TANZANIA VETERINARY ASSOCIATION

PROCEEDING OF THE 35 SCIENTIFIC CONFERENCE OF THE TANZANIA VETERINARY ASSOCIATION HELD AT AICC ARUSHA, TANZANIA ON THE *5TH TO 7TH* DECEMBER, 2017

Volume 35 2017 ISSN 0856 - 1451

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Publishers: Tanzanina Veterinary Association.

Comparative effectiveness of Aloe vera aqueous crude extracts and ivermectin for treatment of gastrointestinal nematodes infection in goats

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SUMMARY

The current study was undertaken to determine the effectiveness of Aloe vera aqueous crude extracts in comparison to Ivermectin in treatment of gastrointestinal nematodes infections in goats at Sokoine University of Agriculture in Morogoro. Goats were examined for GIT nematode infections using modified Mc master technique and those with EPG ≥ 150 were recruited for this trial. Furthermore, the recruited animals were randomly allocated into three groups (@10 animals) that included one control group and two experimental groups. The control group was left untreated while the remaining experimental groups were treated with Aloe Vera aqueous crude extracts and Ivermectin respectively. Faecal samples were collected at day of treatment (day 0) and days 14 and 21 post treatment. The effectiveness of the Aloe Vera and Ivermectin was assessed using Feacal Egg Count Reduction Test (FERT). The anthelmintic was considered to be effective when the calculated FECRT% was ≥ 95% and 95% Lower Confidence Limit (LCL) was ≥ 90%. The day 14 post treatment results of FERT% and LCL for Aloe vera were 97% and 74% while for Ivermectin were 96% and 69% respectively. However, the FERT% and LCL results at day 21 post treatment were 100% for both products. The findings of this study indicate that Aloe vera aqueous crude extracts were effective as Ivermectin in treatment of GIT nematodes infections in goats.

Keywords: Aloe vera, Ivermectin, GIT nematodes, Goats

INTRODUCTION

Gastrointestinal nematodes are responsible for causing huge economic losses in goat productivity worldwide. The GIT nematode parasites that have been reported to infect goat in Tanzania include; *Haemonchus contortus*, *Trichostrongylus* spp, *Oesphagostomum* spp (Connor *et al.*, 1990, Keyyu *et al.*, 2002). *Haemonchus controtus* is ranked as the major constraint to goat productivity in Tanzania (Connor *et al.*, 1990). Control of helminthes infections in domesticated ruminants in the country; largely depend on prophylactic or therapeutic use of broad spectrum anthelmintics (Keyyu *et al.*, 2008). The most commonly used anthelmintics for control of GIT nematode infections in goats by farmers in Tanzania include benzimidazoles (Albendazole), macrocyclic lactones (Ivermectin and imidazothiazoles (levamisole).

The use of anthelmintics as a major means of controlling GIT nematode infections in small ruminants is threatened by development of anthelmintic resistance worldwide (Kaplan 2004; Wolstenholme *et al.*, 2004) including Tanzania where albendazole resistant to *H. contortus* in sheep has been reported (Keyyu *et al.*, 2002). The development of anthelmintic resistance necessitates the searching of new effective alternatives against GIT nematodes (Amhed *et al.*, 2013) and traditional medicinal plants are considered as one of the most promising alternatives (Maphosa *et al.*, 2010).

Worldwide, there are several medicinal plants including Aloe vera are tested for their anthelmintic activity (Eguale *et al.*, 2007). In vitro studies on the anthelmintic activity of aloe vera extracts to the GIT nematode infections in sheep and goats have been reported elsewhere in the world (Maphosa *et al.*, 2010, Ahmed *et al.*, 2013). The later studies indicated that aloe vera extracts had larvicidal and egg hatching inhibition effects for *H. controtus* and the authors recommended for invivo studies on the efficacy of the plant. This study was designed to determine the effectiveness of the aloe vera aqueous extracts on treatment of GIT nematode infections in goats compared to ivermectin as a positive control.

MATERIALS AND METHODS

Preparation of aqueous aloe vera extracts

A crude extract was prepared as described by Kaingu *et al.* (2013) whereby fresh aloe vera leaves were chopped using machete and placed in a rotary blender and blended to slurry. The slurry was then squeezed to give out the crude extract viscous juice which was placed in the glass bottles and stored in a refrigerator.

Experimental design

An experimental study design was adopted in this study. In order to get animals that were used in the selection of groups, all goats at Sokoine University Farms were screened for GIT nematode infections using quantitative floatation method (Modified Mc Master technique). The goats with EPG ≥150 were randomly selected and divided into three groups (control group, aloe vera group and ivermectin group) of 15 animals each.

The control group was left untreated and the remaining two groups were treated with aloe vera aqueous extracts and ivermectin respectively. The concentration of the amount of aloe vera aqueous that was administered to the goats was not established but goats were drenched 5 mls of the prepared extracts. The ivermectin was administered subcutaneously at dosage of 0.2 mg/kg body weight.

Data collection and processing

Faecal samples from each group were collected at day of treatment (day 0) and at days 14 and 21 post-treatment). Collection of samples was per rectum using gloved hand fingers that followed by labeling the samples with permanent maker and eventually the samples were kept in cool and then transported to laboratory for further analysis. In the laboratory the samples were processed by Modified Mc Master method and recovered eggs were examined and counted under compound light microscope so as to establish faecal egg counts for each sample. Identification of eggs aided by using standard morphological keys of GIT nematode eggs of goat (Bowman, 2009). Pre-treatment (day 0) and post-treatment (days 14 and 21) faecal samples were pooled for each group and faecal culture, harvesting and identification of larvae was performed as described by Hansen and Perry (1994).

Data analysis

The analysis of the data was conducted using Faecal Egg Count Reduction Test (FECRT %) as described by Coles *et al*., (1992). In this analysis the post-treatment faecal egg counts of treated groups are compared with that of the control group to compute the percentage reduction of faecal egg counts. The anthelmintic considered to be effective when the percentage reduction is ≥ 95% and 95% Lower Confidence Limit is ≥ 90%.

RESULTS AND DISCUSSION

The pre-treatment faecal culture results indicated that goats at Sokoine University Farms were infected with the following species of GIT nematodes: *Haemonchus contortus* (48.4%), *Trichostrongylus* (21.2%), *Cooperia* (14.8%) species, *Oesophagostomum* (14.4%) and *Strongyloides* (1.2%). Similar results have been recorded in the previous studies at SUA farms by Keyyu et al. (2002, 2003).

This study has indicated that aloe vera aqueous leaves extracts was effective as ivermectin in treatment of GIT nematode infections in goats, as the calculated FECRT% at day 21 was > 95% and 95% LCL was > 90% (Table 1). These findings concur with the previous in vitro studies that reported aloe vera extracts were effective against *Haemonchus contortus* (Maphosa *et al*., 2010, Ahmedi *et al.,* 2012) and *Ascaridia galli* (Kaingu *et al.,* 2012).

Table 1: FECRT% and 95% LCL for aloe vera and ivermectin

Treatment group	Days post treatment	FECRT%	95% LCL
Aloe vera	14	97	74
	21	100	100
Ivermectin	14	96	69
	21	100	100

Moreover, the current study has indicated that ivermectin is still effective for treatment of GIT nematodes infections in goats at SUA Farms. These results agree with previous study at SUA farms indicated that ivermectin was effective against GIT nematode infections in sheep and goats (Keyyu *et al*., 2003). However, GIT nematodes resistance to ivermectin in domesticated ruminants has been reported elsewhere in the world (Geurden *et al.,* 2015). This study clearly indicates that aloe vera aqeous crude extracts had good effects on GIT nematode infections as ivermectin. However, more studies are recommended to evaluate the efficacy of the aloe vera, before it recommended for use to the farmers.

ACKNOWLEDGMENTS

The authors would like to acknowledge Higher learning Student Loan Board and the Late Prof. A.A Kassuku for funding the project. Moreover, authors appreciate the great technical assistance from Mr. L.P Msalilwa and R. Manyesela.

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Prevalence of *Leptospira interrogans* in free range domestic duck (*Cairina moschata*) from selected areas of Morogoro Municipality, Tanzania

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SUMMARY

Leptospirosis is described as the most common and universal zoonotic bacterial disease around the world caused by *Leptospira interrogans* which affects different species of domestic and wild animals. The bacteria may occurs worldwide especially in subtropical, tropical, and wet environment with slightly alkaline soil. The current study was conducted between November 2016 and July 2017 in the selected areas in Morogoro aimed at estimating the prevalence and establishes the common serovars of *Leptospira* in free range ducks. A total of 30 ducks from 12 households were used. Before blood sample collection, the duck biodata was recorded and the owner was asked on the general management system including the scavenging areas and any veterinary intervention in place. The ducks were restrained and blood samples were collected from the branchial vein and left to clot before harvesting the serum. Multiple Agglutination Tests was performed using four serovars namely *Kenya, Grippotyphosa*, *Pomona* and *Hebdomadis* as reference serovars. The results indicated that all the ducks were local breeds (*Cairina moschata*) which scavenged for fed around homestead. The ducks accessed dumping areas; stagnant water, animal houses and the mud around. No veterinary intervention to ducks was reported by all 12 respondents interviewed. Laboratory results indicated that 5 samples (16.7%) were positive at 1:20, 1:40 for Hebdomadis and Kenya serovars which implies that the ducks had been exposed to the specific *Leptospira* serovars. This study reports for the first time the seroprevelence of leptosirosis in ducks in Tanzania. Because of the zoonotic nature of *Leptospira* and sharing of common environment between ducks and humans, the diseases can easily get access to human. Therefore, in the efforts of surveillance and control of leptosisis in humans and animals, ducks should also be involved.

Key words: Leptospirosis, *Leptospira interrogans*, MAT, serovars, ducks

INTRODUCTION

Leptospirosis is the most common and universal zoonotic bacterial disease around the world caused by pathogenic *Leptospira* called *Leptospira interrogans* (Abela- Ridder *et al*, 2010). In Tanzania *Leptospira* infection has been reported in many different animal species including domestic animals such as cattle, sheep, pigs, horses and dogs (Machang`u *et al*, 1997; Mgode *et al.*, 2006). Leptospirosis is transmitted from animal to human through direct and indirect contact with urine, abortion products and other material contaminated with fluids from infected animals (Bharti *et al.*, 2003). A wide variety of wild animal hosts including rodents, bats, possums, deer, mongoose and small insectivores also have been reported to habour the infection (Bharti *et al.*, 2003; Ellis, 2010). Ducks may come into contact with the *Leptospira* in infected stagnant water and in mud while swimming, passing through contaminated water, drinking contaminated water and coming into contact with urine from an infected animal (Mwachui *et al.*, 2015) and outbreaks of disease mainly occur after heavy seasonal rainfall (Lau et *al.*, 2010).

Leptospira are aerobic, Gram-negative spirochetes, with periplasmic flagella, resembling a question mark when viewed through a light microscope and are slow growing. Traditionally, *Leptospira* were divided into two groups; the pathogenic *Leptospira* were all classified as members of *Leptospira interrogans*, and the saprophytic *Leptospira* were classified as *Leptospira biflexa*, (Mohammed *et al*, 2011; Mety and Dikken, 1993). The first step in the pathogenesis of leptospirosis is penetration of tissue barriers to gain entrance to the body. Potential portals of entry include the skin via a cut, genital tract, and the mucous membranes of the conjunctivae or oral cavity swallowing while swimming in contaminated water (Corwin *et a.l*, 1990; Lingappa *et al.*, 2004; Stern *et al.*, 2010). Thereafter, there is hematogenous dissemination: Pathogenic leptospires make their way into the bloodstream and persist there during the leptospiremic phase of the illness (Stern *et al.*, 2010) there after the spirochetes multiply in the

organs mostly the central nervous system, kidney and liver. The symptoms of leptospirosis develop around 7 to 14 days after exposure to *Leptospira* with mild clinical signs namely; chills, high temperature, sudden headache, nausea, vomiting and loss of appetite, muscle pain and conjunctivitis (Beran *et al.,* 1994).

Diagnosis of leptospirosis in human is difficult based on clinical signs, such that may be misdiagnosed and mismanaged as being mistaken for malaria with similar clinical presentation that might contribute to a high rate of infection in the study area. Methods used in detection of leptospires depend on the availability of resources. Detection of *Leptopisra* infection can be done by the use of Microscopic Agglutination Test (MAT) where dark field microscope is used to detect the agglutination. Other methods are culture, Enzyme Linked Immunosorbent Assay (ELISA), staining method and the Polymerase Chain Reaction (PCR).

Morogoro is among the regions with many livestock in Tanzania. The region is also bordered with several wildlife conservations areas which give lot interactions between humans and animals especially in the interface areas. This gives some possibilities for transfer of different diseases causing agents from wildlife to the domestic environment where domestic animals and humans can easily be infected. The average annual rainfall varies between 600 mm and 1800 mm. The eastern part of Uluguru mountain receive high rainfall of about 2850 mm annually, the leeward side of the mountain are generally dry and receive rainfall of less than 600 mm per year (Msanya *et al.,* 2001**).** The nature of the soil in the valleys of Morogoro is nearly neutral to alkaline (Msanya *et al.,* 2001). Residents of Morogoro Municipality also keep a number of animals including ducks which scavenge all over the home environment in search for food. Studies on Leptospira infection in animals has been done in other animals (Machang`u *et al,* 1997; Mgode *et al.,* 2006) but no any study in domestic birds. The purpose of this study was to estimate the prevalence and establish the common serovars of *Leptospira* infection in free range ducks reared in Morogoro Municipality, Tanzania.

MATERIALS AND METHOD

Study area and population

The study was conducted in Morogoro Municipality which is 190 km west of Dar es Salaam. The study flocks were from different suburbs namely Magadu, Falkland, Kididimo and Vibandani. Selection of study site was based on the convenience of accessibility from Sokoine University of Agriculture (SUA) laboratory and generally represented backyard farming of ducks in urban areas in Tanzania. The study population was Muscovy ducks (*Carina moschata*) managed in the backyard and allowed to scavenge freely during the day within the homestead.

Research design and sampling

The purposive sampling method was used during this cross sectional study design. Sampling involved only farmers who willingly agreed to participate in the study. A total of 12 backyard duck flocks were involved in this study. Before sampling, information on the duck biodata, general duck management system including the scavenging areas and any veterinary intervention in place were gathered from the owners. The ducks selected for sampling were manually restrained and 2 ml of blood sample was collected from the brachial vein on the inside of either wings using syringes and needles. The blood samples in the syringes were left at room temperature for one night to clot. The second day serum was harvested into cryovials and stored until analysis.

Laboratory diagnosis of the *Leptospira interrogans*

Microscopic Agglutination Test (MAT) which is considered as a gold standard method for leptospirosis serodiagnosis was carried out. This test was conducted at the Pest Management Center, SUA, and Morogoro, Tanzania. The MAT test was performed using standard laboratory procedure that aimed at detecting the antibodies reaction with the respective local antigens. Live leptospira representing 4 serovars including Kenya, Grippotyphosa local isolate from domestic animals and rodents found in Tanzania, were used. Other serovars that were used as references were *Leptospira* serovar Hebdomadis and Pomona. These serovars were cultured into fresh *Leptospira* EMJH (Ellinghausen and McCullough, modified by Johnson and Harris) culture medium incubated at 30 ^{o}C for 4 to 10 days before using as live antigen in MAT. Antigen density of 300×10^{8} leptospires/ml was used for MAT.

Serum samples were diluted to 1:10 –1:80 with phosphate buffered saline (pH 7.2) in U–bottomed microtiter plate. Live leptospires antigen (50 µl) were added to diluted serum to give final dilutions of 1:20–1:160. The plates with serum–antigen mixture were incubated at 30 ^{o}C for 2–4 hours

before examining for agglutination of leptospires and antibodies under dark field microscope.

RESULTS

General results

A total of 30 adult ducks from 12 households in four streets were sampled for analysis of *Leptospira* infection (Table 1). The ducks appeared apparently healthy. Interviews with owners indicated that all the ducks were local breeds (*Cairina moschata*). There was no formal feeding, rather ducks are left to scavenge for food and in rare occasions they were given kitchen leftovers. The ducks were scavenged for fed around homestead and accessed dumping areas; stagnant water, animal houses and any kind of muddy environment. No veterinary intervention to ducks was reported by all 12 respondents interviewed.

Serological results

The Microscopic Agglutination Test results are shown in Tables 1 and 2. The seropreplevelence of leptospirosis in ducks was 16.7%. Five sera samples ducks were reactive at 1:20, 1:40 for Hebdomadis and Kenya serovars which implies that the ducks had been exposed to the specific *Leptospira* serovars.

Table 1. Number of ducks from each street

Street	Number of households visited	Number of ducks	Number (%) of positive ducks
Folkland	4	6	2 (6.7)
Kididimo	3	10	3 (10.0)
Vibandani	3	8	0 (0.0)
Magadu	2	6	0 (0.0)

Table 2. Seroprevelence of *Leptospira* infection in ducks

Title	Number (%) of positive sera to different serovars				
	Hebdomadis	Kenya	Pomona	Grippotyphosa	Total number (%)
1:20	2 (6.7)	1 (3.3)	0 (0.0)	0 (0.0)	3 (10.0)
1:40	2 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (6.7)
Total	4 (13.3)	1 (3.3)	0 (0.0)	0 (0.0)	5 (16.7)

DISCUSSION

This study was carried out to determine the prevalence of leptospirosis in ducks from Morogoro Municipality. The overall prevalence of leptospirosis in ducks was 16.7%. This shows that a relatively high number of ducks were infected with *Leptospira* in the study areas. There has been a belief that leptospirosis is a disease of mammals only but this study shows that birds are also infected. It is still not clear as to whether the ducks were sick from leptospirosis of were just carriers of the infection since all the screened ducks were apparently healthy. Nevertheless, Beran *et al.* (1994) reported that birds like ducks do not develop clinical leptospirosis when are infected with *Leptospira* but rather develop antileptospiral antibodies. Everand *et al.* (1985) reported a seroprevalence of 11% in chickens. This suggests that it is true that poultry are susceptible to leptospira infections. Whatever the case, ducks in Tanzania are always left to scavenge for food around homestead areas and shed their faecal droppings all over and if infected, *Leptospira* can find their way into the food chain and affect humans. This study showed for the first time the role of ducks as reservoir hosts of *Leptospira* in Morogoro Municipality.

It was further found that 10% the agglutination was observed at the titre of 1:20 and 6.7% the agglutination observed at the titre of 1:40. The titres were extended up to 1:160 but the agglutination was not observed implying that the observed positive results indicated acute infection. The ducks had been recently infected or exposed to *Leptosira* and this shows that the pathogen exists either in the soil, other animals around or in humans. In this case, the ducks can be used as bioindicators of existence of *Leptospira* in the locality.

Of the four serovars tested, Hebdomadis and Kenya reacted positive to some samples. This is the

indication that ducks are more likely to be infected by Herbidomadis and Grippotyphosa serovas. Previous studies in Tanzania have reported the two serovars in humans, fish, domestic and wild animals (Machang`u *et al.*, 1997; Mgode *et al*., 2006). Since the two serovars are already in the surroundings, different animal species will be exposed as has been with the case of ducks. The emergence and endemicity of *Leptospira* in Morogoro Municipality may have resulted from the regular high seasonal rain to the area and due to an increase of pastoral population over the recent years (Machang`u *et al.*, 1997; Mgode *et al*., 2006). The nature of the soil in the valleys of Morogoro is nearly neutral to alkaline (Msanya et al., 2001) which give optimal condition for the survival of the *Leptospira* in the environment.

The questionnaire study further supports the laboratory observation on seropositivity of ducks on *Leptospira*. It was observed that ducks scavenge around areas of homestead and also easily access to dumping areas where every kind of rubbish is thrown including domestic animal manure. Ducks were exposed to muddy environment and wetland areas which may potentially serve as sources of *Leptospira* infections in these birds. Therefore, the intensive management system of ducks can help to minimize the unnecessary exposure of ducks to the contaminated areas which will make them to be safe but also for the affected birds, the chances of contaminating the environment becomes minimal.

It is concluded that ducks have been observed to be seropositive of Leptospira infection in Morogoro municipality which further give evidences of existence of this zoonotic pathogen. Efforts should be put in place to confine the ducks so that to minimize environmental shedding of the pathogens and also to protect the birds from *Leptospira* infection.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support from the Higher Education Student Loan Board HESLB. Sincere thanks are extended to the farmers for accepting to use their ducks for this study samples. Also grateful to laboratory technicians at SUA Pest Management Center for advice and helping in analysis of duck sera samples.

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