchyma of mammary glands. Mastitis is considered the main disease in dairy herds (Kaneen and Bandhard, 1990). The occurrence of disease is an outcome of interplay between three major factors: infectious agents, host resistance, and environmental factors (Gera and Guha, 2011). It is characterized by physical, chemical and, usually, bacteriological changes in milk and pathological changes in glandular tissues (Radostitis et al., 2000). Mastitis is a global problem as it adversely affects animal health, quality of milk and the economics of milk production, affecting every country, including developed ones and causes huge financial losses (Sharma et al., 2007). It is the most important disease in dairy cattle and more affect in economic, the most damaging (Ashish et al. 2000; Sharma et al. 2012; Elango et al. 2010; Mostert et al. 2004). Mastitis is caused by several species of common bacteria, fungi, mycoplasmas and algae (Batavani et al., 2007). Most mastitis is of bacterial origin, with just a few of species of bacteria accounting for most cases.

Mastitis pathogens are categorized as contagious or environmental (Kivaria, 2006). Contagious pathogens live and multiply on and in the cow's mammary gland and are spread from cow to cow, primarily during milking. Contagious pathogens include: *Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma* spp. and *Corynebacterium bovis* (Radostitis et al., 2000). Environmental mastitis can be defined broadly as those intra-mammary infections (IMI) caused by pathogens whose primary reservoir is the environment in which the cow lives (Smith et al., 1985). The most frequently isolated environmental pathogens are Streptococci, other than *S. agalactiae*, commonly referred to as environmental streptococci usually *S. uberis* and *S. disagalactiae* and gram-negative bacteria such as *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. (Hogan et al., 1999).

Tylosin is macrolide antibiotic produced from *Streptomyces fradiae* and related structurally to erythromycin (Plumb, 2002; Giguere, 2013). It is the first antimicrobial of the fluoroquinolones group available to veterinarians, they are bactericidal, their wide spectrum of antimicrobial activity includes various microorganisms such as gram positive, gram negative bacteria, mycoplasma, and chlamydiae (Pyorala et al., 1994). Enrofloxacin is alternative

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drug. The aim of this study to identify the most common causes of bacterial mastitis in cows in Omdurman locality and to measure the effectiveness of Tylosin and Enrofloxacin in the treatment of bacterial mastitis in vitro.

MATERIALS AND METHODS

Study area

Study area is Omdurman city which located at the intersection of latitude 15 degrees 41 minutes north, longitude 32 degrees 37 minutes east, on the west bank of the Nile opposite the coupler with a tributary of the White Nile, and off both Khartoum and Khartoum North, which are linked by the bridge of the White Nile and Shambat bridge. The numbers of dairy cows were more in this locality than the other sites of Khartoum province.

Sampling

A total of 30 suspected cows were examined clinically: took the body temperature, pulse, heart rate, respiration, auscultation and palpation the last examination especially for mammary gland and supra mammary lymph nodes for presence of mastitis. Sixty milk samples from mastitic cows were collected. Mastitis was diagnosed when there were visible or palpable sings of udder, inflammatory changes in milk secretion, or through bacteriological examination of milk. During the study 60 milk sample were encountered from 30 cows suffering from clinical and subclinical mastitis. Milk sample were taken under from infected quarters only under aseptic condition for bacteriological studies. The fore milk was stripped off and about 5 ml of milk were drawn in sterile disposable bottle. All samples collected were immediately placed on ice in a thermo flask after collection.

Isolation and Identification of bacteria

Culture. Milk samples were collected from mastitic cows were cultured in two media: Blood agar and MacConkey's agar. After culturing the plates were incubated for 24 hours at 37°C. Purification was achieved by further subculturing on nutrient agar and incubated at 37°C for 24 hours. After purification, a full loop from purified culture was taken and a smear was made and stained with Gram's stain to differentiate between Gram's positive and Gram's negative bacteria and to see the shape of bacteria. Plates were examined for cultural characteristics and biochemical reactions according to standard keys (Barrow and Feltham, 2003). Staphylococci were studied in particular for haemolysis and coagulase production using human plasma. A positive coagulase test was judged as any degree of clotting from a loose clot suspended in plasma to a solid clot (Barrow and Feltham, 2003).

Purification of cultures. Purification of culture was made by sub-culturing a part of a typical and well isolated colony on nutrient agar. This process was repeated twice. The resulting of growth was checked for purity by examining smears stained with Gram's stain method.

Identification of bacteria. The purified isolated bacteria were identified according to criteria outlined by Barrow and Feltham (2003) which included of: Reaction of Gram's stain, shape of the bacterial colonies, presence or absence of spores, motility, the colonial characteristics on different media, haemolysis of blood agar and biochemical tests. All biochemical tests for identification of isolated bacteria were performed according to Barrow and Feltham (2003).

Antibiotic sensitivity test. Some of the bacteria that isolated through microbiological procedures were subjected to antimicrobial susceptibility test by disc diffusion method to identify the effectiveness of the Tylosin and Enrofloxacin. The sensitivity against Tylosin and Enrofloxacin were determined on Mueller Hinton agar as described by National Committee for Clinical Laboratory Standards (NCCLS, 2002). The results were obtained by measuring the diameter of the growth inhibition zone around the antibiotic disc for each isolated bacterial strain and recorded as sensitive, intermediate and resistant.

RESULTS

Identification of isolates

Out of the 100 isolates 70 (70%) were gram positive, and 30 (30%) were gram negative bacteria. Among the total of the gram positive isolates, 40 (57.1%) were *Staphylococcus* spp., *Bacillus* spp. were 18 (25.7%), *Streptococcus* spp. were 6 (8.6%), *Corynebacterium* spp. were 4 (5.7%), *Actinomyces* spp. were 2 (2.9%) and from gram negative isolates, *Enterobacter* spp. were 26 (86.7%) and *E. coli* were 4 (13.3%) (Figure, 1). The isolated *Staphylococcus* spp. in this study was divided into two groups: Coagulase – positive included *Staph. aureus* and *Staph. hyicus*. Coagulase – negative were *Staph. epidermidis*, *Staph. chromogens*, *Staph. simulans* and *Staph.*

hominis. The total 70 isolates: 18 (25.7%) were found to be bacillus species. There were (6%) isolates of *Streptococcus* spp obtained in this study. Other isolates were: *Corynebacterium* spp., *Actinomyces* spp., *Enterobacter* spp. and *E. coli*. Table 1 shows the quality control limits for antibiotics. The results of sensitivity tests by used Tylosin and Enrofloxacin are shown in Tables 2 and 3. They were affected in *Staph. aureus*, *Staph. epidermidis*, *Enterobacter aerogenosa*, *Enterococcus faecalis*.

Table 1 - Quality control limits for antibiotics

Antimicrobial agent			Zone diameter in mm			<i>Staph. aureus</i>
Potency	Code		S	I	R	
Enrofloxacin	10Mg	EX	>23	22-17	<16	27-34
Tylosin	15Mg	TY	>26	25-23	<23	24-31

S: sensitive; I: intermediate; R: resistant

Table 2 - The efficacy of Tylosin against different types of bacteria.

Isolated bacteria	Zone of inhibition	Remarkd
<i>Staph. aureus</i>	30mm	S
<i>Staph. epidermidis</i>	23mm	I
<i>Enterobacter aerogenosa</i>	24mm	I
<i>Enterococcus faecalis</i>	27mm	S

S: Sensitive; I: Intermediate

Table 3 - The efficacy of Enrofloxacin against different types of bacteria

Isolated bacteria	Zone of inhibition	Remarkd
<i>Staph. aureus</i>	34 mm	S
<i>Staph. epidermidis</i>	26 mm	S
<i>Enterobacter aerogenosa</i>	30 mm	S
<i>Enterococcus faecalis</i>	25 mm	S

S: sensitive

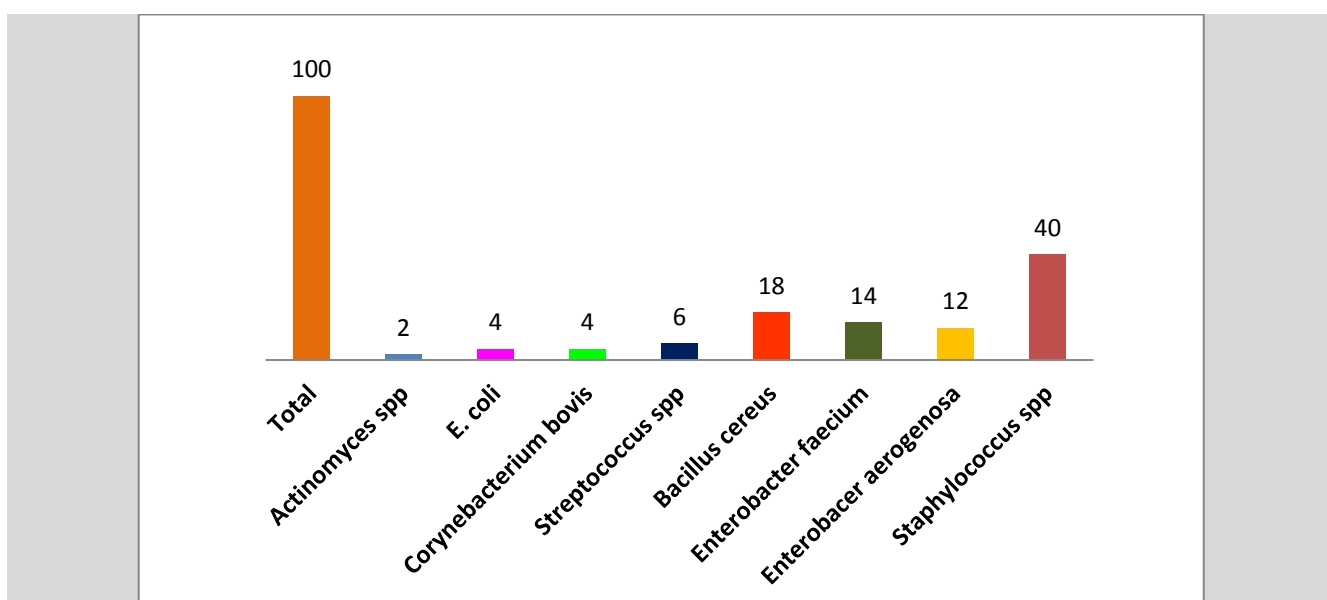


Figure 1 - Total of different isolated bacteria from mastitic milk samples

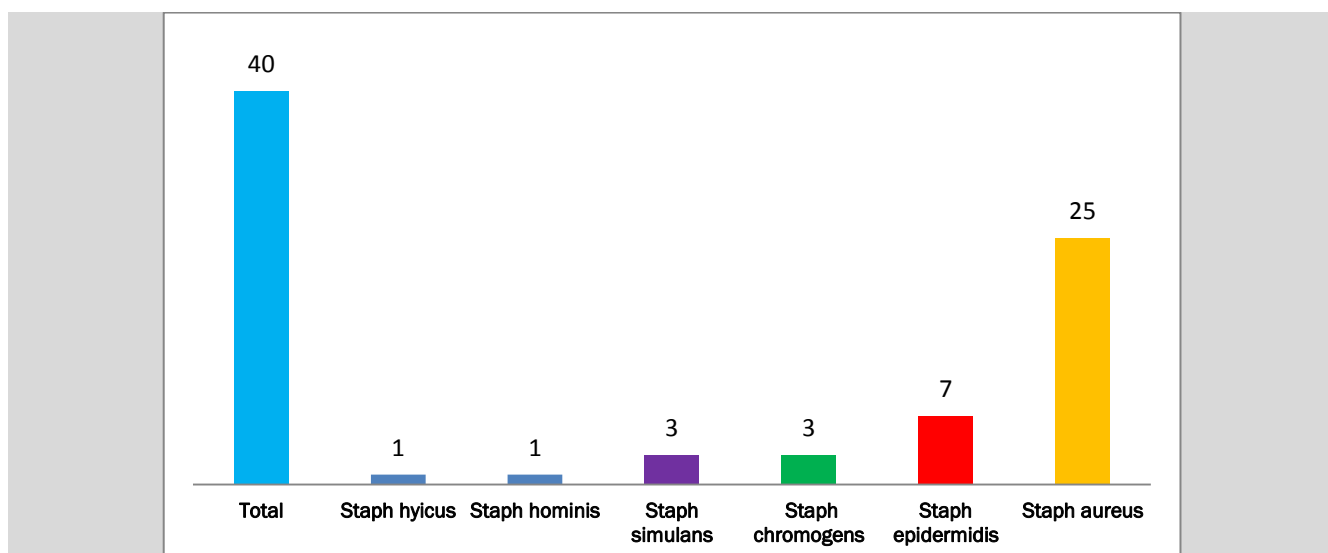


Figure 2 - Species of Staphylococci isolates

DISCUSSION

Bovine mastitis is a common disease entity of dairy cows, accompanied by physical, chemical, pathological and bacteriological changes in milk and glandular tissue (Samad, 2008). It is a harmful disease affecting the dairy industry worldwide and is a matter of great concern for leading milk producing country because of the losses incurred due to high morbidity, discarded milk, treatment costs and reduced milk production, thus drawing in more attention towards its treatment and control (Mohanty et al., 2013).

Apart from the economic losses, mastitis can have serious implications on public health. Mastitis which is mostly caused by the interaction of multiple pathogenic agents (primarily bacteria), can expose human beings to various organisms through infected milk, thus serving as a media for transmission of various zoonotic diseases like T.B, brucellosis, diphtheria, scarlet fever and Q fever (Mahantesh and Kaliwal, 2011).

In fact, *S. aureus* was one of the most frequently isolated staphylococci, supporting the assertion that this microorganism numbers among the main mastitis pathogens in the Czech Republic (Rysanek et al., 2007). In this study the isolation of *E. coli*, *Bacillus* spp., and *Enterobacter* spp., might be attributed to poor or absence of hygiene. This suggestion was supported by the statement of Quinn et al. (2004) who mentioned that *Bacillus cereus* and *E. coli* were isolated from mastitic milk of bovine. These results collectively support our results in this study also the result is in agreement with Sudhan et al. (2005).

The detection of *Actinomyces bovis* in the mastitic milk in this study was in agreement with Quinn et al. (2004) who mentioned that this bacterium among a rarely Gram- positive rod – shaped causing bovine mastitis.

In this study found the isolates were sensitive to tylosin and enrofloxacin sensitive, these antibiotics are used either for treatment of clinical cases to avoid the spreading of the causative agent, in prophylactic measures to eliminate the susceptibility of animals for prevention of new infection or growth promotion in weight gain for fattening programmes. This study is an agreement with Anon (2011), who reported that indicated for the treatment of local signs (inflammation, milk quality and yield) associated with per acute/acute mastitis in lactating dairy cattle. Also the isolates in this study were affected with Tylosin and this is an agreement with (Pyorala et al., 1994).

CONCLUSION

In this study we have showed that the incidence of bovine mastitis is high in Omdurman locality. The most frequent isolated bacteria are *Staphylococci*. In addition, other opportunistic and environmental organisms were isolated from mastitic milk samples. Inadequate stall or pasture management e.g. dirty and wet bedding material or muddy areas as well as in proper milking procedures lead to an increased infection risk.

Recommendation

- 1- In dairy farm hygienic procedure must be from Practice sustainable.
- 2- Antimicrobial sensitivity testing should be practiced before treatment of mastitis with antibiotics.
- 3- Farmers should be aware about what suitable antibiotics to be used for specific mastitis case.
- 4- The usage of antibiotics in dairy farm should be under supervision of veterinarian to avoid missed used which leading to the development of antibiotic resistance bacteria.

DECLARATIONS

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Authors' contribution

All authors contributed equally to this work.

Availability of data

The data can be available to the journal upon request.

Consent to publish

Not applicable

Conflict of interest

The authors declare they have no competing of interests.

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PREVALENCE OF BOVINE GASTRO INTESTINAL PARASITIC INFECTION IN AND AROUND KOMBOLCHA TOWN

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 Supporting Information

ABSTRACT: A cross-sectional study was conducted in and around Kombolcha from October 2017 to April 2018 to determine the prevalence of gastro-intestinal helminthes parasites in cattle. A total of 384 randomly selected cattle were sampled and examined using standard coprological procedure. The overall prevalence was 39.8% of gastrointestinal (GI) helminthes and the prevalent helminthes eggs identified were 15.6% *Paramphistomum* species (spp), 10.4% strongly type eggs, 8.6% *Fasciola* spp., 3.1% *Trichuris* species and 2.1% *Toxocara* species. This result indicated the highest prevalence of *Paramphistomum* spp. eggs than other helminthes egg and the lowest prevalence of *Toxocara* species egg. There was statistically significant difference among the age groups in paramphistomum and strongly infection ($\chi^2=24.960$, $p\leq 0.001$) and ($\chi^2=17.047$, $p\leq 0.001$) respectively. Higher prevalence rate was shown in 2-5 years age of cattle. Between body conditions there was also significant ($p\leq 0.000$ and $p\leq 0.013$) difference in paramphistomum and strongly and which was higher in moderate animals and lower in animals with good body condition. Sex had no significant effect on the prevalence of helminthes parasite, except for strongly type of egg. The present study revealed that there is high prevalence of GI helminthes infection in cattle in the study area. Therefore, strategic prevention should be advocated to prevent the problem in and around Kombolcha.

Keywords: Cattle, Gastrointestinal, Prevalence, Helminthes parasites, Kombolcha

INTRODUCTION

Ethiopia is a home for about 54 million cattle, 25.5 million sheep, 24.06 million goats, 7 million equines, 1.25 million camels and 42.1 million poultry. From the total cattle population 98.95% is local breeds with the remaining bear hybrid and exotic breeds (CSA, 2013). In Ethiopia, livestock play an important role in the livelihood of poor farmers as it provides a vast range of services and products such as meat, milk, skin, hair, horns, bones, manure and urine, security, gifts, religious rituals and medicine (Yami and Merkel, 2008). In spite of the large population of cattle, productivity in Ethiopia is low. According to studies in the country, this is due to poor nutrition, reproduction familiarity, management constraints and prevailing animal diseases (Alsan, 2012).

Gastrointestinal helminthes are one of the main problems causing economic losses and diseases in animals. The effect of infection is determined by a combination of factors of which the varying susceptibility of the host species, the pathogenicity of the parasite species, the host/parasites interaction and the infective dose are the most important. The direct losses caused by these parasites are attributed to acute illness and death, premature slaughter and rejection of some parts during meat inspection. Indirect losses include the diminution of productive potential such as reduction of milk production in dairy cow, decreased growth rate, weight loss in young growing calves and late maturity of slaughter stock (Hansen and Perry, 1994).

The most important helminthes parasites in cattle include nematodes (round worms), trematodes (flukes) and cestodes (tape worms). These parasitic infections are problem for both small- and large-scale farmers worldwide, but their impact is greater in sub-Saharan Africa in general and Ethiopia in particular due to the availability of a wide range of agro-ecological factors suitable for diversified hosts and parasite species (Tesfaye, 2006). A number of helminthes species are known to infect cattle worldwide. The most important ones include nematodes like *Strongyle* species (*Haemonchus*, *Ostertagia*, *Trichostrongylus*, *Cooperia*) and trematodes of economic importance *Fasciola* species (*Fasciola hepatica* and *Fasciolagigantica*) and *Paramphistomum* species (*Paramphistomum cervicei*), while cestodes like *Monezia* species (*Monezia benedeni* and *Monezia expansa*) could also be important constraints in animal production (Onah and Nawa, 2000).

There are many risk factors influencing the prevalence and severity of gastro intestinal (GI) helminthes. These include age, sex, weather condition and husbandry or management practices of host species (Khan et al., 2009). Young animals are most susceptible. The effect of these parasites is strongly dependent on the number of parasites and the nutritional status of the animals they are infecting. The major clinical signs are weight loss, reduced feed intake, diarrhea,

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and mortality reduced carcass quality and reduced wool production or quality (Radiostits et al., 2000). Young animals do not have a great deal of immunity to parasites during their first year at pasture. The second year, they have partial immunity and, although they may appear healthy, they eliminate many eggs. Adult animals are much less susceptible to most parasites, unless they are in poor living conditions (Hansen and Perry, 1994).

Animals are sometimes kept in conditions that make them highly susceptible to parasites. In the case of recently dewormed animals, internal parasites no longer exist. There is thus no equilibrium and such an animal put into a contaminated pasture may be seriously affected. Animals in poor condition (e.g., recent illness, food shortages) are also highly susceptible (Keyyu et al., 2003).

Previous reports on prevalence of helminthes parasites of cattle in different areas of Ethiopia showed that 71%, 82.8%, 50.2%, 54.4%, 47.1% and 77.6% which is reported by Manaye, 2002 from highlands of Asella and its surrounding, Etsehiwot, 2004 in dairy cows in and around Holeta, (Regassa et al., 2006) in Western region of Oromia, Berhanu, 2008 in West Shoa zone, Ephrem (Ephrem, 2007) in Addis Ababa dairy farms and Cherinet, 2009 in small holder dairy farms of Jimma town, respectively. A study conducted in and around Holeta in Ormambia region, Ethiopia, indicated that the overall prevalence parasitic infections of cattle were 82.8%. The predominant helminthes egg identified were trematodes (*Fasciola* and *Paraphistomum species*) (80.6%), *Strongyle* (66.25%), mixed infection (trematodes and *Strongyle*) 63.12%, while others such as *Trichuris* and *Monezia* 1.5% (Etsehiwot, 2004). Therefore, the aim of this study was to determine the current prevalence of Gastro intestinal helminthes parasites of cattle and its associated risk factors.

To identify and determine the major GI helminthes, and its potential risk factors associated with the occurrence of gastro intestinal parasites in affecting cattle's in and around Kombolcha.

MATERIALS AND METHODS

Study area

The study was conducted in Kombolcha town. It is situated in North Eastern part of Ethiopia, at 11°4' 37"N and 39°44' 42"E at a distance of about 375 km from Addis Ababa, the capital of Ethiopia, at south Wollo administration zone of Amhara national regional state. The area has an altitude range of 1500-1840 meter above sea level with three topographic categories 14% high altitude-Dega, 34% mid highland-weinadega, and 52% of low altitude-kola. The area experiences a bimodal rain fall with a minimum annual rain fall of 750-950 mm and a relative humidity from 25-80%. The average monthly recorded minimum and maximum temperature is 11.7°C and 27°C respectively (CSA, 2008).

Study animals

The study was performed on cattle which were randomly selected from those that were brought to the three veterinary clinics that are found in and around Kombolcha. A total of 384 heads of cattle were examined during the study period.

Sample size and sampling methods

Sample was taken from all animals come to three veterinary clinics available in and around Kombolcha town starting from October, 2017 to April 2018 to examine the prevalence of GI parasite infections of bovine in the area. The sample size was determined according to Thrusfield (2005). The other determinants considered in sample size determination were 95% confidence interval and 5% desired absolute precision. Based on the formula a total of 384 cattle were taken as total sample size. Hence the sample size is estimated as:

$$N = \frac{1.96 [P (1-P_{exp})]}{d^2}$$

where;

N = required sample size

P_{exp} = Expected prevalence of nematode parasites

d₂ = desired absolute precision

1.96 = the value of "z" at 95% level of confidence

d = 5% = 0.05

Study design

A cross sectional study was carried out from October, 2017 to April 2018 to estimate the prevalence of bovine GI parasite infection and to identify possible risk factors for the occurrence of GI parasite infection in the area.

Study methodology

Fecal samples were collected directly from rectum of animals using disposable plastic globe. The samples were transferred into a clean fecal sampling bottle carefully and each sample was labeled accordingly and transported to Kombolcha regional veterinary laboratory for parasitological examination. Samples were kept in refrigerator at 4 °C if immediate processing was not possible; however, all samples were processed within 48 hours. During the sample collection different factors like the breeds of animals, age and code given for individual animals as well as sample collection date were recorded for each sampled animals. Also their body conditions were registered. Parasitological

techniques including direct fecal smear, sedimentation and floatation techniques were used to identify the eggs in feces microscopically for presence of parasite ova following their procedures. Identification of the eggs was made on the basis of their morphology. The presence of at least one parasite egg in either of the tests revealed as positive.

Data analysis

The collected data during the study periods were recorded carefully into MS- Excel spread sheet and descriptive statistics was used to determine the prevalence, while Chi-square analysis was employed to test the presence of variation between ages, sex, breed and body conditions of the animals involved in the study. Confidence level was set at 95% with statistical significance tested at $p < 0.05$ was set for significance. All statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software package version 20.0.

RESULTS

A total of 384 cattle were sampled and examined for GI helminthes parasites and 153 (39.8%) were found to infected with one and/or more parasites. The prevalence of different type of parasites in cattle recorded were 60 (15.6%) *Paramphistomum* spp., 40 (10.4%) Strongyle type eggs, 33 (8.6%) *Fasciola* spp., 12 (3.1%) *Trichuris* species and 8 (2.1%) *Toxocara* species. The present study indicated that a higher prevalence of *Paramphistomum* species.

The results showed association between prevalence of GI helminthes parasite and gender of the animal. Out of 130 male animals examined, prevalence of *Paramphistomum*, *Strongyle*, *Trichuris* *Fasciola*, and *Toxocara* were reported as 25 (6.5%), 9 (2.3%), 7 (1.8%), 3 (0.8%), (0.3%); whereas 254 female animals examined were infected with *Paramphistomum* 35 (9.1%), *Strongyle* 31 (8.1%), *Fasciola* 26 (6.8%), *Trichuris* 9 (2.3%), and *Toxocara* 7 (1.8%) parasite. There was relatively higher occurrence of all GI helminthes in female animals than male animals. But sex had no significant effect on the prevalence of helminthes parasite, except for strongyle type egg. $P \leq 0.001$. The prevalence study in the different age groups was also conducted and it was observed to be 9.6%, 20.9% and 9.3% in age categories of less than 2 years, 2 year to 5 year, and greater than 5 years respectively (Table 1). Higher prevalence rate was shown in 2- 5 years age of cattle. There was statistically significant p-value difference among the age groups in paramphistomum and strongyle ($\chi^2=24.960$, $P \leq 0.001$) and ($\chi^2=17.047$, $P \leq 0.001$). Comparison of different breeds of animals showed that there was significance difference only in strongyle type eggs ($\chi^2=6.163$, $P \leq 0.001$) with the prevalence of helminthes parasites (Table 3).

Table 1 - Prevalence of the helminthes parasite between sexes of animals

Items	Male	Female	Total	χ^2	p value
Paramphistomum	25 -6.50%	35 -9.10%	60 -15.60%	1.938	0.164
Strongyle	9 -2.30%	31 -8.10%	40 -10.40%	17.047	0
Fasciola	7 -1.80%	26 -6.80%	33 -8.60%	2.577	0.108
Trichurus	3 -0.80%	9 -2.30%	12 -3.10%	0.434	0.51
Toxocara	1 -0.30%	7 -1.80%	8 -2.10%	1.664	0.197
Total	45 -11.70%	108 -28.10%	153 -39.80%	—	—

Table 2 - Prevalence of helminthes parasite in different age groups

Items	<2 age	2-5 age	>5 age	Total	χ^2	p value
Paramphistomum	9 -2.30%	43 -11.20%	8 -2.10%	60 -15.60%	24.96	0
Strongyle	15 -3.90%	17 -4.40%	8 -2.10%	40 -10.40%	17.047	0
Fasciola	9 -2.30%	16 -4.20%	8 -2.10%	33 -8.60%	5.138	0.077
Trichurus	3 -0.80%	3 -0.80%	6 -1.50%	12 -3.10%	1.939	0.379
Toxocara	1 -0.30%	1 -0.30%	6 -1.50%	8 -2.10%	4.445	0.108
Total	37 -9.60%	80 -20.90%	36 -9.30%	153 -39.80%	—	—

Table 3 – Prevalence of different GI helminthes in animals of different body condition						
Items	Poor	Moderate	Good	Total	Total	χ^2
Paramphistomum	20 -5.20%	35 -9.10%	5 -1.30%	60 -15.60%	18.512	0
Strongyle	20 -5.20%	20 -5.20%	0 0.00%	40 -10.40%	6.163	0.013
Fasciola	5 -1.30%	26 -6.80%	2 -0.50%	33 -8.60%	5.867	0.053
Trichurus	1 -0.30%	9 -2.30%	2 -0.50%	12 -3.10%	1	0.607
Toxocara	2 -0.50%	5 -1.30%	1 -0.30%	8 -2.10%	0.697	0.706
Total	48 -12.50%	95 -24.70%	10 -2.60%	153 -39.80%	–	–

Table 4 - Prevalence of different GI helminthes between cattle breeds					
Items	Male	Female	Total	χ^2	p value
Paramphistomum	51 -13.30%	9 -2.30%	60 -15.60%	2.67	0.102
Strongyle	37 -9.60%	3 -0.80%	40 -10.40%	6.163	0.001
Fasciola	22 -5.70%	11 -2.90%	33 -8.60%	2.091	0.148
Trichurus	7 -1.80%	5 -1.30%	12 -3.10%	2.378	0.123
Toxocara	6 -1.60%	2 -0.50%	8 -2.10%	0.015	0.902
Total	123 -11.70%	30 -28.10%	153 -39.80%	–	–

DISCUSSION

The overall prevalence of helminthes infection of cattle in the present study was 39.8%. This result is very close to the report on gastrointestinal helminthes prevalence rate of 41.2% (Ephrem, 2007) and 26.3% (Darsema, 2009) in Western Amhara region, Ethiopia respectively. In addition, Keyyu et al. (2006) reported an overall prevalence of 44.4 and 37.0% for large and small scale dairy cattle, respectively in Tanzania. In contrast, the present study was lower as compared to the prevalence of GI helminthes obtained in dairy cows by Cherinet (2009) and Etsehiwot (2004) who indicated 77.6% in small holder dairy farms of Jimma town and 82.8% in dairy cows in and around Holeta respectively. Differences in the prevalence of GI parasite (Table 4) between the different studies could be due to variation in management system, topography, deworming practices, and climatic condition that favor the survival of infective stage of the parasite and intermediate hosts.

According to the current study result which indicated the prevalent helminthes egg were 60 (15.6%) *Paramphistomum* spp., 40 (10.4%) Strongyle type eggs, 33 (8.6%) *Fasciola* spp. 12 (3.1%) *Trichuris* species and 8 (2.1%) *Toxocara* spp. In this result, the *Paramphistomum* species were highly prevalent than other parasite.

The present study showed that, there was higher occurrence of all GI helminthes in female 108 (70.6%) animals than male 45 (29.4%) animals. But sex (Table 1) had no influence on the prevalence of helminthes parasite. Insignificant difference between sexes is similar with previous results reported (Teku, 2008; Manaye, 2002) except significant difference between sexes on the prevalence of strongyle species which was 31 (12.2%) in females and 9 (6.9%) in males.

A significant variation was observed between different age (Table 2) groups in which young animals were higher number of eggs than adults particularly for paramphistomum and strongyle. This might be due to a limited previous exposure and immaturity of the immune system that resulted in higher development of the parasite. This finding is in harmony with reports of (Manaye, 2002) on bovine GI helminthes in Asella and its surrounding highlands. Watson and Gill (1991) reflected common ground which young animals are believed to be more susceptible to parasitic and non-parasitic infections.

The coprological examination of collected fecal sample revealed there was significant difference among body condition) of paramphistomum and strongyle. Which was higher in lower body condition animal's and lower in good body condition animals. This finding contradicts the findings of Manaye (2002) who reported absence of significant difference on the prevalence of helminthes in animals of different body condition. This might be that the animal in previous study

done by Manaye (2002) could be in the good plane of nutrition that enables them to support parasite infection without showing clinical helminthiosis. But animals in the current study were possibly feed on crop residue like wheat and teff straw that is less nutritious, and infected animals can easily show clinical helminthiosis.

CONCLUSION AND RECOMMENDATIONS

Based on the current study the most predominant GI helminth parasites identified in this study were *paramphistomum*, *strongyle*, *Fasciola*, *Trichuris* and *Toxocara*. Geographical location of the study area, body condition, age, sex, and anthelmintic therapy status considered as risk factors for helminthes infection; and had a varying degree of contribution for helminthes infection. The overall prevalence and the prevalence of the different types of parasites of cattle recorded in the current study are high enough to limit and constraint cattle production of the district. Based on the above conclusion, the following recommendations are forwarded:

- Intensive emphasis should be given for prevention of GI helminthes parasites in and around Kombolcha as the prevalence was found high.
- Cattle should be treated with effective broad spectrum anthelmintic as there were many co-infection cases in the study areas.
- Young cattle should receive great attention as they were found significantly susceptible categories to helminthiosis.
- This study did not consider the management and feeding systems, seasonal helminthes dynamics, and identification of parasite to species level. Therefore, future detailed works should be undertaken.

DECLARATIONS

Consent to publish

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

MB conceived the study, coordinated the overall activity, and carried out the statistical analysis, drafted the manuscript and participated in the design of the study, and reviewed the manuscript. All authors read and approved the final manuscript. AY participated in drafting and reviewing the manuscript. MA conceived the study, coordinated the overall activity, and reviewed the manuscript and participated in drafting and reviewing the manuscript.

Availability of data and materials

Data will be made available up on request of the primary author

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COMPARATIVE ADVANTAGES OF CERVICAL INSEMINATION OVER NATURAL MATING ON PRODUCTION OF CROSSBRED LAMBS FOR EXPORT MARKET

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✉Supporting Information

ABSTRACT: A study was conducted at Debre Birhan agricultural research center to evaluate the success of artificial insemination (AI) following oestrous synchronization in Washera ewes. A total of 32 ewes were selected from this center. All experimental animals were synchronized using single injection of 1 ml Enzaprost® intramuscularly at unknown stage of estrous cycle. Then ewes were divided into Dorper and Awassi fresh semen cervical insemination. Fixed time cervical insemination was performed in estrous ewes 48-51 hrs following 1 ml Enzaprost® injection with 0.25 ml fresh diluted Dorper and Awassi semen. Out of 32 ewes synchronized, 22 (68.8%) of ewes exhibited overt sign of oestrous within 48 hrs of hormone administration. Ewe's body condition score (BSC) wasn't found to be a significant factor determining oestrous response to hormone treatment. The overall pregnancy, lambing and fecundity rates for cervical insemination and natural mating were 63.64, 77.27, 121.43% and 57.46, 61.57, 107.14%, respectively. There was no significant difference in pregnancy, lambing and fecundity rates among ewes mated with fresh semen cervical insemination and controlled ram mating ($P < 0.05$). The pregnancy, lambing, and fecundity rates for Awassi and Dorper semen were 58.3, 75.0, 128.57% and 70.0, 80.0, 114.29%, respectively. No significance differences were observed in pregnancy, lambing and fecundity rates among the genotypes ($P < 0.05$). The current work strongly indicated that artificial insemination following oestrous synchronization has a tremendous impact on terminal crossing, that allows us to import fewer exotic genotypes and producing large number of crossbreed lambs from imported breed with in few years.

Keywords: Artificial insemination, Commercial lamb production, Washera, Enzaprost

INTRODUCTION

Ethiopia is home for diverse small ruminant population, numbering 30.7 million sheep and 30.2 million goat head (CSA, 2017). They account for 40% of cash income earned by farm households, 19% of the total value of subsistence food derived from all livestock production, and 25% of total domestic meat consumption (Hirpa and Abebe, 2008). Despite the large numbers and the multiple roles small ruminant productivity in Ethiopia, is considered low as compared to productivity levels in developed livestock industries of the world (Gizaw et al., 2010). This low level of productivity could be attributed to several factors, chief among them is low genetic potential of the animals (Abebe et al., 2010; Kosgey et al., 2006; Tibbo et al., 2006).

Ethiopia meat exporters are not competent in the world market due to lack of supply from the traditional sheep husbandry that meets the export market standard. Since 2012 G.C., Debre Birhan agricultural research center (DBARC) has conducted research to evaluate combining ability of local ewes (Washera and Bonga sheep) with Awassi rams in growth and carcass traits. The experiment was designed to utilize the fast growth and larger carcass of exotic breed to satisfy the required export market standard. The study revealed that crossbred lambs that were finished at early age (on seven month of age) could satisfy the required export market standard (DBARC unpublished data).

However, reproduction data recorded in DBARC from 2013 to 2017 G.C., under terminal crossing of Washera ewes using Awassi rams showed that lambing was occurred in scatter manner (within 140 days of the commencement of lambing). This has great impact on effectiveness of terminal crossing and management of lambs to finish at early age. However, artificial insemination (AI) following oestrous synchronization could be a solution for achieving planned and concentrated lambing without significantly affecting pregnancy. In addition AI could be a solution to import fewer exotic genotypes and allows us to produce large number of crossbreed lambs from imported exotic sheep breed. Therefore, the objective of this study was to evaluate success of artificial insemination following oestrous synchronization in Washera sheep.

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MATERIALS AND METHODS

Experimental protocol was approved by the Amhara Agricultural Research center (ARARI), Bahir Dar, Ethiopia. This experiment was conducted on on-station, called Debre Birhan Agricultural research Center (DBARC), Debre Birhan, Ethiopia.

Location of study area

DBARC is located 120 km North-east of Addis Ababa at an altitude of 2,765 m.a.s.l. and at a latitude of 09°36'23"N and longitude of 39°39'10"E. Debre Birhan is characterized by a bi-modal rainfall pattern, where the main rainy season is from June–September accounting for 75% of the total rainfall, an erratic unreliable short rainy season is February, March–April, May and a dry season October–January. Based on the meteorological data obtained from DBARC, the average annual rainfall is 923 mm. The mean annual maximum temperature is 19.87 °C occurs between March and June, while the mean annual minimum temperature is 6.59 °C occurs between October and January.

Experimental animals and their management

A total of 32 Washera ewes were used for AI experiment. Ewe's age ranges from 4 to 6 years and that gave at least one birth, which their body condition score (BCS) are 2.5 and above and ewes not suckling were used in this trial. Whereas, in natural controlled mating system all ewes were sired by pure Awassi in a group of 26-30 ewes per ram and mating were lasted on average between 51 and 90 days. All experimental ewes were housed in the night and allowed to graze during the day on natural pasture daily for 6 hrs. In addition, the experimental animals were supplemented with 200g head/day mixed commercial concentrate consists of 33% noug (*Guizotia abyssinica*) cake, 65.5% wheat bran, 1% limestone and 0.5% salt. The animals had free access to fresh water twice a day. As a routine flock health management practice of the research center the experimental animals were drenched against internal parasites (Rafoxanide at 1ml/4kg body weight, Chanelle pharmaceuticals manufacturing Ltd., Ireland and Tetraclozash-900® at 1 bolus/30kg body weight, Ashish life Science Pvt. Ltd., India) and were vaccinated against Ovine Pasteurellosis, Peste des Petitis Ruminants (PPR), Sheep and Goat Pox, Blackleg and Anthrax (National veterinary institute, Debrezeit, Ethiopia) in the different seasons of the year before the beginning of the experiment. Moreover, the experimental animals were sprayed against ectoparasites (Diazinol 60% E.C at 1ml/1Lit. of clean water, Kafr EL Zayat pesticides and chemicals CO., Egypt) in July 2017.

Oestrous synchronization

All experimental ewes received single injection of 5 mg of the PGF2α analogue dinoprost (1 ml Enzaprost®; CEVA laboratories, Libourne, France) intramuscularly at unknown stage of estrous cycle. Then ewes were subdivided into two groups randomly by blocking the animals for BCS and allocated to Awassi and Dorper fresh semen cervical insemination. Ewes were observed for oestrous at 4 hrs intervals for a period of 48 hrs. Ewes were kept indoor the whole days during oestrous identification. Ewes at heat were identified using proven ram fitted with an apron. Standing to be mounted was the key sign used to determine oestrous response.

Semen collection and artificial insemination

Semen was collected from Awassi and Dorper rams trained to serve an artificial vagina (AV), after allowing the rams to mount in estrus ewes. Collected semen was immediately evaluated for volume, appearance (color and contamination), sperm cell concentration and mass motility. The volume of semen was measured with a calibrated collection glass and sperm cell concentration was evaluated using AccuRead IMV Technologies SA, 232 Spectrophotometer. Sperm mass motility was estimated subjectively by using phase contrast microscope (Scope Technology Scope Photo 3.0.12). The qualified semen to be used for AI was diluted with OviXcell extender (preservation medium for ovine semen, IMV Technologies, France). Fixed time cervical AI was performed in estrus ewes 48-51 hrs following 1 ml Enzaprost® administration with 0.25 ml diluted fresh semen (approximately 150×10⁶ sperm/straw and its mass motility score 3 and above) using a speculum equipped with a light source and an insemination gun, slowly releasing the semen into the first fold of the cervix.

Statistical analysis

The data were analyzed using Statistical Package for the Social Sciences (IBM SPSS version 20). Effects were considered to be significant when the level of probability was 5% or less.

Oestrous response, pregnancy, lambing, and fecundity rate were calculated by using the following formula:-

$$\text{Oestrous response} = \frac{\text{ewes show oestrus}}{\text{ewes administered hormone}} \times 100$$

$$\text{Pregnancy rate} = \frac{\text{number of ewes pregnant}}{\text{number of ewes inseminated}} \times 100$$

$$\text{Lambing rate} = \frac{\text{number of lambs born alive}}{\text{number of ewes inseminated}} \times 100$$

$$\text{Fecundity rate} = \frac{\text{number of lambs born alive}}{\text{number of pregnant ewes}} \times 100$$

RESULTS AND DISCUSSION

Oestrous response

As indicated in Table 1, out of 32 ewes treated with single injection of PGF2 α analogue dinoprost (1 ml Enzaprost®), 22 (68.8%) ewes exhibited overt signs of oestrous within 48 hrs. of hormone administration. The current result revealed that oestrous could be effectively synchronized using single injection of PGF2 α analogue dinoprost (1 ml Enzaprost®) for achieving concentrated lambing and producing large number of crossbred lambs using AI within few years. Oestrous response attained in this trial was comparable to values (65%) reported by Mekuriaw et al. (2015) in Menz sheep synchronized with single injection of PGF2 α (2 ml Lutalyse® and 1 ml Synchronate®). Findings of the current study were however, higher than those obtained by Gizaw et al. (2016) who reported 57.5% oestrous response in Tigray highland sheep synchronized with single injection of PGF2 α (2 ml lutalyse®) within 96 hrs. post hormone administration. However, it was lower than values reported by Gizaw et al. (2016) who recorded oestrous response of 80% in Washera sheep treated with single injections of PGF2 α (2 ml Lutylase®). The differences in oestrous responses reported in different studies might be due to differences in breed, season and overall management conditions of the animals. In the current study, ewe's BSC wasn't found to be a significant factor determining oestrous response to hormone treatment. This may be due to ewes included in this experiment had a BCS of 2.5 and above. Ewes BCS of 2.5 and above are recommended BCS for achieving good reproduction in sheep. Santoralia et al. (2011) reviewed the factors affecting efficiency of synchronization indicated that high BCS has been associated with an increase of ovulation, with recommended BCS of 2.5–3.0 and a score of <2 resulting lowest pregnancy rates in sheep.

Table 1 - Estrus response of Washera ewes to estrus synchronization protocol

Parameter	n	Oestrous response (%)	X2	P value
Body condition			1.663	0.265
Between 2.5 and 3.0	17	58.8		
3.5 and above	15	80.0		
Overall	32	68.8		

Pregnancy, lambing and fecundity rates

To evaluate the comparative advantages of cervical insemination following oestrous synchronization over natural mating on production of crossbreed lambs, five years reproduction data ($n=268$) recorded under controlled natural mating at DBARC were used as a comparison. The overall pregnancy, lambing and fecundity rates recorded in the natural mating were 57.46, 61.57 and 107.14% respectively, while the overall pregnancy, lambing and fecundity rates recorded following oestrous synchronization and AI were 63.64, 77.27 and 121.43%, respectively. There was no significant difference in pregnancy, lambing and fecundity rates among ewes mated with fresh semen cervical insemination and controlled ram mating ($P < 0.05$). Moreover pregnancy, lambing and fecundity rates for Awassi \times Washera and Dorper \times Washera were 58.3, 75.0, 128.57% and 70.0, 80.0, 114.29%, respectively. No significance differences were observed in pregnancy, lambing and fecundity rates among the genotypes ($P < 0.05$).

The current pregnancy, lambing and fecundity rate falls in an acceptable range. According to Allaoui et al. (2014) in sheep, fertility rates ranges from 60 and 100% qualified as acceptable performance, in this regard the present study revealed that implementation of fresh semen cervical insemination in Awassi \times Washera terminal crossing could be a solution for achieving concentrated lambing and also allows us to producing large number of crossbreed lambs from imported breed. The overall pregnancy and lambing rates achieved in the current study under fresh semen cervical insemination was comparable to values reported by Allaoui et al. (2014); Kumar et al. (2015); Pervag et al. (2010); Najafi et al. (2014). However, it was lower than values obtained by Mekuriaw et al. (2005) who reported pregnancy rates of 70.6 and 70.4% in Dorper sheep synchronized with MAP and FGA sponges respectively. Similarly Fornazari et al. (2018) reported pregnancy rates of 76.5% in Assaf sheep synchronized with progestagen sponges. However, the current result was higher than value obtained by Olivera-muzante et al. (2011) who reported a pregnancy rate of 51% synchronized with two doses of PGF2 α 7 days apart and inseminated cervically with fresh semen. Pregnancy rate under fresh semen cervical insemination was not significantly influenced by BCS of ewes on the other hand, ewes BCS was found to be a significant factor determining lambing and fecundity rates (Table 2). Study in Suffolk ewes showed that BCS of ewes was not significantly affected pregnancy rate (Fukui et al., 2010). However, it is contrary to the findings of Gizaw et al. (2016) who reported the significant implication of BCS on fertility rates of ewes. Under natural mating experiment, effect of ewe's BCS on pregnancy, lambing and fecundity rates were not considered because in case of natural mating ewes were allocated to rams based on their live body weight without scoring their BCS. However, as routine flock management of the research center ewes that had poor BCS were not included in the mating group.

Table 2 - Pregnancy, lambing and fecundity rates (%) in Washera sheep

Factors	n	Pregnancy rates	Lambing rates	Fecundity rates
P-value		ns	ns	ns
Natural mating	268	57.46	61.57	107.14
Artificial insemination	22	63.64	77.27	121.43
Genotype		ns	ns	ns
Awassi × Washera	12	58.3	75.0	128.57
Dorper × Washera	10	70.0	80.0	114.29
Body condition score		ns	*	*
Between 2.5 and 3.0	10	70.0	110.0 ^b	157.14 ^b
3.5 and above	12	58.3	50.0 ^a	85.71 ^a

^{a, b} On the same column, numbers bearing the same superscript are not statistically different at $p < .05$; ns: not significant.

CONCLUSION

The current work strongly indicated that oestrous could be effectively synchronized using single injection of 1ml Enzaprost®) for achieving concentrated lambing in Washera sheep. This study revealed that no statistical significant difference was observed in pregnancy, lambing and fecundity rates among ewes mated with fresh semen cervical insemination and controlled ram mating. AI could be thought possible as a strategy in production of crossbred lambs for export market. Fresh semen AI has a tremendous impact on terminal crossing, allows us to import fewer exotic genotypes and producing large number of crossbred lambs from imported breed within few years.

Recommendations

For successful oestrous synchronization application ewes that have a body condition score of 2.5 or above should be considered. In addition to the use of fresh semen AI, the success rate of AI with chilled and frozen ram semen in Washera sheep needs to be further investigated.

DECLARATIONS

Authors' contribution

Shanbel B contributed to the research design, analysis, interpretation of the data and writing the manuscript. Chekol D contributed to prostaglandin administration. Asfaw B, Ayele A, Aschalew A, Shenkute G and Tesfaye Z contributed to field implementation of the research work.

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Conflict of interest

None of the authors have any conflict of interest to declare.

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HEMATO-BIOCHEMICAL PROFILES OF SHEEP INFECTED WITH FASCIOSIS IN COMPARISON WITH HEALTHY CONTROLS

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✉ Supporting Information

ABSTRACT: Fasciolosis can cause considerable change in hematological parameters and liver driven serum enzymes. Hence, the aim of this research is to assess the hematological and biochemical changes in sheep infected with Fasciolosis in comparison with healthy controls. A total of 52 local breed (26 from naturally infected and 26 from healthy control group) matched with sex and age were enrolled in the study. Five ml blood using EDTA vacutainer tube for hematology and another 5 ml blood by serum separating tubes for serum biochemical profiles were taken and analyzed using Sysmex automated hematological analyzer and Vegasys chemistry analyzer respectively. The hematological analysis indicated there were significant mean reductions ($P < 0.001$) in tRBC, hemoglobin, hematocrit, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH); Mean corpuscular hemoglobin concentration (MCHC) in case group (5.62 ± 1.15 , 8.92 ± 1.66 , 20.12 ± 5.78 , 26.05 ± 4.16 , 9.444 ± 2.00 and 29.72 ± 2.55) than control group (10.27 ± 1.36 , 12.68 ± 1.66 , 38.9 ± 6.05 , 40.50 ± 3.35 , 13.100 ± 1.35 and 34.327 ± 3.06) respectively. On the contrary the mean value of tWBC and eosinophil were significantly higher in the infected group (11.27 ± 2.7 and 7.19 ± 2.81) than the flock free of Fasciolosis (7.12 ± 2.61 and 2.82 ± 1.07) respectively. Regarding biochemical parameters, the significant mean elevation ($P < 0.001$) in serum ALT (125.92 ± 20.71) and AST (34.73 ± 11.97) in the infected sheep were observed than ALT (77.04 ± 13.96) and AST (23.69 ± 7.37) of the control groups. Whereas, the serum total protein and glucose level (5.17 ± 1.05 and 21.15 ± 11.08) in Fasciola infected sheep were significantly lower ($P < 0.001$) than healthy control (7.33 ± 1.06 and 32.62 ± 9.48 , respectively). The alteration in hemato-biochemical profile suggested a great impact of Fasciolosis on liver physiology leading to loss of production and productivity in sheep industry.

Keywords: Biochemical Profile, Fasciola, Hematology, Sheep.

INTRODUCTION

The liver plays a central role in the body of organisms. Hepatic pathology may result in significant disturbances and changes in blood components, carbohydrate and protein metabolisms as well as bile flow and composition. Certain hemato-biochemical changes are essential feature hepatocytes infection (Grunwaldt et al., 2005).

In Fasciolosis, the metabolic processes of the liver are gradually reduced (Doaa et al., 2007). As a result, the biochemical molecules like blood glucose, liver derived serum enzyme, serum proteins, and others are altered following injury and damage of hepatocytes (Behm and Sangster, 2006).

Liver enzymes are known to have intracellular action and their levels in the blood are very low under normal condition. Any increments in the systemic circulation are evidence of enzyme release due to tissues damage (Grunwaldt et al., 2005). The migration of larvae in the liver parenchyma is the primary cause for hemorrhage and severe tissue damage responsible for alteration of hematological and biochemical parameter related to the Fasciolosis (Mas-Coma et al., 2009). Consequently, exposed animals are predisposed to secondary bacterial infections (Anosike et al., 2005).

The aim of the study was to assess the hematological and biochemical changes in sheep naturally infected with Fasciolosis in comparison with health control groups.

MATERIALS AND METHODS

Study population

Fifty-two local breed sheep above 2 years of age from both sex reared under extensive farming system enrolled to this study. Sheep naturally infected with Fasciolosis were considered as case group while animals which were free of Fasciolosis during the study period were taken as healthy control group.

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Sampling methods

The study animals were purposively selected by faecal sedimentation techniques to confirm the presence/ absence of *Fasciola*'s eggs. Sheep with the EPG (Egg count per gram of faeces) above 200 were selected as infected group and sheep which were negative at the time of examination were considered as health control group with additional treatment with single dose of Triclabendazole 10 mg/kg to avoid infection with immature stage. Both groups were kept under similar diet and management system.

Analysis of hematological parameters

Five milliliters of venous blood were taken from the jugular vein into Ethylene diamine tetra acetic acid (EDTA) (HiMedia Laboratories LTD®, Mumbai, India) tubes and stored at +4 °C. The CBC (complete blood count) were measured using an automated Sysmex hematology blood analyzer.

Analysis of biochemical parameters

Another five milliliters of blood were drawn from jugular vein into serum separating tubes and allowed to stand in undisturbed and slanted position for three to four hours. The clot was retracted to separate the serum. The collected serum was stored at -20°C in serum vials, which were properly capped and labelled. Serum level aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP) and glucose were measured according to standard procedures by Vegasys Clinical chemistry analyzer using enzymatic methods at 37 °C.

Data analysis

Data analysis was done by STATA software ver.12. Significant differences on the hematological and biochemical parameters between infected and control group were determined by t-test. Results are expressed as mean \pm SD (standard deviation). Differences were considered as significant when $P < 0.05$.

Ethical statement

Ethical clearance was obtained from the Research and Ethical Committee of University of Gondar. Permission approval was also obtained from the district and verbal consent from each owners during selection of the animals. The infected animals were ethically euthanized and the health control group was transferred to Anatomy department for teaching learning process.

RESULTS AND DISCUSSION

Hematological profile

Fasciolosis causes hematological changes on the host that harbor the parasites. The current study indicated the change in hematological values of RBC and its components such as total red blood cell (tRBC), hemoglobin (Hb) and hemotocrite (Hct). The infected sheep showed significant mean reduction ($P < 0.001$) in tRBC ($5.62 \pm 1.15 \times 10^6/\mu\text{l}$) compared to the control group ($10.27 \pm 1.36 \times 10^6/\mu\text{l}$). Accordingly, the mean value in Hb and Hct were significantly reduced ($P < 0.001$) in *Fasciola* infected sheep than non-infected groups. There was significant mean variation in Hb and Hct in infected group (8.92 ± 1.66 g/dL, 12.68 ± 1.66 %) and health controls (20.12 ± 5.78 g/dL, 38.9 ± 6.05 %) respectively (Table 1). Fasciolosis were also induced significant change in all RBC indexes. The infected sheep demonstrated significant mean reduction ($P < 0.001$) in MCV, MCH and MCHC (26.05 ± 4.16 fl, 9.444 ± 2.00 pg and 29.716 ± 2.55 g/dL) compared to the non-infected sheep (40.50 ± 3.35 fl, 13.100 ± 1.35 pg and 34.327 ± 3.06 g/dL, respectively) (Table 2). Regarding to leukocytes, the total white blood cells (tWBC) and eosinophils count were significantly elevated ($P < 0.001$) in case groups than controls. The mean count of tWBC was significantly raised from 7.12 ± 2.61 to $11.27 \pm 2.7 \times 10^3/\mu\text{l}$ and eosinophils from 2.82 ± 1.07 to 7.19 ± 2.81 % in non-infected group and infected groups respectively. However, there were no significant changes in neutrophils, lymphocytes, basophils and monocytes count between the infected and non-infected group (Table 3).

Table 1 - Erythrocyte values of *Fasciola* infected sheep and control group (n=52)

No	Parameter	Case group (n=26) Mean \pm SD	Control group (n=26) Mean \pm SD	P-value
1	tRBC ($\times 10^6/\mu\text{l}$)	5.62 ± 1.15	10.27 ± 1.36	0.001
2	Hb(g/dl)	8.92 ± 1.66	12.68 ± 1.66	0.001
3	Hct (%)	20.12 ± 5.78	38.9 ± 6.05	0.001

Table 2 - Value of RBC indexes in *Fasciola* infected sheep and control group (n=52)

No	Parameter	Case group (n=26) Mean \pm SD	Control group (n=26) Mean \pm SD	P-value
1	MCV (fl)	26.05 ± 4.16	40.50 ± 3.35	0.001
2	MCH (pg)	9.444 ± 2.00	13.100 ± 1.35	0.001
3	MCHC (g/dL)	29.716 ± 2.55	34.327 ± 3.06	0.001

Biochemical test results

The biochemical analysis of the present study showed the serum level of AST and ALT were significantly increased ($P < 0.001$) in sheep naturally infected with Fasciolosis than the control group. The mean value of serum AST and ALT in animals infected with Fasciolosis were (125.92 ± 20.71 and 34.73 ± 11.97 IU/L) indicated higher elevation compared to the mean value in health controls (77.04 ± 13.96 and 23.69 ± 7.37 IU/L) respectively (Table 4). In contrast, the total protein and glucose determination revealed there were significant mean reduction ($P < 0.001$) in total protein and glucose in case group as compared to the controls. The mean reduction in total protein value from 7.33 ± 1.06 to 5.17 ± 1.05 g/dL and glucose from 32.62 ± 9.48 to 21.15 ± 11.08 mg/dL were observed in comparison between health control and infected groups respectively (Table 4).

Table 3 - Leukocytes values of Fasciola infected sheep and control group (n=52)

No	Parameter	Case group (n=26) Mean \pm SD	Control group (n=26) Mean \pm SD	P-value
1	WBC ($10^3/\mu$ l)	11.27 \pm 2.7	7.12 \pm 2.61	0.001
2	Neutrophils %	65.65 \pm 6.33	68.64 \pm 3.78	0.73
3	Lymphocytes %	21.03 \pm 4.81	20.43 \pm 3.67	0.61
4	Basophils %	0.77 \pm 0.48	0.77 \pm 0.60	0.98
5	Eosinophil %	5.16 \pm 1.22	1.82 \pm 1.07	0.001
6	Monocytes %	7.19 \pm 2.81	8.34 \pm 0.90	0.97

Table 4 - Biochemical profiles of Fasciola infected and control sheep (n=52)

No	Parameter	Case group (n=26) Mean \pm SD	Control group (n=26) Mean \pm SD	P-value
1	AST(IU/L)	125.92 \pm 20.71	77.04 \pm 13.96	0.001
2	ALT(IU/L)	34.73 \pm 11.97	23.69 \pm 7.37	0.001
3	Total protein (g/dl)	5.17 \pm 1.05	7.33 \pm 1.06	0.001
4	Glucose (mg/dl)	21.15 \pm 11.08	32.62 \pm 9.48	0.001

DISCUSSION

Liver fluke infestation in ruminants has important implications on animal health and welfare, farming economics and food production all over the world. It causes heavy loss in production and productivity since the animals became under stress condition.

The current study indicated various changes in hematological parameters. The tRBC, Hb, Hct, showed significant mean reduction in infected groups than non-infected groups. This result is supported by other study conducted by [Pandya et al. \(2015\)](#) who reported severe anemia in sheep infected with Fasciolosis. The lower mean values of total erythrocytes, Hct and Hb in infected sheep might be due to hemorrhage following severe damage by extensive migration of young fluke and blood sucking activity of the adult fluke ([Radostits et al., 2007](#)). Furthermore, the continuous drainage of iron stores and depression of erythropoiesis due to chronic inflammation of liver were thought to be responsible for reduction in total red blood cell and related components ([Berry and Dagie, 1978](#); [Chandra et al., 2011](#)).

The infected sheep demonstrated significant mean reduction in MCV, MCH and MCHC compared to those of control. The finding is in line with the result of [Pandya et al., \(2015\)](#) who recorded microcytic and hypochromic anemia in Fasciola infected animals. However, the current result contrasts with the study conducted by [Egbu et al. \(2013\)](#) who reported high level of MCV, MCH and MCHC in the infected group. The variation could be due to ecological and nutritional differences that greatly affect the hematological profiles. The possible reason for microcytic and hypochromic anemia is related to depletion of iron stores due to damage of liver parenchyma ([Beesley et al., 2018](#)).

The eosinophilia observed in the present study supported by various researchers [Egbu et al. \(2013\)](#); [Pandya et al. \(2015\)](#) and [Matanovic et al. \(2007\)](#) who had similar observation. The elevation of eosinophils is an indication of body defense mechanism against parasitic infection ([Duffus et al., 1980](#)). This is due to inflammation and reaction by secretory antigenic substances released by fluke and cell mediated immunity ([Radostits et al., 2007](#)).

In the present study, the presence of leukocytosis is supported by previous findings reported by [Sykes et al. \(1980\)](#) and [Zhang et al. \(2005\)](#). The net increase in total leukocytes count (WBC) in Fasciola infection may also be due to marked eosinophilia which is associated with parasitic infection. The present study also revealed that there were certain biochemical and enzymatic change related to Fasciolosis. The serum level of liver enzyme (ALT and AST) significantly increased in case group than the control. The findings are in line with corresponding studies conducted by [Pandya et al. \(2015\)](#); [Edith et al. \(2012\)](#); [Yasuda \(1988\)](#) and [Sheikh et al. \(2006\)](#) who mentioned significant increase in mean value of

AST and ALT in Fasciola infected animals. The possible cause is that Fasciola can release reactive oxygen species that can produce damage to cell wall and hepatic tissue which is responsible to the release of intracellular enzyme to the circulation that can increase their serum level (Hodzic et al., 2013).

The significant mean reduction of serum glucose and protein level in sheep infected with Fasciolosis was observed than the flock free of Fasciolosis in this study. This finding agreed with the report of Phiri et al. (2007), who revealed serum glucose and protein values were lower in infected animals than normal. The lower glucose level could be because of inhibition of hepatic glucogenic pathways due to the migration of flukes that causes severe liver pathology and death of hepatocytes (Sharon, 2013).

Similarly, the reduction in total protein level attributed to the damage of hepatocytes by the parasite. The flukes reside in the liver disruption the protein synthesis leading to the development of hypoproteinemia (Martina and Jozica, 2012).

DECLARATIONS

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Authors' contribution

All the five authors reviewed the paper and contributed in developing the content.

Availability of data

The data can be availed to the journal upon request.

Consent to publish

Not applicable

Conflict of interest

The authors declare they have no competing of interests.

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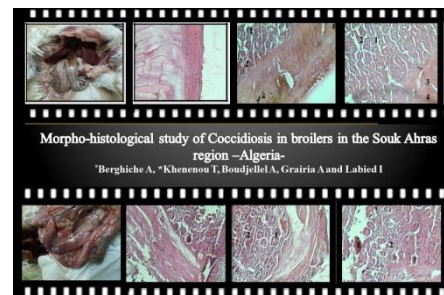
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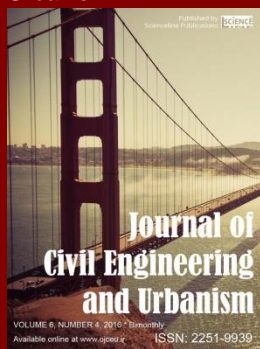
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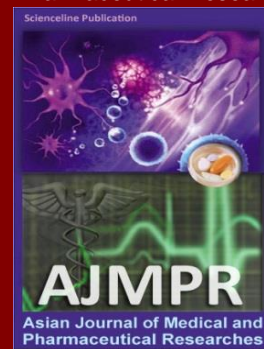
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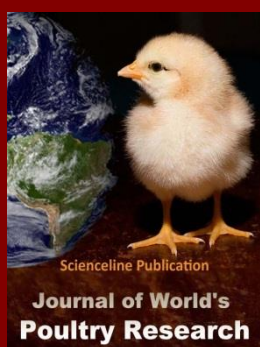
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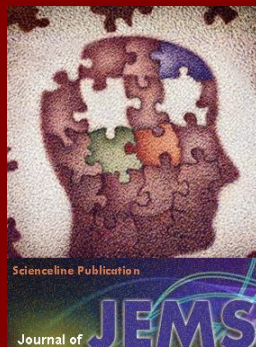
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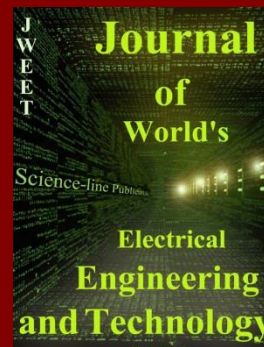
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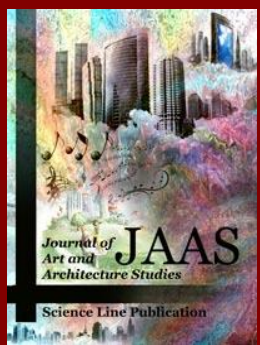
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