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Review

Review on camel trypanosomosis (surra) due to Trypanosoma evansi: Epidemiology and host response

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Trypanosomosis is the most important and serious pathogenic protozoal disease of camel caused by Trypanosoma species. Trypanosoma evansi parasite has a wide range of distribution throughout tropical and subtropical regions of the world. Mostly, camels suffer from trypanosomosis caused by T. evansi that is transmitted mechanically, non-cyclically, by haematophagus flies such as horseflies (Tabanus) and stable flies (Stomoxys) which are endemic in Africa, Asia and South America, although in America the vampire bat also acts as a vector as well as reservoir hosts. The disease manifests itself in different forms: acute, sub-acute, chronic and in-apparent. Anaemia appears to be a major component of the pathology of surra and generally the degree of anaemia might be considered as an indicator of the disease severity. Control of camel trypanosomosis depends mainly on the use of curative and prophylactic drugs even though this strategy is faced with various problems. Surra has a wide host spectrum, the main host species varies with the geographical region. In Africa, beyond the northernmost limits of the tsetse fly belt, and in parts of East Africa, camels are the most important host, whilst in Central and South America the horse is principally affected. In Asia, a much wider range of hosts is involved, including cattle, buffalo and pigs. The disease is most severe in horse, donkey, mules, camels, dogs and cats. T. evansi, like other pathogenic trypanosomes induce a generalized immune-suppression of both humoral antibody response and T cell-mediated immune responses. As a result, in the long term, the host’s immune responses fail and it succumbs to either the overwhelming parasite load or to secondary infection, consequently leading to occurrence of the trypanosome-induced immunopathology. This paper reviews the epidemiology of the disease and host response against the parasite.

Key words: Trypanosomosis, Trypanosoma evansi, flies, hosts.

INTRODUCTION

Trypanosoma evansi (T. evansi), the protozoan parasitic cause of camel trypanosomiasis (Surra), constitutes one of the major veterinary problems worldwide (Omer et al., 2004). The disease is an important single cause of economic losses, causing morbidity of up to 30.0% and mortality of around 3.0% camels in Ethiopia (Njiru et al., 2001; Tekle and Abebe, 2001), in which, as in most dry lands of Africa and Asia, camels are the principal source of income and food for millions of pastoralists and has a population of approximately over 0.807 million heads of dromedary camels (Central Statistical Agency of Ethiopia (CSA), 2010), placing it at the third position in camel rearing countries after Somalia and Sudan, then followed by Mauritania and Kenya in that order (Food and Agricultural Organization of the United Nations (FAO), 2008).

T. evansi is transmitted mechanically, non-cyclically, by haematophagus flies such as horseflies (Tabanus) and stable flies (Stomoxys) which are endemic in Africa, Asia and South America; although in America the vampire bat
also acts as a vector as well as reservoir hosts (Urquhart et al., 1996). Surra is manifesting itself both in acute and chronic forms. Affected camels show fever, anorexia, marked generalized edema and deteriorate rapidly and die; the chronic form is characterized by progressive loss of body weight, intermittent high fever, marked generalized muscular atrophy, pale mucous membranes and occasionally abdominal edema. Affected camels also may exhibit a characteristic sweet odour due to an increase of urinary ketone. The chronic form is most common and is likely to present an association with secondary infection due to immune-suppression caused by *T. evansi* infection (Olaho-Mukani et al., 1993; Ahmed, 2008).

Treatment recommendations and strategies for chemotherapeutic control depend on information of trypanosomosis risk and the prevalence of trypanocidal drug resistance in the area. Sensitive diagnostic techniques are required to detect the parasite and the efficacy of trypanocidal drug treatment. Parasitological methods used in the diagnosis of *T. evansi* in camels are considered easy, rapid and economic. However, they are not sufficient to detect all trypanosome infected animals, especially in case of low parasitaemia and also in the chronic form of the disease (Ahmed, 2008). The serological test such as the card agglutination test (CATT) is used for the detection of antibodies circulating in the serum of infected camels, the test could be used under both laboratory and field conditions. It is a quick, simple and easy to perform and sensitive method. However, serological techniques are not always distinguishing current from past infection due to the prolonged persistence of antibodies in the blood of treated animals (Luckins et al., 1988). Particularly in these cases of treatment success evaluation, DNA based techniques, as polymerase chain reactions (PCR) are useful. These DNA tests are considered sensitive and specific. With the introduction of molecular diagnostic techniques, several diagnostic assays based on the detection of trypanosomal DNA by PCR have been developed.

Surra has a wide host spectrum, the main host species varies with the geographical region. In Africa, camels are the most important host, whilst in Central and South America the horse is principally affected. In Asia, a much wider range of hosts is involved, including cattle, buffalo and pigs. The disease is most severe in horse, donkey, mules, camels, dogs and cats. In Ethiopia, the occurrence of Surra reported as it has been associated with camel rearing areas (Tekle and Abebe, 2001; Getachew, 2005; Basaznew et al., 2012). Because of the range of agro-ecological zones and the diverse farming systems in which the disease occurs, and its debilitating effects on a variety of livestock, surra has attracted international attention in recent years, with a focus on formulating and implementing effective control strategies aimed at increasing productivity and achieving a decrease in mortality and morbidity (Obihiro, 1998). Therefore, in this review, the epidemiology of surra and host response to *T. evansi* infection are presented, as researchers provide both historical perspective and summarize the latest discoveries which will help in the design of effective diagnosis, treatment and control of camel trypanosomosis.

**CAMEL TRYPANOSOMOSIS (SURRA)**

**History and origin of the disease**

The causative agent, *T. evansi*, was discovered by Griffith Evans, in 1880 in infected camels and horses in India. The local Indians had a local name for the disease – Surra, meaning emaciated (Al-Rawashdeh et al., 2000). Since then, studies have shown that *T. evansi* is related to the African trypanosomes and is thought to have evolved from *T. brucei*, the cause of nagana in animals in Africa.

It is thought that *T. evansi* evolved from its ancestors along the edges of the tsetse fly belt in Africa and from there was spread via infected camels used for trade with India (Hoare, 1972). Continuous mechanical transmission by blood-sucking flies in the absence of *Glossina* caused the loss of cyclic transmissibility and gave rise to a predominance of slender parasite forms.

**The parasite**

**Taxonomy and identification**

Trypanosomes are unicellular flagellar protozoa belonging to phylum Sarcomastigophora, the order of Kinetoplastidae, family of Trypanosomatidae and the genus of trypanosome, under the *Salivarian* group. The sub-genus Trypanozoon includes the pathogenic species *T. evansi, T. brucei* and *T. equiperdum* (FAO, 2000). Because the trypanosome shows variation in its antigen coat, there are antigenic differences between isolates of *T. evansi*. There is limited, equivocal information concerning the existence of strains of *T. evansi* of different pathogenicity (Queiroz et al., 2000). However, some strains are referred to colloquially as highly pathogenic’ but this may be a result of host- vector factors, such as stock and insect densities and the susceptibility of host species (Hoare, 1972). *Trypanosoma evansi* is morphologically identical with and indistinguishable from slender forms of other members of the subgenus *Trypanozoon* and is described as monomorphic but may be pleomorphic in some strains with length of 15 to 34 μm. Leaf-like slender forms are characterized by a long free flagellum, which may be up to one half of the length of the organism with narrow and drawn out posterior end (Queiroz et al., 2000).
Replication of the trypanosome occurs by longitudinal binary fission both in the host and in the vector with the flagellum and kinetoplast dividing together (Liu-Liu et al., 2005), but in the noncyclically transmuted *T. evansi* developmental stages were not observed in any of the mechanical vectors. Consequently a procyclic or insect stage (epimastigotes) does not exist in *T. evansi* which is attributed to lack of maxi circles in the kinetoplast DNA (Ellie et al., 1999) (Figure 1). The non-cyclical transmission of trypanosomes is aided by biting flies and thus, in the absence of *Glossina*, the transmission is maintained in the ecosystem. Biting flies, such as *Tabanids* (horse flies), *Stomoxys and Hippoboscids* transmit *T. evansi* mechanically through their mouthparts when they feed on more than one host within a short interval because the trypanosomes remain infective for only a short period (Evans et al., 1995).

**Clinical manifestations**

*T. evansi* can infect a variety of hosts and causes a species-specific pathology. In camels trypanosomosis occurs both in chronic and acute forms (Payne et al., 1990). The acute form of the disease in camel may last for up to three months and is characterized by irregular fever, reduced appetite and water intake, as the disease progresses hump disappear (FAO, 2000), recurrent kerato conjunctivitis and urticarial plaques on the neck and flank, dependent oedema under the belly, marked depression, dullness, loss of condition, the hair coat become dull and rough with loss of hair at the tail and often rapid death (Luckins, 1998). There is also pallor of mucous membranes of the eye, a fluctuating temperature with initial peaks of up to 410C and the urine usually has a characteristic smell increases in body temperature correspond with peaks of parasitaemia (Kohler-Rollefson et al., 2001). Anaemia was observed to be a major
clinical finding in camel Trypanosomosis in Morocco (Rami et al., 2003). Chronic cases are most common and develop recurrent episodes of fever. Some camels develop oedema in their dependent parts of the body, urticaria plaques and petechial haemorrhages in serous membranes. Death finally ensures if untreated however; some may harbour trypanosomes for 2-3 years thus constituting reservoirs of infection to susceptible camels and hosts. Other well documented field reports are death, abortion, and nervous signs like circling movement and trembling, unusual aggressiveness, running aimlessly and sudden collapse in severely stressed and over worked camels (Luckins, 1998).

Diagnosis

Trypanosomiasis is diagnosed by demonstrating the parasite. However, because dromedaries are usually far away from laboratory facilities, a tentative diagnosis can be reached without microscopy, by taking into account the owner's observations and clinical examination of camels in the field. The chronic form is most common in camel and may present an association with secondary infections due to immunosuppressant caused by T. evansi infection, and this complicates clinical diagnosis (Luckins, 1992). The parasites can be detected in blood 13 to 16 days after an infective fly has had a meal to confirm infection. Parasitological diagnosis is mainly carried out by the direct microscopic examination of wet or stained blood films. However, the test has a poor sensitivity. One often less than 50% due to parasitaemia is intermittent (Yadvendra et al., 1998). The implication of this is that in most situations T. evansi is under-diagnosed and the level of infection may be greater than what is frequently reported. In these circumstances, concentration methods such as theuffy coat or haematocrit centrifugation technique are necessary, as they increase the sensitivity of microscopic examination (Reid et al., 2001). Mini-anion exchange centrifugation technique (mAECT) is also one of the most sensitive method for trypanosome detection in blood and is based on a purification technique and adapted for diagnosis of animal infections with T. brucei and T. evansi (Gutierrez et al., 2004).

Serological and molecular tests have strengthened the diagnosis of camel trypanosomosis. Antibody techniques including complement fixation test (CFT), enzyme-linked immunosorbent assays (ELISA) have been used (Reyna-Bello et al., 1998). Others like indirect fluorescent antibody test (IFAT) and card agglutination test (CATT) can also be employed (Connor, 1994). The CATT/T. evansi based on the RoTat 1.2 VAT is a quick and easy test which can be performed under field conditions for serological diagnosis of surra in dromedary camels. Even though Ab-detection tests are sensitive, it cannot distinguish current from cured or past infections (Luckins, 1988). Demonstration of trypanosomal antigens in the blood of the infected animal would be synonymous with parasitological diagnosis (Voller and Desavitgny, 1981). With the introduction of molecular diagnostic techniques, several diagnostic assays based on the detection of trypanosomal DNA by PCR have been developed. PCR is reported to be more sensitive than conventional parasitological techniques in a number of hosts and has the advantage that it can identify parasites at the species level (Gutierrez et al., 2004).

Treatment and control

Treatment of surra depends largely on four drugs: suramin, diminazene aceturate (Berenil), melarsomine (cymelarsan) and quinapyramine. Suramin and quinapyramine have been used for the treatment of T. evansi infection in camels, and only recently melarsomine (cymelarsan) was introduced for the treatment of surra in camels because of the problem of drug resistance. Most drugs are either not curative such as homidium bromide, or are too toxic for camels such as diminazene aceturate (Bourdichon, 1998). Treatment of T. evansi infected camels in Morocco with melarsomine (cymelarsan) reduced the sero prevalence level from 58 to 19% within a year (Rami et al., 2003). Drug resistance is known to occur amongst T. evansi isolates and there have been reports of its occurrence in several different countries in Africa and Asia. In Sudan, from a place named Kassala (near the west border of Ethiopia), an isolate of T. evansi Kassala/4 stock was found to be resistant to the curative action of Suramin even at the maximum tolerated dose of Suramin for mice (Abebe et al., 1983), which was attributed to an extensive and repeated use of Suramin in that area a similar problem may be expected in the adjacent Metema areas of Ethiopia.

The aim of prevention is to break the vector transmission cycle in camels and should be directed towards elimination of trypanosomes from the blood of animals or elimination of the vectors from the environment (Luckins, 2000). However there is no obvious way of developing exclusion zones for animals on grazing land and limited likelihood that vector control will be successful, compared to tsetse-transmitted trypanosomes (Jones and Davila, 2001) as the population of biting flies are extremely numerous, widely distributed, and difficult to deal with various options proposed to limit the impact of biting flies are application of chemicals like malathion or sumithion to stable walls or/and managing the grazing periods for stock at peak period of biting activity of the flies since they are most active in the middle of the day in sunlight; housing animals during the day would offer them protection, allowing animals to have access to field shelters could also help separate hosts from vectors (Coetzer et al., 1994).
Table 1. Prevalence of camel Trypanosomosis in some countries based on serological tests.

<table>
<thead>
<tr>
<th>Country</th>
<th>Prevalence (%)</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>Nigeria</td>
<td>27</td>
<td>Losos (1980)</td>
</tr>
<tr>
<td>Mauritania</td>
<td>24</td>
<td>Dia et al. (1997)</td>
</tr>
<tr>
<td>Niger</td>
<td>29</td>
<td>Pacholek et al. (2001)</td>
</tr>
<tr>
<td>Kenya</td>
<td>28</td>
<td>Njiru et al. (2001)</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>21</td>
<td>Zeleke and Bekele (2001)</td>
</tr>
<tr>
<td>Jordan</td>
<td>33</td>
<td>Alrawashdeh et al. (2000)</td>
</tr>
<tr>
<td>India</td>
<td>22</td>
<td>Pathak et al. (1993)</td>
</tr>
<tr>
<td>Sudan</td>
<td>33</td>
<td>Elamin et al. (1999)</td>
</tr>
<tr>
<td>Iran</td>
<td>10</td>
<td>Zarif-Fard et al. (2001)</td>
</tr>
</tbody>
</table>


EPIDEMIOLOGY

Host range and geographic distribution

Although trypanosomiasis is often referred to as African trypanosomiasis, certain trypanosomes do cause infections outside this continent. *T. evansi*, the causative agent of surra occurs not only in Africa, but also in Central and South America, the Middle East, and Asia. Surra has a wide host spectrum, the main host species varies with the geographical region. In Africa, beyond the northernmost limits of the tsetse fly belt, and in parts of East Africa, camels are the most important host, whilst in Central and South America the horse is principally affected (Dia et al., 1997). In Asia, a much wider range of hosts is involved, including the Bactrian camel and dromedaries, cattle, buffalo, horses and pigs (Pacholek et al., 2001). This is contrary to observations in Africa and South America, where there is little evidence to suggest that domesticated livestock other than camels and horses, respectively, are clinically affected or infected with *T. evansi* (El-Sawalhy and Seed, 1999).

The disease is most severe in horse, donkeys, mules, camels, dogs and cats. Camels, horses, dogs and Asian elephant are more susceptible than sheep and goat, which are more susceptible than bovines and pigs. Rats and mice are highly susceptible as experimental hosts for detecting subclinical (non patent) infection (Reid and Husein, 2001). It has been suggested that, unlike in tsetse-transmitted trypanosomiasis, wildlife reservoirs of infection are unimportant with *T. evansi*, although it is possible that South American coatis and capybaras are an exception to this (Herrera and Dvila, 2004). The ability to be transmitted by blood-sucking insects other than Glossina, has enabled *T. evansi* to extend its range into African areas north of the Sahara desert, into Asia Minor, Pakistan, India, the USSR, China, Sumatra, Java, the Philippines, Mauritius, Madagascar, and South and Central America. It was introduced by camels into Australia, North America and South-West Africa. Introduction of the parasite to new areas is generally characterized by a high prevalence of infection, with mortality reaching 30 to 100% (Elamin et al., 1999).

Occurrence and prevalence

Trypanosomes are insect-borne and their occurrence depends on vector dynamics. Majority of camels suffer from trypanosomosis caused by *T. evansi* that is spread mechanically and independently of tsetse flies. Camels are also affected to a lesser extent by the tsetse-transmitted trypanosome species *T. brucei* (Evans et al., 1995). *T. evansi* parasite is cosmopolitan wherever camels are reared and camel trypanosomosis is endemic in most camel herds and 95% of camel trypanosomosis has been associated with *T. evansi* in Africa (Table 1) (Njiru et al., 2000).

Camel trypanosomosis in Ethiopia

*T. evansi* causing surra in camels is common in the southern and eastern regions of the country (Table 2). In Ethiopia, the distribution of *T. evansi* coincides with the distribution of camels in the semi-desert environment of the country. This trypanosome also occurs in the dry country of the North West near the Sudan border. In Southern Ethiopia (Borena), the disease caused by *T. evansi* is well known to the breeders by the local name “Dhukane” and is given the first priority in its order of importance among camel diseases (Demek, 1998; Tekle and Abebe, 2001).

Course of infection and risk factors

The sequel to infection with the trypanosomes is not always a disease, some may affect self cure, but some individual animals may come down with the disease of...
different stage. In camel trypanosomosis, the period between initial infection and the onset of clinical signs is extremely variable, but generally ranges between 5 and 60 days - although longer periods (such as 3 months) have been recorded. The interval between infection and the demonstration of parasites in the blood is usually less than 14 days (AHA, 2005). Factors that affect the incubation period include the initial infective dose (equivalent to the number of infective insect bites), and stress. Stress occurs in late pregnancy and early in lactation in animals that are more susceptible (Getahun and Demeke, 1998). Inter-current infections (helminthiosis), also stressful, may accentuate the severity of the disease. Trypanotolerance may also be reduced by low plane of nutrition or when animals have to trek for long distances in search of water and pasture in the dry season. This is especially common in the nomadic pastoral communities. Surra affects camels of all ages with a higher incidence of disease in sub-adult camels shortly after weaning (Evans et al., 1995). There is usually a build-up of fly vector populations (Tabanids, Hippoboscids, Stomoxys) during the rains due to a good humid environment for breeding hence resulting in increase of new infections. During the dry season, pastoralists usually take their animals to riverine or swampy areas, which are also favourable grounds for these flies. The degree of risk depends on the challenge, that is, the number of vector fly bites that an animal experiences in a given time. T. evansi has adapted to an entirely mechanical, non-cyclical mode of transmission by bloodsucking flies other than tsetse and infects a wide range of animal hosts compared to cyclically transmitted trypanosomes (Evans et al., 1995).

**HOST RESPONSES**

**Pathology and pathogenesis**

The earliest clinical sign of infection with *T. evansi* in any host is the development at the fly bite of a chancre: a cutaneous swelling in which the first trypanosomes multiply (Luckins et al., 1992). This initial replication increases the establishment of infection, while at this spot also the first interactions take place between the host immune system and the trypanosomes. After formation of a chancre, trypanosomes invade the blood stream, which is accompanied by pyrexia. The parasitaemia may remain high for 4 to 6 days after which it declines with remission of the temperature (Murray et al., 1998). Anaemia is a major component of the pathology of surra and of African trypanosomosis, generally the degree of anaemia might be considered as an indicator of the disease severity. The parasitaemia causes a large number of red blood cells (RBCs) to be removed from circulation by cells of the mononuclear phagocytic system (MPS) in the spleen, bone marrow, and haemal lymph nodes. The removal of a large number of RBCs leads to a fall in packed red cell volume (PCV) to below 25% or even to as low as 10%. This results in affecting animal camel with anaemia and it became dull, anorexic, listless, with ocular discharges, and loss of body condition (Evans et al., 1995).

In the late stages, anaemia continues to be a major factor, with probably additional causes. However, irrespective of the cause of anaemia the primary abnormality of function are the anoxic conditions created by the persistent anaemia tissue anoxia, which results in a fall in tissue pH and vascular damage (Connor, 1994). Following this are signs of dysfunction which appear in the various organs. An increase in cardiac output due to increases in stroke volume and heart rate and a decrease in circulation time are obvious manifestations. The central nervous system is reported to be most susceptible to anoxia with consequent development of cerebral anoxia (FAO, 2000). In camels suffering from surra on postmortem, the carcass is generally emaciated, pale and may be icteric sometimes. The lymph nodes are enlarged and oedematous on incision. There is hydrothorax, hydropericardium and ascites. In acute

### Table 2. Prevalence of *T. evansi* in camel by parasitological method in Ethiopia.

<table>
<thead>
<tr>
<th>Region</th>
<th>Location</th>
<th>Sample size</th>
<th>Prevalence (%)</th>
<th>Sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oromia</td>
<td>Borena</td>
<td>391</td>
<td>10.9</td>
<td>Tekle and Abebe (2001)</td>
</tr>
<tr>
<td></td>
<td>Yabello</td>
<td>294</td>
<td>31.9</td>
<td>Lakew (1993)</td>
</tr>
<tr>
<td></td>
<td>Dello-Mena and Sawena</td>
<td>619</td>
<td>12.12</td>
<td>Hagos et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>24.88*</td>
<td>-</td>
</tr>
<tr>
<td>Somali</td>
<td>Ogaden</td>
<td>321</td>
<td>6.5</td>
<td>Wossene (1988)</td>
</tr>
<tr>
<td></td>
<td>Somali</td>
<td>336</td>
<td>7.7</td>
<td>Issa (1998)</td>
</tr>
<tr>
<td>Tigray</td>
<td>-</td>
<td>280</td>
<td>5</td>
<td>Hailu (2000)</td>
</tr>
<tr>
<td>Afar</td>
<td>Issa</td>
<td>327</td>
<td>0.3</td>
<td>Tefera (1985)</td>
</tr>
</tbody>
</table>

*CATT/T.evansi. Source: Hagos et al. (2009) and Alekaw (2004).*
cases, the spleen is enlarged but in chronic cases, it is atrophic, but these changes are not considered patognomonic for disease (Dargantes et al., 2005).

It is known that *T. evansi* is a member of the Brucel group of trypanosomes, which have a known preference for connective tissues of a host, where they disrupt the collagen bundles and destroy the fibroblasts which produce and maintain the collagen (Boid, 1980). This disruption of host connective tissues, along with the vascular damage attributable to brucel group trypanosomes, would be expected to release large quantities of cytoplasmic and mitochondrial enzymes into the serum, thereby causing further tissue damage. The fever characterized by high temperature might be due to the effects of toxic metabolites produced by dying trypanosomes (Wellde et al., 1989). In addition, the oedema reported in the dependant parts of the body during the chronic stage could be due to a significant decrease in the albumin levels, resulting in alterations in osmotic pressure of the blood (Dargantes et al., 2005).

**Immune response**

Trypanosomiasis is a disease affecting the immune system of the host animal (Lutje and Mertens, 1995). Although the immune system is designed to protect a host from pathogens, it can sometimes be overwhelmed, respond inappropriately or result in immune mediated disease with clinical signs (Stijlemans et al., 2007). Circulating trypanosomes are rarely observed in individuals suffering from chronic disease and studies have failed to show a correlation between the intensity of inflammation and the level of parasitaemia (Olivares-Villagomez et al., 1998). This may be because the immune response is directed against both parasites and self antigens. The parasites might achieve this through molecular mimicry or inflammation and tissue damage leading to the release of tissue proteins which stimulate formation of self antigens (Soares and Santos, 1999). *T. evansi* as purely extracellular parasites survives, multiplies and differentiates in extracellular fluids of the mammalian host including the aggressive vascular environment. Thus, these parasites are permanently confronted with the multiple components of the host's immune system ranging from innate to adaptive immune defences. Among many molecules, the trypanosomal DNA and the GPI anchor of the VSG that might be released from the dead trypanosomes has been shown to activate macrophages to secrete proinflammatory molecules like TNF-α, IL-6, IL-1, IL-10 and NO as the first response of the host immune system that are involved in the control of the first peak of parasitemia by the toxic nature of TNF and NO for both the host cell and the parasite (Stijlemans et al., 2007).

However as a prototype of extracellular parasites, these pathogens defy humoral immunity through a subtle mechanism of antigenic variation whereby they sporadically vary their main exposed membrane surface glycoprotein (termed variable surface glycoprotein or VSG) to elude antibody (Ab) recognition. IL-6 (secreted by activated macrophages) receptors are only present on directly activated B-cells which result in an increase in IgM and IgG antibodies (Pays, 2006).

The polyclonal B cell activation induced by *T. evansi* infections is characterised by a predominantly IgM response with limited IgG production (Sacks et al., 1980). Sacks et al. (1980) hypothesized that penetration of IgM into tissues where trypanosomes replicate may be impeded because it is a larger molecule compared to IgG and that this may lead to chronic infections, because of the presence of tissue reservoirs, whilst preventing uncontrolled growth by the parasite in the circulation. IgM is detectable during parasitaemia but IgG levels are detectable only after remission of parasitaemia (De-Aquino et al., 1999). Therefore elimination of the infecting VAT appears to be associated mainly with an IgM response, although both IgM and IgG responses to the variable surface glycoproteins (VSG) occur during infection. The antibodies directed against the specific surface exposed epitopes of the VSG coat opsonize the parasites and the immune complexes are efficiently phagocytosed and destroyed, mainly in the liver, by the macrophages. A role for complement-mediated lysis in parasite clearance has been proposed but could not be confirmed because *T. evansi*-infected complement (C5)-deficient AKR mice control successive parasitaemia waves as efficiently as complement-competent strains (Vincendeau and Bouteille, 2006).

An increase in gamma-globulin (IgM) during both acute and chronic *T. evansi* infections in camels has been reported (Naessens, 2006) but this is not protective, as the majority of the antibodies are auto antibodies. In the acute phase of the disease, lymph nodes and spleen are remarkably reactive. This may account for the generalized lymphoid tissue hyperplasia characteristic of *T. evansi* infections, while in the late stages the immune system becomes depleted of lymphoid cells (Raes et al., 2002).

**Antigenic variation and immune evasion**

The blood stream form of African trypanosomes are entirely covered by 5 × 10^6 dimers of variable surface glycoproteins (VSG), which is the most abundant surface protein in the blood stream form of the trypanosomes. It forms a dense surface coat of 12 to 15 nm over the entire surface of the trypanosome and accounts for about 15 to 20% of the total protein content of the bloodstream form of the parasite (Field and Carrington, 2009). This surface coat is attached to the outer membrane of the trypanosomes by glycosylphosphatidyl linositol (GPI) anchors, which make the variable surface antigen water-
insoluble and may contribute to the host's immune response to trypanosome infection (Pays and Nolan, 1998). It is likely that the VSG repertoire of *T. evansi* is smaller than that of trypanosomes with a tsetse fly intermediate host because exchange of genetic information and rearrangement of VSG repertoires occurs in this vector (Engstler et al., 2007). During the ascending phase of the parasitemia, the majority of parasites are of the same antigenic type (called homotype). The host immune system recognizes this homotype and makes antibodies against it. As the parasites of the major variable antigenic type (VAT) are eliminated the parasitemia goes in descending phase but at the same time, the parasites expressing the heterotype or the minor VATs are multiplying and one of them overgrows others. As a result, this one becomes the new homotype, leading to a new wave of parasitemia and resulting in a long-lasting chronic infection. So expression of the VSG is central in the antigenic variation process and eventually for exhausting the host immune system in the benefit of the parasite (Field et al., 2009).

**Immunosuppression**

Pathogenic trypanosomes induce a generalized immunosuppression of both humoral antibody response and T-cell-mediated immune responses. As a result, in the long term, the host's immune responses fail and it succumbs to either the overwhelming parasite load or to secondary infection, consequently leading to occurrence of the trypanosome-induced immunopathology. Various studies have shown that polyclonal B cell activation, generation of suppressor T-cells and macrophages and altered antigen handling and presentation are all mechanisms that could be involved in trypanosome mediated immunosuppression (Sileghem and Flynn, 1994). It seems that macrophages are central to immunosuppression and that upon activation of these cells a variety of factors and cytokines are released which cause a range of effects such as B-cell activation and T-cell suppression. During trypanosome infections, TNF which are secreted by classically activated macrophages are involved both in parasitemia control and infection associated pathology like anemia, organ lesion and fever. Trypanose-induced immunosuppression is also appeared to be due to the action of trypanosome enzymes. Trypanosome enzymes, such as phospholipases, neuraminidases and proteases have all been implicated in membrane fluidity and cellular damage (Fung et al., 2007).

**Current alternative anti disease strategy**

Beside treatment, effective vaccination strategy is a second approach for the control of any infectious diseases. In the case of trypanosomiasis, all conventional anti-parasitic vaccination efforts undertaken so far, that used dominant surface protein, have failed due to the antigenic variation of the trypanosomes surface coat. Therefore, an alternative strategy of the vaccination is demanding. As alternative vaccination approaches, different parasitic molecules have been attempted (Fung et al., 2001). The GPI-anchor of the VSG as one of the major parasitic components causing the inflammatory response associated to the infection has been identified (Taylor et al., 1999). In one of the studies, this information has been used to evaluate GPI based vaccination as an alternative strategy with antidiisease potential. Using liposomes as slow delivery system, the GPI administered prior to the infection had been shown to result in a better control of the parasitemia and a longer lifespan of the infected mice. These trials were successful in reducing weight loss, liver damage, acidosis and anaemia during *T. brucei* and *T. evansi* infection models; this reduction in pathology was associated with a reduced TNF production and an increased level of IL-10, along with the expression of alternatively activated macrophage. Due to increased level of IL-10, CD4/Th-cells activated and secrete IL-4, IL-10 and IL-13 which are responsible for T-cell dependant B-cells activation (Naessens, 2006).

**CONCLUSION AND RECOMMENDATIONS**

Camel trypanosomosis is a disease of major economic importance in many countries of Africa, Asia and South America. Because of the wide geographic range of surra, its control has attracted international attention, vector control seems not the solution for surra as a range of non-related biting flies should be targeted, each with its own biology, while unlike tsetse flies most other flies are prolific breeders, and as such vector populations are difficult to control. Anaemia is a major component of the pathology of surra and of African trypanosomosis. Trypanosomiasis is a disease affecting the immune system of the host animal. *T. evansi* as purely extracellular parasites are permanently confronted with the multiple components of the host's immune system ranging from innate to adaptive immune defences. Among many molecules, the trypanosomal DNA and the GPI anchor of the VSG that might be released from the dead trypanosomes has been shown to activate macrophages to secrete proinflammatory molecules as the first response of the host immune system. However as a prototype of extracellular parasites, these pathogens defy humoral immunity through a subtle mechanism of antigenic variation.

The polyclonal B cell activation induced by *T. evansi* infections is characterised by a predominantly IgM response with limited IgG production. The vaccination approaches by using dominant surface proteins have not been successful, mainly due to antigenic variation of the
parasite surface coat. On the other hand, the chemotherapeutic drugs in current use for the treatment of surra are toxic and problems of resistance are increasing. Therefore, there is an urgent need for alternative control approaches against camel trypanosomiasis. Therefore, the following points are recommended:

1. Biology of the parasite as well as the host-pathogen interaction needs to be studied for each specific geographical area as there might be variations in the strains of the parasites and the responses of camels to the disease.

2. The dynamics of mechanical transmission of camel trypanosomiasis in endemic areas has to be thoroughly studied by including those factors contributing to occasional outbreaks.

REFERENCES


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Full Length Research Paper

Epidemiological studies of gastrointestinal parasitic infections in ruminants in Jakiri, Bui Division, North West Region of Cameroon

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This study was undertaken to determine the prevalence, intensity of infection and management systems associated with gastrointestinal (GIT) parasites in grazing ruminants (cattle, sheep and goats). Faecal samples were collected from 277 cattle, 104 sheep and 94 goats, from different areas in Jakiri. Samples were analysed using the Formol-ether concentration technique. 318 samples were found positive with one or more parasites giving an overall prevalence of 66.9%. Goats recorded the highest (90.4%) prevalence of GIT parasites, followed by sheep (73.1%), and the least prevalence was observed in cattle (56.7%). Concerning the various management techniques, prevalence of GIT parasites were higher in tethered animals (88.1%) followed by free range grazing animals (60.9%). Animals confined in paddocks had the least prevalence (45.5%). Eimeria species recorded the highest prevalence (20.9%) among the various species of parasites encountered during the study in cattle, Trichostrongylus species and Eimeria spp. in sheep (28.8%) while the highest prevalence in goats was Trichostrongylus spp. (55.8). Mixed infections of Trichostrongylus spp., Eimeria spp. and Haemonchus species were most prevalent in all the animal species. The prevalences of Fasciola species and Monezia species were significantly low in all the three animal groups in the study area. Adults were more infected compared to young stock animals (lambs and kids). This work provides an important step to minimize economic losses in ruminants by providing information that will help farmers practice the right traditional management techniques.

Key words: Gastrointestinal parasites, ruminants, prevalence, management systems, Jakiri, Cameroon.

INTRODUCTION

Gastrointestinal tract (GIT) parasites are known to be widespread in Cameroon (Ndamukong, 1985) and limit livestock production in many areas and countries of the world (Vlassoff and Leathwick, 2001; Ng’ang’a et al., 2004). Studies have shown that helminth parasites are by far the most serious causes of production losses in farmed ruminants and the nematodes are indisputably the cause of serious production losses to ruminants in sub-Saharan Africa, and indeed worldwide (Ng’ang’a et al., 2004; Odoi, 2007; Kanyari et al., 2009).

Despite the relative importance of nematode parasites in ruminants worldwide, other gastrointestinal parasites like...
like the trematodes, cestodes and coccidians have also shown higher prevalence rates in most countries of the world. The trematodes of traditional veterinary and medical significance are almost all digenetic flukes that require a mollusc or snail as the first intermediate host. Prevalence studies reveal that *Fasciola* species are by far the most economically important trematodes of ruminants in the tropics (Maingi et al., 1997). According to Food and Agriculture Organization (FAO)/World Health Organization (WHO) (1999), intense fascioliasis has been reported in the following African countries: Northern Nigeria, Kenya, Lake Chad, Zaire, Zambia, Ivory coast, Zimbabwe and Cameroon. The occurrence of flooding, water pans and swamps are important habitats for propagation of the snail intermediate hosts of these flukes. Some of the regions in Cameroon have their borders to the west of the Atlantic Ocean which makes them marshy and swampy, thus suitable for survival of snail intermediate hosts.

Ruminants, the most widespread livestock in Cameroon, are reared in traditional systems. Cattle, goats and sheep rearing systems are: nomadic or pastoral, mixed farming and the peri-urban systems. Production and management systems vary from free range in less populated areas, to year-round confinement and cut-and-carry feeding in densely populated areas. Ruminants under extensive systems rely on natural grazing. Because of shortage of water and forage, malnutrition is often the major limiting factor for profitable production of ruminants particularly during the dry season. Grazers of the Fulani tribe in the North West region of Cameroon seek refuge during dry periods in Wasi-ber, Bangolan of Babesi and Bambalan of Ndop. Animals suffer from stressful and disease effects especially during these transhumance periods.

In most semi arid and arid regions of sub-Saharan Africa, ruminants play a vital role in rural economies through the provision of meat, milk, household income, manure and skin (Mulugete et al., 2011). In most cases, the animals are run in large flocks or herds, concentrated in confined areas or tethered on pegs where they are likely to pick up infective larva or oocyst from contaminated pastures (Kanyari et al., 2009). These poor management systems have contributed massively to economic losses of ruminant production in sub-Saharan countries (Mulugete et al., 2011). As a result, most of the rural farmers and livestock farmers pay keen attention to parasites that may likely cause the death of their animals.

**MATERIALS AND METHODS**

**The study area**

The study was conducted in Jakiri, Bui Division (Figure 2). This rural village is situated at Latitude, 6.1°N and Longitude 10.65°S about 89 km from the capital city Bamenda of the North West region of Cameroon (Jocelyn, 1982). The climate in the area is characterized by a long rainy season from April to October, with annual average rainfall ranging from 1,500 to 2,000 mm and an altitude of about 1,100 m above sea level (Jocelyn, 1982). The dry season stretches from November to March, with monthly average temperatures in June reaching a maximum of about 21°C. Jakiri is a typical mountainous area covered with grass on the hills and valleys which constitute the major natural resource that the ruminant population of livestock depends on.

Mixed crop/livestock production system is the main form of agriculture. Most families are also involved in livestock farming, especially goats and sheep. Flock sizes under the tethering system in Jakiri are in the order of 1 to 10 goats or sheep per household. Cattle herds of large sizes are mostly owned by the Fulani tribes and they form separate communities in the upland grazing quarters of Jakiri.

**Selection of study sites and farms**

The study sites (Figure 1) were selected on the basis of having a higher concentration of livestock. The sites included small locations (quarters) in Jakiri village: Tan, Sodpea, Vekovi, Nkar, Kiform, Weinamah and Shiy.

**Study subjects**

A total of 475 ruminants consisting of 131 males and 344 females were examined for intestinal parasites, out of which 277 were cattle, 104 sheep and 94 goats. Also 335 of these animals were adult ruminants, 57 heifers and 83 kids/lambs. For animals to be qualified as subjects, the sheep, goats and cattle must have been living in Jakiri and its environs for at least three months. Samples were collected from ruminants of both sexes. The ages of the animals were determined from interviews with the farmers. Animals with ages ranging from one month to a year were classified as young stock (lambs for sheep, kids for goats) while those from one year and above were categorized as adults. The criteria for cattle were different. Cattle with ages ranging from one month to a year were classified as calves while those from one to three years were classified as heifers and those above three years were categorized as adults.

**Study design**

A preliminary survey was carried out prior to sample collection to sensitize interested farmers on the objectives of the study. Questionnaires were administered to all the farmers whose animals were to be examined. It included information on the age/sex/breed of the animal, farm management practices and health conditions of the animals. Oral interviews were also conducted to obtain other relevant information about the ruminants and the study site.

**Sampling and faecal analysis**

Faecal samples were collected directly from the rectum using plastic gloves and put into clean, dry, leak-proof, transparent plastic bottles. The samples were labelled and transported to the laboratory of the National Veterinary Training school for Livestock and Animal Husbandry in Jakiri where they were examined immediately for parasite eggs and oocysts. Stool samples not observed on the same day were treated and stored in the
refrigerator for subsequent examination the next day. The formol-ether concentration technique as described by Christensen et al. (1984) was used to detect the presence of helminth eggs and coccidian oocysts. The overall proportion of infective larvae from faecal cultures among management systems was equally determined. Strongyle species were identified based on standard criteria. The eggs per gram (EPG) of feces were quantitatively analyzed to determine the intensity of infection using the modified McMaster technique and the coccidian oocysts quantified (MAFF, 1977).

Statistical analysis

Data was entered into Ms Excel® 2003 (Microsoft corporation, USA) and analysis were conducted using the statistical package statistical package for social sciences (SPSS) version 12. Prevalence was calculated as a percentage of d/n where d is the number of animals

Figure 1. Study sites.

Figure 2. Map showing rate and distribution of cattle, sheep and goats in Jakiri sub-division.
infected and \( n \) = Total number of animals examined. The association between independent factors (age, and area of origin) and continuous dependent variables (EPG, oocyst numbers per gram (OPG) and intensity of infection) was calculated using one way analysis of variance (ANOVA). The association between the independent factors and the prevalence of the various parasites were evaluated using the Chi-square test \((\chi^2)\). In all the analysis, confidence level was held at 95%, and \( P \leq 0.05 \) set for significance.

RESULTS

Out of the 475 ruminants examined, 318 were found positive with one or more parasites, giving an overall prevalence of 66.9%. Goats recorded the highest prevalence of 90.4%, followed by sheep, 73.1% and the least prevalence was observed in cattle, 56.7%. The study revealed a significant difference \((P < 0.05)\) in the prevalence of the gastrointestinal parasites among the ruminants in the study area (Table 1).

Gastrointestinal parasites identified from faecal samples in the study along with their prevalences are shown on Tables 2 and 3. Strongyle nematodes and *Eimeria* spp. were the most prevalent parasites recorded in all the three groups of animals. From the results obtained, goats recorded the highest prevalence rates in *Trichostrongylus* spp. 55.8%, followed by sheep 28.8%, and the least prevalence was recorded in cattle 9.7%. Equally, goats recorded the highest prevalence of *Haemonchus* spp. 49.5%.

Concerning the intensity of infection of the nematode species, the study revealed that, mean egg per gram was notably high for almost all the Strongyle nematodes observed in small ruminants (sheep and goats). However, faecal egg counts revealed overall low egg per gram in all of the recovered worm egg types in cattle (Table 2). Most of the animals had mix infections, with most of the combinations being *Trichostrongylus* spp./*Strongyloides* spp. (Table 4).

Village based prevalence revealed that, in cattle; gastrointestinal parasites had the highest prevalence in Wainamah (80.6%). Infection rates in goats were highest in Vekovi (97.8%) and in sheep; Shiy recorded the highest prevalence (87.5%). It was found that in all the three groups of animals, statistically significant difference \((P \leq 0.05)\) was not observed in the prevalence with respect to the various villages (Tables 5, 6 and 7).

A total of 335 adult ruminants, 57 heifers and 83 kids/lambs were examined during the study. Out of these lots, adult goats recorded the highest prevalence of gastrointestinal parasites (93.3%), followed by the young goats (kids) (78.9%), and the least prevalence was observed in adult cattle (53.1%). However, the overall prevalence of gastrointestinal parasites among the different age groups showed that generally, the youngest animals (calves, lambs and kids) had the highest prevalence (71.2%) (Figure 3). Chi square value however revealed no significant differences among the different age groups \((P \geq 0.05)\).

The present study also revealed details on the prevalence of gastrointestinal tract (GIT) parasites in animals kept under different traditional management systems. It was found that, animals confined in paddocks recorded lower prevalence rates compared to free range grazers and tethered animals. For both sheep and goats, tethered animals had highest infection rates of 85.4 and 90.4%, respectively. Cattle and sheep that grazed in confined paddocks had prevalence rates of 37.7 and 56.8%, respectively. Free range grazers had prevalence rates of 61.2 and 73.3% for cattle and sheep, respectively. A significant difference in prevalence was observed in both cattle and sheep practising the different grazing systems (Tables 8, 9 and 10).

DISCUSSION

The present study revealed an overall prevalence of GIT parasites in the ruminants to be 67.45%, with 56.7, 73.1 and 90.4% in cattle, sheep and goats, respectively. These results are in line with the findings of Fikru et al. (2006) and Biu et al. (2009). The high prevalence of GIT parasites in small ruminants as a whole agrees with most reports (Odoi et al., 2007; Fufa et al., 2009; Kanyari, 2009; Mulugete et al., 2011). The higher prevalence rate in goats and sheep in the study area might be due to poor management systems. In Jakiri, mixed crop livestock production predominates where few numbers of small ruminants are kept together. Majority of the sheep and goats are tethered on farm lands. As a result of this, most of the animals are re-infected due to pasture contamination as they graze within a confined region for several months. Ticks also posed a major health problem to ruminants in Jakiri. Seven out of 15 farmers from whom we collected faecal samples complained of their animals passing out blood tinged urine which is a sign of babesiosis; a tick-borne infection. Under such conditions, gastrointestinal parasites thrive best due to reduced immunity in the ruminants. This led to increased mortality rates in ruminants prior to the research. The higher prevalence of GIT parasites in goats compared to sheep is in agreement with the report of Ndamukong (1985) in Momo division, North West Region of Cameroon. This result however, contradicts the findings of Kanyari (2009) whose assertions explained that the grazing habits of sheep (grazing closer to the earth soil) warrants these animal species to be more infected than goats. However, in the present survey, the difference in philosophy with the previous findings may be because the majority of the goats are kept under poor veterinary infrastructure and medication. More importantly, this may be due to low or
Figure 3. Age related prevalence of GIT parasites in ruminants in Jakiri.

Table 1. Overall prevalence of gastrointestinal parasites of ruminants in the study area.

<table>
<thead>
<tr>
<th>Ruminant</th>
<th>No. examined</th>
<th>Infected no.</th>
<th>Prevalence (%)</th>
<th>( \chi^2 ) (P value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>277</td>
<td>157</td>
<td>56.7</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>104</td>
<td>76</td>
<td>73.1</td>
<td></td>
</tr>
<tr>
<td>Goats</td>
<td>94</td>
<td>85</td>
<td>90.4</td>
<td>38.4 (0.001)</td>
</tr>
<tr>
<td>Total</td>
<td>475</td>
<td>318</td>
<td>66.9</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Prevalence and intensity of gastrointestinal nematodes in cattle, sheep and goats in study area (N = total number of animals examined).

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Infected no. (%)</th>
<th>Intensity</th>
<th>Infected no. (%)</th>
<th>Intensity</th>
<th>Infected no. (%)</th>
<th>Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichostrongylus</em> spp</td>
<td>27 (9.7)</td>
<td>5.19±23.81</td>
<td>30 (28.8)</td>
<td>179.4±1007.2</td>
<td>53 (55.8)</td>
<td>693.4±1903.3</td>
</tr>
<tr>
<td><em>Haemonchus</em> spp</td>
<td>16 (5.7)</td>
<td>2.6±12.47</td>
<td>24 (23.1)</td>
<td>75.4±413.6</td>
<td>47 (49.5)</td>
<td>344.2±1261.7</td>
</tr>
<tr>
<td><em>Oesophagostomum</em> spp</td>
<td>18 (6.5)</td>
<td>11.7±50.7</td>
<td>3 (2.9)</td>
<td>4.4±30.3</td>
<td>5 (5.3)</td>
<td>11.7±50.6</td>
</tr>
<tr>
<td><em>Ostertagia</em> spp</td>
<td>8 (2.9)</td>
<td>1.2±9.0</td>
<td>12 (11.5)</td>
<td>13.8±73.9</td>
<td>30 (31.5)</td>
<td>41.2±97.0</td>
</tr>
<tr>
<td><em>Strongyloides</em> spp</td>
<td>25 (9.0)</td>
<td>4.2±21.0</td>
<td>26 (25.0)</td>
<td>86.7±409.8</td>
<td>46 (48.9)</td>
<td>324.9±831</td>
</tr>
<tr>
<td><em>Trichuris</em> spp</td>
<td>51 (18.4)</td>
<td>6.8±18.3</td>
<td>8 (7.7)</td>
<td>7.7±27.0</td>
<td>13 (13.9)</td>
<td>14.8±48.9</td>
</tr>
<tr>
<td>Other nematodes</td>
<td>17 (6.1)</td>
<td>-</td>
<td>9 (8.7)</td>
<td>-</td>
<td>3 (3.2)</td>
<td>-</td>
</tr>
</tbody>
</table>

N = number of animals examined.

slow development of immunity in goats to GIT parasites as compared to sheep and cattle.

The prevalence of GIT parasites in cattle in the study area was generally low compared to small ruminants, with a majority of the cattle having light infections. These results are in line with the findings of Adrien et al. (2001), Waruiru et al. (2005) and Kenyu et al. (2006). The reason might be due to frequent drenching habits of the farmers.
Table 3. Prevalence of Trematodes, Cestodes and Eimeria species in cattle, sheep and goats in the study area.

<table>
<thead>
<tr>
<th>Parasite specie</th>
<th>Cattle (N=277)</th>
<th>Sheep (N=104)</th>
<th>Goats (N=94)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected No. (%)</td>
<td>Infected No. (%)</td>
<td>Infected No. (%)</td>
</tr>
<tr>
<td>Fasciola spp</td>
<td>17 (6.1)</td>
<td>3 (2.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Entamoeba spp</td>
<td>5 (1.8)</td>
<td>1 (1.0)</td>
<td>13 (13.7)</td>
</tr>
<tr>
<td>Moniezia spp</td>
<td>10 (3.6)</td>
<td>8 (7.7)</td>
<td>4 (4.3)</td>
</tr>
<tr>
<td>Eimeria spp</td>
<td>58 (20.9)</td>
<td>30 (28.8)</td>
<td>45 (47.9)</td>
</tr>
</tbody>
</table>

Table 4. Prevalence of mixed infections in ruminants in the study area.

<table>
<thead>
<tr>
<th>Mixed infections</th>
<th>Cattle (N=277)</th>
<th>Sheep (N=104)</th>
<th>Goats (N=94)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected No. (%)</td>
<td>Infected No. (%)</td>
<td>Infected No. (%)</td>
</tr>
<tr>
<td>Trichostrongylus spp./Strongyloides spp./Eimeria spp.</td>
<td>3 (1.08)</td>
<td>26 (12.60)</td>
<td>36 (38)</td>
</tr>
<tr>
<td>Trichostrongylus spp./Haemonchus spp.</td>
<td>5 (1.80)</td>
<td>45 (22.61)</td>
<td>12 (12.7)</td>
</tr>
<tr>
<td>Strongyloides spp./Eimeria spp.</td>
<td>6 (2.1)</td>
<td>36 (18.09)</td>
<td>4 (4.2)</td>
</tr>
<tr>
<td>Strongyloides spp./Trichostrongylus spp.</td>
<td>8 (2.88)</td>
<td>56 (28.14)</td>
<td>7 (7.4)</td>
</tr>
<tr>
<td>Haemonchus/Eimeria</td>
<td>2 (0.7)</td>
<td>1 (0.5)</td>
<td>3 (3.2)</td>
</tr>
<tr>
<td>Trichostrongylus spp./Eimeria spp.</td>
<td>5 (1.80)</td>
<td>25 (12.56)</td>
<td>11 (11.7)</td>
</tr>
</tbody>
</table>

N=number of animals examined.

Table 5. Prevalence of gastrointestinal parasites in cattle in the various villages surveyed in the study area.

<table>
<thead>
<tr>
<th>District</th>
<th>No. examined</th>
<th>No. infected</th>
<th>Prevalence (%)</th>
<th>$\chi^2$ (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiform</td>
<td>68</td>
<td>41</td>
<td>60.3</td>
<td></td>
</tr>
<tr>
<td>Nkar</td>
<td>24</td>
<td>15</td>
<td>62.5</td>
<td></td>
</tr>
<tr>
<td>Sodepa</td>
<td>53</td>
<td>20</td>
<td>37.7</td>
<td></td>
</tr>
<tr>
<td>Vekovi</td>
<td>36</td>
<td>18</td>
<td>50.0</td>
<td></td>
</tr>
<tr>
<td>Shiy</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>4.573 (0.47)</td>
</tr>
<tr>
<td>Wainamah</td>
<td>31</td>
<td>25</td>
<td>80.6</td>
<td></td>
</tr>
<tr>
<td>Tan</td>
<td>65</td>
<td>38</td>
<td>58.5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>277</td>
<td>157</td>
<td>56.7</td>
<td></td>
</tr>
</tbody>
</table>

Also during the dry seasons, larva may develop successfully to infective stages in faeces but might not emerge until moisture levels are optimal. Infected faeces continue to be passed out by the cattle until moisture is available when pasture contamination can then rise rapidly.

The most prevalent GIT parasites were the Strongyles, Strongyloides and Eimeria oocyst. This result corroborates many findings in Africa (Ndamukong, 1985; Ndamukong and Sewell, 1992; Odoi, et al., 2007; Fufa et al., 2009; Kanyari, 2009; Mulugete et al., 2011). Strongyle nematodes were of the genera Trichostrongylus, Haemonchus, Oesophagostomum, Ostertagia, Cooperia, Charbata and Nematodirus. The climatic conditions of Jakiri (warm moist) are highly suitable for survival of strongyles and transmission of the parasites. Another contributing factor towards the high prevalence of strongyle nematodes may be due to poor farm management techniques including constructions, feeding, watering systems and generally poor hygienic conditions of the farms.

The prevalence of Fasciola spp. in the study area for all the three animal groups was extremely low. This may be due to the vegetation cover of Jakiri. The typical mountainous area covered with grass on the hills does not favour propagation of the snail intermediate hosts. It is probable that, the few ruminants infected with Fasciola gigantica might have gotten their infection during transhumance in Wasiber, Bangolan of Babesi and Bambalan of Ndop, a period during which there is scarcity of pasture.
Table 6. Prevalence of gastrointestinal parasites in goats in the various villages surveyed in the study area.

<table>
<thead>
<tr>
<th>District</th>
<th>No. examined</th>
<th>Infected No. (%)</th>
<th>( \chi^2 ) (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiform</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nkar</td>
<td>27</td>
<td>24 (88.8)</td>
<td></td>
</tr>
<tr>
<td>Sodepa</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Vekovi</td>
<td>45</td>
<td>44 (97.8)</td>
<td>0.377 (.0825)</td>
</tr>
<tr>
<td>Shiy</td>
<td>22</td>
<td>17 (77.3)</td>
<td></td>
</tr>
<tr>
<td>Wainamah</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tan</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>85 (90.4)</td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Prevalence of gastrointestinal parasites in sheep in the various villages surveyed in the study area.

<table>
<thead>
<tr>
<th>District</th>
<th>No. examined</th>
<th>Infected No. (%)</th>
<th>( \chi^2 ) (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiform</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nkar</td>
<td>43</td>
<td>34 (79.1)</td>
<td></td>
</tr>
<tr>
<td>Sodepa</td>
<td>37</td>
<td>21 (56.8)</td>
<td></td>
</tr>
<tr>
<td>Vekovi</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Shiy</td>
<td>24</td>
<td>21 (87.5)</td>
<td>1.343 (0.511)</td>
</tr>
<tr>
<td>Wainamah</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tan</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>76 (73.1)</td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Prevalence of gastrointestinal parasites in ruminants confined in paddocks.

<table>
<thead>
<tr>
<th>Ruminant</th>
<th>No. examined</th>
<th>Infected No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>53</td>
<td>20 (37.7)</td>
</tr>
<tr>
<td>Sheep</td>
<td>37</td>
<td>21 (56.8)</td>
</tr>
<tr>
<td>Total</td>
<td>90</td>
<td>41 (45.5)</td>
</tr>
</tbody>
</table>

Table 9. Prevalence of gastrointestinal parasites in tethered ruminants.

<table>
<thead>
<tr>
<th>Ruminant</th>
<th>No. examined</th>
<th>Infected No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat</td>
<td>94</td>
<td>84 (89.3)</td>
</tr>
<tr>
<td>Sheep</td>
<td>48</td>
<td>41 (85.4)</td>
</tr>
<tr>
<td>Total</td>
<td>142</td>
<td>125 (88)</td>
</tr>
</tbody>
</table>

and water in Jakiri. The distribution of the snail intermediate host (*Lymnea* spp.) in this area of transhumance is not well understood. The only cestode observed in the study area was *Moniezia* spp with sheep having the highest prevalence of all the three animal groups. These results are in line with the findings of Sissay et al. (2008) and Kanyari (2009). The pathogenic significance of this parasite is not well understood. However, occurrence of this parasite in the tropics is associated with the ingestion of oribatid mites infected with cysts of *Moniezia* spp.

Locations in Jakiri where farmers practiced the traditional management systems showed higher prevalence rates and
intensities of infection compared to areas managed by the government parastatal called "Societe de Developments des Petite Ruminant" (SODEPA) under the semi-intensive management system. The low prevalence rate in SODEPA could be explained by the fact that the parastatal has a curved out vast grazing land reserved only for ruminants of the parastatal. Animals kept by SODEPA are well catered for, frequently drenched, well fed with supplemental feed and constantly monitored for any irregularities that might lead to death of the animal.

Upland grazing areas in Jakiri recorded the highest prevalence of gastrointestinal parasites in cattle as compared to lowland grazing areas which had higher prevalence of GIT parasites in small ruminants. The upland grazing areas are occupied mostly by the Fulani Tribes while the vast lowland grazing areas are occupied mainly by the indigenes of Jakiri for crop farming. The Fulani tribes form the minority group and often are faced with a problem of limited grazing land. They often pitch their tents and small huts closer to their cattle herds on mountainous grazing areas for proper supervision of their animals. Most of them rear cattle and few sheep inherited from their parents. They do not keep goats since they attach more religious significance to sheep during Ramadan festivities. This therefore implied that the low prevalence of GIT parasites in small ruminants in the upland grazing community was not due to absence of parasites on contaminated pastures but rather might have been due to a relatively small sample size of small ruminants kept by the Fulani community.

Though infection rates were higher in traditionally managed animals (tethered goats and sheep), a study carried out in Mankon in the North West Region of Cameroon (Ndamukong, 1985) showed that mortality rates were relatively low for all animals reared under the traditional management systems. The reason behind this could be that, local breeds of small ruminants and cattle in the North West region of Cameroon (Cameroon Dwarf goats, Red Sokoto, red Fulani cows, and Dwarf Forest sheep) have acquired strong immunity to infection of GIT parasites due to recurrent infections.

Generally, young stock animals had a slightly higher prevalence rate of GIT parasites compared to the adults. This result is in line with the findings of Ndamukong (1985), Githigia (2001), Almalaik et al. (2008) and Kanyari (2009). Calves, lambs and kids are more susceptible to infection than adults due to low levels of immunity. Higher prevalence in young stock may also be due to failure in separating young stock from the adults at pre weaning age, overgrazing of infested pastures coupled with inappro-priate and inadequate use of anthelmintics (Ndamukong, 1985).

**Conclusion**

The study clearly indicates that control measures should make use of the variations in helminth prevalence and intensity among management systems and age groups to achieve rational use of anthelmintics. Also, tethered animals should not be allowed to graze on a particular spot continuously for several weeks. Grazing spots should be rotated to reduce the chances of ruminants being re-infected from contaminated pastures. Field veterinarians should assist farmers in strategic deworming with broad spectrum anthelmintics used at the beginning and after the end of the rainy season. Finally, farmers should be educated on the importance of using dry season feed reserves as means to ensure safe feed for zero-grazed ruminants.

**REFERENCES**


**Table 10. Prevalence of gastrointestinal parasites in free range grazing ruminants.**

<table>
<thead>
<tr>
<th>Ruminant</th>
<th>No. examined</th>
<th>Infected No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>224</td>
<td>134 (59.8)</td>
</tr>
<tr>
<td>Sheep</td>
<td>19</td>
<td>14 (73.7)</td>
</tr>
<tr>
<td>Total</td>
<td>243</td>
<td>148 (60.9)</td>
</tr>
</tbody>
</table>


Isolation time of brooding chicks play an important role in the control of Marek’s disease

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Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka, Nigeria.

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This work reported the use of a combination of measures for the control of an outbreak of Marek’s disease (MD). Post-introduction vaccinations of young chickens in a farm that had an outbreak over a period of six years did not yield good result. The disease was therefore controlled by brooding new birds in isolated pens far away from the other one, use of biosecurity measures and brooding birds during the rainy season when the environment was damp with minimal dust in the air. Result showed marked reduction of infection during the first one year of trial. By the second and third years of trial, no infection was detected in the birds. This study therefore recommends isolation brooding, biosecurity measures, brooding during the rainy period and completely avoiding brooding during the harmattan period as good methods of controlling cases of MD outbreaks in Nigeria.

Key words: Marek’s disease, isolation brooding, vaccination, biosecurity measures, young chickens, Nigeria.

INTRODUCTION

Marek’s disease (MD) is attributed to a renowned veterinarian Dr. Joseph Marek, who in 1907 described the disease as polyneuritis during a research endeavour in four adult paralysed cockerels (Gallus domesticus) at Royal Hungarian Veterinary School Budapest (Goyal et al., 2008). MD is one of the commonest causes of economic loss in the development of poultry industries of many countries (Baigent et al., 2006). The estimated annual global loss to the disease is about $2 billion (Smith et al., 2011). The disease is a lymphoproliferative disease of the domestic chicken in which mononuclear infiltration of the visceral organs as well as infiltration and demyelination of the peripheral nerves are common features (Payne, 1999; Osterrieder et al., 2006). It is caused by a cell associated alpha herpesvirus classified in the family Herpesviridae and Marek’s disease virus (MDV) is the prototype of the group designated as serotype I (Calnek, 2001; Singh et al., 2012). MD manifests in chickens of about 4 weeks of age, but clinical disease is most common in chickens between 12 and 24 weeks, though older chickens may be affected (Payne, 1985).

MD is a ubiquitous infection of poultry throughout the world and outbreaks in farms resulting in significant losses are very common (Payne, 1985). Prior to the use of vaccine, it constituted a serious economic threat to the poultry industry in the world and it still causes great economic losses in areas where vaccinations are not routinely practiced (Schat, 1998; Lobago and Woldemeskel, 2004). The variability of syndromes and types of MD, its wider host range and the propensity of the virus to evolve in time have created a great impact on the diagnosis and control of the disease (Witter, 1997). MD has been described in Nigeria and it constitutes a problem to poultry production (Okwor and Eze, 2011). Nigeria is a tropical country with two successive seasons annually. These are the rainy season that occurs between March and October and the dry season that occurs between November and February. The rainy season is associated with rains, damp and humid atmosphere and minimal dust.
while the dry season is associated with dry environment, dust and wind especially during the harmattan period. Poultry wastes are drier and easily suspended in the air and could constitute an important mode of spread of airborne infections. Control strategy for MD requires an understanding of the epidemiology of the disease especially as it relates to virus shedding and spread (Atkins et al., 2011). MDV is an airborne pathogen with infection occurring via inhalation and virus shedding occurs by infected feather follicles epithelium (Islam et al., 2008; Atkins et al., 2011). Transmission of MD is mostly by inhalation of infectious dander and poultry dust. The follicular cells of the feathers are the most important source of infection and are responsible for the infectivity of dander, poultry house dust and litter; the virus particles in these materials are able to survive for up to one year or more at room temperature (Payne, 1999). The resulting dust and dander from dead stratified cells and moulted feathers can remain in the environment and act as reservoirs of infection for chickens (Atkins et al., 2011). The infection tends to persist in commercial farms for long periods and spreads from one batch of birds to another through horizontal transmission, (Anderson et al., 1998). Control of MD can be possible through reduction and restriction of dust circulation within the farm, since transmission of MD is mostly by infectious dander circulating in poultry dust and the environment. This work therefore reported the use of isolation brooding and brooding during periods of low atmospheric dust concentrations to control measures to outbreaks of MD.

MATERIALS AND METHODS

Case history/Outbreak

Persistent outbreaks of a disease were reported in a farm located in Nsukka, South East Nigeria. The birds were reared on deep litter system. The first outbreak was among a batch of 300 brown shaver pullets from Zartech Hartchery Ibadan at the age of 12 weeks. The disease was diagnosed to be MD by clinical signs and post mortem lesions and later confirmed by agar-gel precipitation test as described by Sharma (1998). The disease has lingered in the farm for six years as attempts to control it by vaccination of subsequent batches of chicks introduced into the farm only did not yield the desired result as the birds still shed the virus and infected subsequent batches. The vaccine used was the commercially available cell-free freeze-dried Herpesvirus of turkeys (HVT) vaccine. A single dose of the vaccine was administered to each bird subcutaneously in the thigh muscle. The disease presented both acute and classical conditions and occurred in other batches of pullets (batches varied between 300 and 1800 birds) reared in the farm within the period. The age incidences in all cases were between 10 and 14 weeks. The severity of the disease varied among the batches with some of the breeds showing better resistance than others.

Outbreaks were noticed to be more severe and more generalized in birds reared during the dry harmattan period than in those reared during the rainy season. A combination of isolation of the brooder birds reared away from the older virus shedder birds and timing of introduction of the brooder birds during the rainy season were tried as measures to control and possibly eradicate the disease.

Experimental design

The study covered the period of May 2009 to April 2011. The isolation brooding and laboratory studies were carried out as shown in Table 1.

Table 1. Experimental protocol.

<table>
<thead>
<tr>
<th>Year</th>
<th>Description</th>
<th>End of Year I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year I</td>
<td>Isolation brooding of a total of 1800 during rainy periods with strict biosecurity measures</td>
<td>Random serum sample collection (50) for AGID test</td>
</tr>
<tr>
<td>Year II</td>
<td>Isolation brooding of a total of 1500 during rainy periods with strict biosecurity measures</td>
<td>Random serum sample collection (50) for AGID test</td>
</tr>
<tr>
<td>Year I</td>
<td>Isolation Brooding of a total of 1800 during rainy periods with strict biosecurity measures</td>
<td>Random serum sample collection (50) for AGID test</td>
</tr>
</tbody>
</table>

Isolation brooding

The first control strategy against the disease was done using isolation brooding. Here, the brooding house which was fairly located away from other pens that housed older productive birds was demarcated with wood and palm front fencing. The windows of the brooding house facing the direction of these other pens were closed permanently. Entry into the brooding premises was restricted to the attendants who must disinfect themselves, their clothings and foot wears before entry. Strict biosecurity measures were applied both during brooding and upon introduction of the birds into the pens. A combination of ISOL® and IZAL® were used to disinfect the brooding premises on a regular basis. Other biosecurity measures were washing of hands and feet on arrival, changing into the farm apron and foot wears, matching of leg in foot dip before entering the farm.

The birds were left in the brooding house from day-old to 24 weeks (6 months) before they were transferred to their laying pen. The fencing was replaced with new ones each time a new batch of birds was introduced.

Timing of brooding

In addition to the isolation brooding and biosecurity measures mentioned earlier, the pullets were introduced between March and
September. This coincided with the period of rain when the environment was relatively damp and dusts were minimal in the air. No bird was introduced between October and February.

Clinical examination and sample collection

The birds were observed on a daily basis for clinical signs of MD. Serum samples were collected and used in agar-gel immunodiffusion test. Blood was collected randomly from the birds.

Agar gel immunodiffusion test (AGID)

The conventional MD diagnosis was done following that described by Sharma (1998). Briefly, feather tips and follicle epithelium from a flock already confirmed to have MD using specific immune serum was used to prepare the antigen. Agar gels were prepared in petri dishes to a thickness of 2 to 3 mm and holes were cut in the agar using templates with a centre well and 6 wells spaced at equal distances around the centre well. The antigen was placed in the centre well and the test serum samples were placed in other wells and incubated for 24 h at 37°C. Formation of a precipitin line showed positive reaction. When two positive samples were placed in adjacent wells, a continuous line of identity was formed.

RESULTS

During the first year, out of a total of 1800 pullets that were procured at day old and reared up to 24 weeks of age, none showed clinical disease during the brooding and rearing period. When they were introduced into laying pen after the isolation period, 83 birds were observed to have clinical signs of MD between 25 and 90 weeks of age giving 4.6% (Table 2). Figure 1 shows classical clinical sign (paralysis of limb and wing) in layers seen during one of the experiment. At the end of the first year of study, out of the 50 samples collected, 8 were positive while 42 were negative giving 16% seropositive (Table 3).

During the second year of study, the 1500 pullets introduced did not show clinical signs of Marek’s during the brooding and rearing periods. When introduced at 24 weeks into the laying pen, no birds was observed with clinical signs of MD, thus giving 0% morbidity from MD (Table 2). At the end of this year, out of the 50 serum samples collected, one sample was positive while 49 samples were negative thus giving 2% seropositive (Table 3).

During the third year of studies, out of a total of 1800 pullets that were procured at day old and reared up to 24 weeks of age, none showed clinical disease during the brooding and rearing period. When introduced at 24 weeks into the laying pen, no birds was observed with clinical signs of MD, thus giving 0% morbidity from MD (Table 2). Serum samples collected from this last batch of layers showed no detectable antibodies against MDV giving 0% seropositive (Table 3).

DISCUSSION

Among the factors that have been associated with host resistance to MD are genetic constitutions, prior infection or vaccination with avirulent herpesvirus strains, and old age (Anderson et al., 1971; Crittenden et al., 1972). The result of the aforementioned trial showed an effective control of infection by a combination of the practices mentioned earlier. These are in agreement with the report of Payne (1999) who observed that isolation of growing chicks from sources of infections, use of genetically resistance strains and vaccination are good measures for the control of MD. Vertical or transovarian transmission is unimportant in MD (Vietitz, 1987). The first strategy or method is keeping birds away as much as possible from sources of infection since horizontal transmission through infectious dander and dusts are the most important means of spread and transmission of the virus (Anderson et al., 1998). The second strategy was also tried or aimed at rearing the birds when it is damp as there is less dust in

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of birds introduced</th>
<th>No. of birds showing clinical signs of MD (25-90 weeks)</th>
<th>Percentage morbidity resulting from MD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1800</td>
<td>83</td>
<td>4.6</td>
</tr>
<tr>
<td>2</td>
<td>1500</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>1800</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of birds introduced</th>
<th>No. of samples examined</th>
<th>No. positive</th>
<th>No. negative</th>
<th>Percentage seropositive</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1800</td>
<td>50</td>
<td>8</td>
<td>42</td>
<td>16</td>
</tr>
<tr>
<td>2</td>
<td>1500</td>
<td>50</td>
<td>1</td>
<td>49</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>1800</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>0</td>
</tr>
</tbody>
</table>
the poultry farms during the raining seasons. This will minimize the quantity of virus being transmitted through aerosol.

Anderson et al. (1971) has used filtered air and positive pressure houses in the control of MD. MD is spread mostly by inhalation of infectious dander carried in poultry dusts and litter and this being a ubiquitous agent can survive in the dust and remain infective for months (Witter, 1998; Calnek and Witter, 1997). Many birds also act as carriers shedding the virus in the environment (Payne, 1985). Moreover, vaccination greatly reduces clinical disease but not persistent infection by MD virus. The vaccine viruses are also carried through the life of the fowl and are continually shed which results in the ubiquitous presence of the virus (Office International des Epizooties (OIE), 2010).

Experiments by many scientists have shown age-related resistance to MD as is observed in older birds being refractory to infection with increased resistance to tumor formation (Witter and Gimeno, 2006; Anderson et al., 1971). Witter et al. (1973) demonstrated that birds 20 to 22 weeks of age and free of prior infection, were substantially more resistant to mortality and tumor induction caused by exposure to the virus than one day old chicks. It therefore means that keeping young bird away from sources of infection up to when they become older could be a good way of controlling MD. As shown in this work, the birds were isolated from infectious sources in the farm and also reared when there was less dust circulating in the air. It yielded good result within three years and therefore supported the claim that MD can be controlled by isolation brooding. This delay of contact was done up to 24 weeks in other birds to make sure that the birds were mature enough to resist the infection.

It should be noted that most hatcheries in Nigeria do not vaccinate day old chicks against MD. Normally, birds are vaccinated at day old in hatcheries in order to protect them before resistance to MD is developed. Attempts to eradicate MD in this poultry farm by post-exposure vaccination did not yield the desired result; because it did not prevent the shedding of the virus and transmission of infection. Pre-exposure vaccinated chickens may also shed the virus and transmit infection, although tumors and deaths will be reduced. Vaccinated birds can be vireamic and can persistently transmit the virus (Hlozanek et al., 1977). MD vaccines especially Serotype 1 vaccine

**Figure 1.** Paralysis of limb and wing in classical Marek's disease.
strains (Rispens) need special storage facilities (liquid nitrogen) and this can only be maintained mainly by hatcheries and research institutes. Hatcheries are in better positions to vaccinate day old chicks before exposure to the field virus.

Conclusion

Isolation brooding, biosecurity measures and brooding during periods with low proportion of dusts in the air is an effective way of controlling MD in cases of outbreaks. Brooding completely outside the farm may even be a better way of control. The use of concrete fencing in place of woody and palm fronts may enhance control by isolation brooding. Efforts to procure vaccinated birds from hatcheries will help in the control of the disease. Moreover, in infected farms, brooding of birds during the hamattan period should be discouraged.

REFERENCES

Full Length Research Paper

Sero-prevalence of small ruminants’ brucellosis in four districts of Afar National Regional State, Northeast Ethiopia

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A cross-sectional study was conducted in four districts of Afar region to determine the prevalence of brucellosis in small ruminants. One thousand fifty sera were tested using modified Rose Bengal plate test (mRBPT) and complement fixation test (CFT) as screening and confirmatory tests, respectively. The results showed that 7.1 and 13.6% of sheep and goats were sero-positive, respectively. District level sero-prevalence ranged from 3.6 to 13.6% and 3.3 to 6.7% in sheep and goats, respectively. The logistic regression model for small ruminants identified goats (odds ratio (OR) = 2.36; 95% CI: 1.46 to 3.82) are at higher risk of brucellosis as compared to sheep. In addition, small ruminants greater than two years (OR = 3.132; 95% CI: 1.6 to 6.15), and larger flock size (OR = 2.04; 95% CI: 1.35 to 3.1) are at higher risk of brucellosis than their counter categories. The results of this study demonstrated that livestock brucellosis is widely prevalent in the study areas. Hence, the study suggests the need for implementing control measures and raising public awareness on prevention methods of brucellosis.

Key words: Afar region, brucellosis, complement fixation test, modified Rose Bengal precipitation test, small ruminants.

INTRODUCTION

Brucellosis is considered by the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World animal Health Organization as the second most important zoonotic disease in the world accounting for the annual occurrence of more than 500,000 cases (Schelling et al., 2003; Pappas et al., 2006). The disease can affect almost all domestic and wild mammal species and cross transmission can occur between cattle, sheep, goats, camels and other species (Ghanem et al., 2009). It is an important zoonotic disease and causes significant reproductive losses in sexually mature animals (Radostits et al., 1994). The disease is manifested by late term abortions, weak calves, still births, infertility and characterized mainly by placentitis females, epididymitis and orchitis in males, with excretion of the organisms in uterine discharges and milk in female animals. It also causes considerable loss of productivity through high morbidity (Pappas et al., 2006). Brucella melitensis, Brucella abortus, and Brucella suis are zoonotic pathogens which can infect humans. Brucella canis may cause infections in immune-suppressed individuals (Young, 2000).

Globally, this disease is under-reported because of its vague clinical symptoms, difficult laboratory diagnosis and lack of familiarity by the medical professionals (Corbel, 2006). It has been stated that in Sub-Saharan

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Africa (SSA), the epidemiology of brucellosis in humans and livestock are not well understood and available data are limited (McDermott et al., 1999; Mangen et al., 2002; McDermott and Arimi, 2002; Schelling et al., 2003). Ministry of Agriculture and Rural Development (MoARD) estimated that pastoralists own 7.06 million (73%) goats, 4.25 million (25%) sheep, 7.70 million (20%) cattle and 100% of the camels (MoARD, 2008). The rest of livestock is reared by highland mixed crop-livestock production system farmers. Under Ethiopian context, livestock of different species usually share pastures and dwellings. This may play a role in maintenance and transmission of endemic diseases such as tuberculosis and brucellosis (Amenu et al., 2010). Moreover, brucellosis is common in rural areas because farmers live in close contact with their animals and often consume raw dairy products. However, the vending of dairy products may also bring the disease to urban areas (Mantu and Amarnath, 2008).

Very few reports have been published about brucellosis in small ruminants of Ethiopia as compared to its economic and public health importance. Ashenafi et al. (2007) revealed 9.4 and 4.8% prevalence using Rose Bengal plate test (RBPT) and complement fixation test (CFT), respectively. Teshale et al. (2006) documented 1.9% (n = 38) positive using RBPT and 9.7% (n = 193) positive by enzyme linked immunosorbent assay (iELISA) in pastoral areas.

In Afar region activities such as habit of consuming raw milk, unsafe handling of aborted materials and other infected excretions of animals, rearing of diversified animal species together, herding of large number of animals collectively is widely practiced. Moreover because of repeatedly occurring natural phenomenal such as concurrent drought, earth quake and flooding in the region the introduction of new animals from outside the districts as a replacement stock by different non-governmental organizations (NGOs) and food security programs for vulnerable communities may also be causes of introduction of infection to the area. There is paucity of current situation about the disease in livestock and humans in the region. Therefore the objectives of this study were to determine the sero-prevalence of brucellosis in sheep and goats and assess the possible risk factors for the disease.

MATERIALS AND METHODS

Description of study area

The Afar National Regional State (ARS) is located in the northeast part of Ethiopia. Administratively, the region is divided into five zones which are further subdivided into 32 districts and 358 peasant associations (PAs). Pastoralism and agro-pastoralism are the two major livelihood ways practiced in the region. The population is estimated to be 1.2 million of which 90% are pastoralists and 10% agro-pastoralists. The total surface area of the region is estimated to be 97,970 Km² (ARFEB, 2007). The study was conducted in four districts namely, Afambo, Assayita, Teru and Awura.

Assayita and Afambo are parts of the administrative Zone 1 located at about east of Semera town. The district has 11 and 8 rural PAs and 2 and 1 urban PAs, respectively. Assayita and Afambo have altitudes of 350 and 280 to 850 m above sea level (masl), respectively. Assayita is a destination for pastoralists from zone four and neighboring districts of zone one in search of pasture for their livestock as it is endowed with several large scale irrigation farms using Awash River which attracts the pastoralists to feed agricultural leftovers. Afambo district is known by Lake Abbi which is the final destination for Awash River (ARFEB, 2007).

Awura and Teru districts are found in Zone 4 and located 250 and 350 km from Semera town and established with eleven and twelve PAs, respectively. Some PAs practice agricultural activity and they are on the transit to agro-pastoral arena. The climate of Teru is arid with minimum and maximum temperatures of 28 and 50°C, respectively. The climate of Awra is generally arid to semiarid, with high temperature. The districts have two main rainy seasons, karma (July to September) and sugum (March to April) and also short rainy season called Dadaa within January. There are dry seasons called Gillil and Hagay. The annual rainfall is from 200 to 800 mm. There are no perennial rivers in these districts. The main water sources are seasonal rivers, Ela and Pond. The districts are covered by sparse Acacia species and extensive grazing land (ARFEB, 2007).

Study population

The approximate number of sheep and goats in Awesi-Resu (zone one) were 687,551 and 1,083,567, respectively (Community-supported agriculture (CSA), 2010, 2011); while in Fanteyna Resu (zone four) approximately 418,206 sheep and 398,127 goat populations exist in the area and considered as study population (ARFEB, 2007). Sheep and goats which were above 6 months of age, with no history of vaccination against brucellosis were included in the study. Then individual animal age, species, sex category and flock size were recorded. Afterwards, herd size per household was classified as ≤ 30 and > 30. Moreover, based on their sexual maturity animals were classified into ≤ 2 years and > 2 years, respectively.

Study design

A cross-sectional study design was conducted from November, 2011 to April, 2012, to determine the sero-prevalence of brucellosis in sheep and goats across selected pastoral and agro-pastoral residents of the Afambo, Assayita, Awura and Teru districts and to identify potential risk factors associated with sero-positivity. Four districts and about 30% PAs per district were selected purposively based on easier accessibility as well as past experience. The districts are on the transit to agro-pastoral areas and are endowed with several large scale irrigation farms using Awash River which attracts the pastoralists to feed agricultural leftovers. Afambo district is known by Lake Abbi which is the final destination for Awash River (ARFEB, 2007).

Experimental

Multistage sampling technique was used in the survey of sheep and goat brucellosis. The PA was considered as primary unit, the herds as secondary units and individual animals as tertiary units. An average of 30% of small ruminants aged 6 month and above were picked randomly from each selected herd until the calculated sample size was achieved.

Sheep and goats herds in 13 PAs from four districts were sampled during the study based on the livestock population of each...
district. In order to determine the desired sample size there was no previous reports of prevalence in the districts except the 0% (n = 32) report by Ashenafi et al. (2007) in Assayita. Hence, the average expected prevalence rate was assumed to be 50% for the area within 95% confidence intervals (CI) at 5% desired accuracy as stated by Thrusfield (2005) as shown in the formula below:

\[ n = \frac{1.96^2 \times P_{ex} \times (1 - P_{ex})}{d^2} \]

Where \( n \) = sample size, \( d \) = desired absolute precision (0.05), \( P_{ex} \) = expected prevalence (50%), thus the desired sample size for \( P_{ex} = 0.5 \) is \( n = 384 \). But, we inflate the sample size to 1,050 to increase the representativeness of the samples to the wider population. Hence, \( n = 1,050 \) goats and sheep were sampled. Sampling was proportionally distributed based on the total small ruminant population in the study districts and PAs. Blood samples were collected from jugular vein of each animal of selected herds using plain vacutainer tubes and allowed to clot at room temperature. Serum was separated from clotted blood by decanting to other tubes and stored at -20°C until laboratory test was performed.

**Complement fixation test (CFT)**

Positive sera with RBPT were further tested with CFT for confirmation using standard Brucella abortus antigen (New Haw, Addleston, Surrey KT15 3NB, UK). The CFT test proper and reagent preparation procedures were following the procedures outlined by OIE (2004). Sera with strong reaction, more than 75% fixation of complement (3+) at a dilution of 1:5 or at least with 50% fixation of complement (2+) at a dilution of 1:10 and above were classified as positive (OIE, 2004).

**Data processing and statistical analysis**

The data were entered into Microsoft Excel 2007 and coded data were stored and finally transferred to SPSS® version 16 for statistical analysis. Descriptive statistics were used to analyze the sero-positivity at individual animal and flock levels. Logistic regression and Chi-square test (\( \chi^2 \)) were employed to see the association of risk factors with that of sero-positivity to Brucella antibody and the degree of association was computed using Odds ratio (OR) and 95% confidence interval (CI). A test value was considered as statistically significant when \( P < 0.05 \). Odd ratio (OR) was used to indicate the degree of risk factor association with the disease occurrence as defined by 95% confidence intervals. Variable reduction was performed by fitting univariate logistic regression for each covariate and variables with \( p \)-value > 0.25 were dropped.

### RESULTS

The sero-positivity of sheep and goats in study districts was 7.1 and 13.6%, respectively (Table 1).

### Effect of risk factors on sero-prevalence of brucellosis in small ruminant

Higher prevalence was seen in goat than sheep with a statistically significant difference (\( X^2 = 24.91, \ P < 0.05 \)).

**Table 1. Herd level sero-prevalence of small ruminants brucellosis.**

<table>
<thead>
<tr>
<th>Zone</th>
<th>District</th>
<th>Species</th>
<th>Number tested</th>
<th>RBPT positive (%)</th>
<th>CFT positive (%)</th>
<th>Herd Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Number Tested</td>
<td>Positive (%)</td>
<td></td>
</tr>
<tr>
<td>Afambo</td>
<td></td>
<td></td>
<td>100</td>
<td>12 (12)</td>
<td>9 (9)</td>
<td>43</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>237</td>
<td>34 (14.35)</td>
<td>30 (12.66)</td>
<td></td>
</tr>
<tr>
<td>Assayta</td>
<td></td>
<td></td>
<td>69</td>
<td>5 (7.24)</td>
<td>4 (5.8)</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>193</td>
<td>32 (16.58)</td>
<td>30 (15.54)</td>
<td></td>
</tr>
<tr>
<td>Awura</td>
<td></td>
<td></td>
<td>110</td>
<td>4 (3.64)</td>
<td>4 (3.64)</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>128</td>
<td>18 (14.06)</td>
<td>15 (11.71)</td>
<td></td>
</tr>
<tr>
<td>Teru</td>
<td></td>
<td></td>
<td>45</td>
<td>6 (13.33)</td>
<td>6 (13.33)</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>168</td>
<td>25 (14.88)</td>
<td>24 (14.28)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>324</td>
<td>27 (8.33)</td>
<td>23 (7.1)</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>726</td>
<td>109 (15.0)</td>
<td>99 (13.64)</td>
<td></td>
</tr>
</tbody>
</table>

**Rose Bengal plate test (RBPT)**

The modified Rose Bengal plate test (mRBPT) was done in Semera Regional Veterinary Laboratory in order to screen positive samples by RBPT using RBPT antigen (Institut Pourquier 325, rue de la galèra 34097 Montpellier cedex 5, France). Positive sera were then retested using complement fixation test (CFT) of same origin at the National Veterinary Institute (NVI), Debre Zeit. Samples were considered positive for brucellosis if they were positive for RBPT and CFT. For the mRBPT, the procedure described by Alton et al. (1975) was followed with little modification by Blasco et al. (1994). Reactions were categorized as 0, +, ++, ++++, according to Nielsen and Dunkan (1990), where: 0 = means no agglutination, + = barely perceptible agglutination (using magnifying glass), ++ = fine agglutination, some clearing, and +++ = clumping, definite clearing. Those samples identified with no agglutination (0) were regarded as negative, while those with +, ++ and +++ were regarded as positive.

*J. Vet. Med. Anim. Health*
Table 2. Association of risk factors with brucellosis reactivity in small ruminants.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>No. sampled</th>
<th>Complement fixation test</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone</td>
<td>Afambo</td>
<td>337</td>
<td>39 (11.6)</td>
<td>0.90</td>
<td>0.764</td>
</tr>
<tr>
<td></td>
<td>Assayita</td>
<td>262</td>
<td>34 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Awura</td>
<td>238</td>
<td>19 (8)</td>
<td>4.8</td>
<td>0.187</td>
</tr>
<tr>
<td></td>
<td>Teru</td>
<td>213</td>
<td>30 (14.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>Goat</td>
<td>726</td>
<td>99 (13.6)</td>
<td>9.32</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>324</td>
<td>23 (7.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>204</td>
<td>16 (7.8)</td>
<td>3.52</td>
<td>0.061</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>846</td>
<td>106 (12.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>≤2 years</td>
<td>200</td>
<td>10 (5)</td>
<td>10.54</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>&gt;2 years</td>
<td>850</td>
<td>112 (13.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flock size</td>
<td>≤30 animals</td>
<td>462</td>
<td>37 (8)</td>
<td>10.47</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>&gt;30 animals</td>
<td>588</td>
<td>85 (14.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. Association of risk factors with brucellosis reactivity in goats in the study areas.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>No. sampled</th>
<th>Complement fixation test</th>
<th>Chi-square</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone</td>
<td>1</td>
<td>430</td>
<td>60 (14)</td>
<td>0.90</td>
<td>0.764</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>296</td>
<td>39 (13.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>District</td>
<td>Afambo</td>
<td>237</td>
<td>30 (12.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Assayita</td>
<td>193</td>
<td>30 (15.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Awura</td>
<td>128</td>
<td>15 (11.7)</td>
<td>23.43</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>Teru</td>
<td>168</td>
<td>24 (14.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>169</td>
<td>13 (7.7)</td>
<td>6.61</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>557</td>
<td>86 (15.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>≤2 years</td>
<td>158</td>
<td>9 (5.7)</td>
<td>10.81</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>&gt;2 years</td>
<td>568</td>
<td>90 (15.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flock size</td>
<td>≤30 animals</td>
<td>313</td>
<td>31 (9.9)</td>
<td>6.50</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>&gt;30 animals</td>
<td>413</td>
<td>68 (16.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The association of large flock size and sero-positivity was statistically significant ($X^2 = 10.47$, $P < 0.05$) (Table 2). Moreover, the difference in sero-prevalence between the sex groups of goats was statistically significant ($X^2 = 6.61$, $P < 0.05$) (Table 3). In sheep only flock size has significant difference ($P < 0.05$).

Univariate logistic regression analysis of risk factors

The univariable logistic regression analysis of the putative risk factors showed statistically significant ($P < 0.05$) difference on brucella reactivity between small ruminants with small and large flock size (Table 4).
Table 4. Effects of risk factors on the overall sero-prevalence of small ruminants' brucellosis using CFT.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zone</td>
<td>4*</td>
<td>-</td>
<td>-</td>
<td>0.508</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.14</td>
<td>0.78-1.67</td>
<td></td>
</tr>
<tr>
<td>Teru*</td>
<td>-</td>
<td>-</td>
<td>0.194</td>
<td></td>
</tr>
<tr>
<td>District</td>
<td>Afambo</td>
<td>1.25</td>
<td>0.75-2.09</td>
<td>0.387</td>
</tr>
<tr>
<td></td>
<td>Assayita</td>
<td>1.09</td>
<td>0.65-1.87</td>
<td>0.725</td>
</tr>
<tr>
<td></td>
<td>Awura</td>
<td>1.89</td>
<td>1.03-3.47</td>
<td>0.04</td>
</tr>
<tr>
<td>Species</td>
<td>Sheep*</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>2.07</td>
<td>1.29-3.32</td>
<td>0.003</td>
</tr>
<tr>
<td>Sex</td>
<td>Male*</td>
<td>1.68</td>
<td>0.97-2.92</td>
<td>0.063</td>
</tr>
<tr>
<td></td>
<td>&lt;2 years*</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;2 years</td>
<td>2.88</td>
<td>1.48-5.61</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>&lt;30*</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;30</td>
<td>1.94</td>
<td>1.29-2.92</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*Reference category.

Multivariable stepwise logistic regression analysis of risk factors for brucellosis reactivity

The difference for age-group, sex and herd size based infection rates observed in goat in the initial logistic regression model were also found evident after the control of confounding factors in the stepwise multivariable logistic regression analysis. On the other hand, in sheep only flock size was used while sex and age of animals were excluded from the model indicating that both were not significantly affecting reaction to brucellosis. Table 5 shows the result of stepwise multivariate logistic regression analysis of risk factors and brucellosis reactivity of sheep and goat in the study area.

DISCUSSION

The overall sero-prevalence in this finding is slightly lower than reports previously by Teshale et al. (2006) and Kaoud et al. (2010) in Ethiopia and Egypt, respectively. The variation might be due to geographical differences, number of animals included and methods implemented. Brucellosis was detected in all the four districts of the two zones. The difference in prevalence between zones and among the districts was not statistically significant. It could be due to the similarity in the agro-ecological conditions and livestock management system in the area. The herd level prevalence is higher than individual animal level and this characterizes the nature and importance of the disease in the large flock size. This signifies that brucellosis has significant economic implication in its ability to bring about morbidity at flock level.

Epidemiology of the disease at individual and herd level show wider spread of the disease in different species of animals. In Afambo and Assayita districts of zone one, animals are kept in confinement around cultivation fields than the other two districts, as the districts are largely dominated by agricultural irrigation using Awash River. This may be responsible for the high prevalence in zone one as infection is easily transmitted within the entire herd under this management system. Teru and Awura districts are mostly pastoralist settings and are dominated by free range management system.

In sheep, the study is fairly in agreement with different findings. Shehu et al. (1999) reported a prevalence of 6.6% in sheep in Nigeria. However, the findings disagree with that of Yesuf et al. (2010) who reported a sero-prevalence of 1.5% in south Wollo, Teshale et al. (2006) and Ashenafi et al. (2007) who reported sero-prevalence of 14.6 and 3.2% in Mille and Dalifage districts of Afar region and in Afar region, respectively. Such differences might be attributed to methodologies followed by number of animals and geographical and management differences. In other countries, Bale et al. (1982) reported 15.9% prevalence in a study conducted in Northern Nigeria.

Higher prevalence in goats compared to this finding was reported by Teshale et al. (2006) (16.45%), Bale et al. (1982) (34.8%) and Ojo et al. (2007) (45.75%) in Afar region of Ethiopia, northern Nigeria and Abeokuta, respectively. However, a lower prevalence of 5.8% was reported by Ashenafi et al. (2007). The high prevalence and wide distribution are not surprising since small ruminants are not being vaccinated against brucellosis, coupled with the traditional practice of communal grazing in most part of the region.

Statistically significant difference in sero-prevalence was observed between sheep and goats where goats were found to be at higher risk than sheep. This finding is in agreement with results of Omer et al. (2000) and Radostitis et al. (1994). The higher prevalence in goats than in sheep may be in part due to the greater susceptibility of goats to Brucella infection than sheep and partly it may be due to the fact that sheep unlike goats do not excrete the Brucella organisms for longer periods of time. This can reduce the potential of the spread of the disease among sheep flock (Radostitis et al., 1994).

There is no statistically significant difference between male and female animals. Hirsh and Zee (1999) have reported that male animals are less susceptible to Brucella infection, due to the absence of erythritol. However, in support of the present findings, Teshale et
brucellosis is common in very old aged goats and animals within large flock size. Thus, there is a need to design and implement control measures aiming at preventing further spread of the disease in the region. Critical assessment of the economic impact of the disease, which emanates from its effect on reproductive and production performance of animals, is worthy. Studies to investigate the link between livestock and human brucellosis and cross infection among species in the region should be conducted to devise appropriate preventive mechanisms. Isolation and identification of the biotypes of brucella responsible for infection in the region should be carried out to look for effective vaccine and treatment. Herd vaccination program should be implemented to prevent the impact of the disease on economy as well as human health hazards.

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**Table 5. Multivariable stepwise logistic regression analysis of risk factors for brucellosis in small ruminants.**

<table>
<thead>
<tr>
<th>Species</th>
<th>Variable*</th>
<th>Complement fixation test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR</td>
</tr>
<tr>
<td>Goat</td>
<td>Female</td>
<td>2.075</td>
</tr>
<tr>
<td></td>
<td>&gt;2 years</td>
<td>3.061</td>
</tr>
<tr>
<td></td>
<td>&gt;30 flock size</td>
<td>1.91</td>
</tr>
<tr>
<td>Goat and sheep</td>
<td>Goat</td>
<td>2.361</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1.785</td>
</tr>
<tr>
<td></td>
<td>&gt;2 years</td>
<td>3.132</td>
</tr>
<tr>
<td></td>
<td>&gt;30 flock size</td>
<td>2.036</td>
</tr>
</tbody>
</table>

Reference categories were omitted to avoid repetitions.

---

Conclusion

The sero-prevalence described in this study shows that brucellosis is a widespread and well-established infection among goats and sheep across the two zones and all the study districts of Afar region. The most important risk factors identified for individual animal sero-prevalence were age, species and flock size. Sero-prevalence of brucellosis was observed between age and healthy animals. Statistical significant difference was observed between age and sero-positivity in goats but not in sheep. In the latter, the reason may be few number of the young sheep included in the sampling. This result was consistent with Ashenafi et al. (2007). Brucellosis infection may occur in animals of all age groups but persists commonly in sexually mature animals (Radostitis et al., 1994). Younger animals tend to be more resistant to infection and frequently clear infections although few latent infections may occur (Radostitis et al., 1994; Walker, 1999).

The prevalence has increased in Assayita district when it is compared with a previous study done by Ashenafi et al. (2007) who found 0% in small ruminants. This increase may be due to the seasonal migration of livestock from Chiffera, Awura, Teru and Golina to Assayita and Dubiti districts of the region in search of cotton, maize and sorgume leftover as an animal feed. Mixing of the different species during migration, at watering or night enclosures (resting) among different species is a common practice in Afar area. The other contributing factor to the spread of brucellosis may be the movement of animals for grazing and watering as aggregating the animals around watering point will increase the contact between infected and healthy animals thereby facilitating the spread of the disease. The disease is a herd wide problem rather than individual animals and this should call our attention to its economic impact on the region and the nation at large.
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2393.
Prevalence of gastrointestinal parasitism of cattle in Gedebano Gutazer Wolene district, Ethiopia

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This study was carried out to determine the prevalence and monthly distribution of helminth parasites of cattle in Gedebano Gutazer Wolene district, Southern Ethiopia, from November 2008 to May 2009. A total of 406 faecal samples were collected and subjected to qualitative and quantitative coprologic parasitological examination. From the cattle examined an overall prevalence of 39.6% (n=161) was recorded. The study revealed an overall prevalence of 37.9% Strongyle, 22.4% Toxocara species, 16.1% Fasciola species, 13.7% Trichuris species and 9.9% Paramphistomum species, Strongyle and Toxocara species were the most prevalent parasites encountered in the study area. Statistically significant difference (P < 0.05) in the prevalence of helminthosis between season and different age groups of cattle was noted. The prevalence of helminth parasites during wet season was significantly higher (P < 0.05) than the dry season. Out of 61 Strongyle egg type positive cattle, 14 (22.95%) were massively, 29 (47.55%) moderately and 18 (29.50%) were lightly affected. Intensity of Strongyle infection in terms of egg per gram (EPG) showed no variations when different age group and sex are compared. The current study indicated that season and age of animals are important factors associated with helminth parasites of cattle of the study area. The study identified high prevalence of parasitism demand due attention to enhance the productivity of cattle. Therefore, strategic control approach by using effective broad spectrum anthelmintics at the beginning of rainy season is recommended and awareness creation to the farmers should be instituted in the study area.

Key words: Ethiopia, cattle, gastrointestinal parasitism, prevalence.

INTRODUCTION

Ethiopian economy is predominantly based on agriculture which is considered as a primary factor in securing food self-sufficiency, generating employment and income for the poor (Coppock, 1994). Livestock sub sector plays a vital role which contributes 33% of agricultural GDP and 19% to the export earnings. In addition, crop production is almost exclusively dependant on livestock especially draft power of cattle. Cattle production, among the sector of livestock production systems, is a critical issue in Ethiopia. In spite of all this, full exploitation of cattle potential is mainly constrained and impeded at a great extent by parasitic diseases (Sintayehu, 1999; Ayele et al., 2003; Zegeye, 2003).

Infectious diseases especially gastrointestinal parasites and exo-parasites are considered as the major diseases of cattle in the district. Helminth parasite infections in cattle are of the major importance in many agro-ecological zones and are a primary factor in the reduction of productivity. This is further aggravated in small holder farmers due to the limited availability of land and feed resources. Year round utilization of communal grazing and watering places shared livestock kept by smallholder are a major source of infection. The prevention and control of helminth parasites is not based on disease epidemiology rather is targeted at sick individual (Alekaw, 2000). According to previous study reports, the
prevalence of helminth parasites in cattle of many African countries including Ethiopia is found to be high. For instance, Etsehiwot (2004) and Fikru et al. (2006) reported 82.2 and 50.2% in Central Ethiopia and Western Oromia, respectively. In Tanzania, Keyyu et al. (2006) obtained prevalence of 44.4 and 37% for large and small scale dairy cattle, respectively. Similarly, Charlotte and Madsen (1998) reported that gastrointestinal parasites are among the constraints in dairy farms of Zimbabwe.

Though many constraints due to parasitic infections in cattle have been out faced, no any previous study prevalence was conducted and there is scarce information in the district. Therefore, the availability of recent information on helminth parasites of cattle in the district has paramount importance to design appropriate strategies for prevention and control of helminth parasite diseases of cattle in the area. In other words, there seems to be an urgent demand to assess the extent and magnitude of gastrointestinal helminth parasites of cattle in Gedebero Gutazer Wolene district, Southern Ethiopia. Therefore, the objectives of this study were to determine the major helminth parasites and assess seasonal occurrence and distribution of helminth parasites of cattle in the study area.

MATERIALS AND METHODS

Study area

The study was conducted in Gedebero Gutazer Wolene district located in Gurage zone at about 120 km from Addis Ababa. The district has a total area of 54,000 ha, and the altitude ranges from 1800 to 3500 m above sea level. The annual rain fall ranges from 780 to 1200 mm and temperature varies between 7 and 25°C. The wet season is from March to August and dry season ranges from September to February. The total livestock population of the district is 56,369 cattle, 39,058 sheep, 12,124 goats and 16,794 equine that are kept in mixed type of agriculture. The study was carried out between November 2008 and May 2009 in four peasant associations, namely Tilamo, Deneb, Enge and Jimma which were accessible for vehicles.

Study design

For this study, indigenous breed (zebu) of all age and sex were selected on the study animals. Simple random sampling method was used to select each study animal. The sample size was determined based on the expected prevalence of 50% and absolute desired precision of 5% at confidence level of 95% according to the methods provided by Thrusfield (2005).

Sample collection

Study animals were categorized into three age groups (<1 year: calf, 1 to 3 years: young and ≥3 years: adult). A total of 58 cattle were sampled monthly. Fecal samples were collected per rectum using plastic gloves in a sterile bottle. All the specimens were clearly identified, labeled, kept in an ice box and were submitted to the Wolkite veterinary clinic laboratory and stored at 4°C until it was processed.

Sample storage and processing

Specimens were stored in refrigerator at 4°C for some delayed samples. Nematode eggs were identified by floatation technique in saturated NaCl solution and Trematodes were examined by sedimentation methods. Fasciola species and Paramphistomum species eggs were distinguish by their morphological and colour differences. Strongyle positive fecal samples were subjected to modified McMaster egg counting technique and the degree of infection was identified based on Soulsby (1982), Urquhart et al. (1996) and Maff (1997). The animals were then categorized as lightly, moderately and severely (massively) infected according to their egg per gram (EPG) of faces counts. Egg counts between 100 and 250, >250 and 500, and more than 500 EPG of feces were considered as light, moderate and massive infection, respectively (Soulsby, 1982; Urquhart et al., 1996; Maff, 1997).

Data analysis

Data was entered into Ms Excel sheet 2003 and descriptive statistics was used to determine the prevalence, while Chi-square analysis was employed to test the presence of variation between age, sex, season and peasant association of the district involved in the study. Confidence level was held at 95% and P < 0.05 was set for significance. All statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software package version 15.0.

RESULTS

Out of the total 406 cattle examined, 161 (39.6%) were found to be harboring one or more gastrointestinal parasite eggs in their feces. The prevalence of different type of parasites in cattle recorded were 61 (37.9%) Strongyle type eggs, 36 (22.4%) Toxocara species, 26 (16.1%) Fasciola spp., 22 (13.7%) Trichuris species and 16 (9.9%) Paramphistomum spp. Different prevalence of gastrointestinal parasites were found for different months with mixed infection: 21 (36.2%) November, 20 (35.1%) December, 17 (29.3%) January, 12 (20.7%) February, 38 (65.5%) March, 29 (50.0%) April and 24 (41.4%) May. Strongyle type eggs were identified as the predominant in almost all months of the study period with the exception of November in which Fasciola spp. was higher. Toxocara spp. was noted as the second most prevalent helminth parasite encountered in this study (Table 1).

The relative prevalence of helminth parasites in different sex and age groups of cattle is shown in Table 2. The prevalence of helminth parasites in young animals of less than one year age was significantly different (P < 0.05) from infections recorded in adult animals of greater than three years age. In wet season infection rate 48 (68.5%) was higher as compared to dry season 113 (33.6%). There was no statistical significance difference prevalence value (P > 0.05) detected between sex in a proportions of 40.7 and 39.1% for male and female animals, respectively (Table 2). Among the different peasant associations (PAs), the highest gastrointestinal parasite prevalence of 57 (45.7%) was found to be in Tilamo, whereas the lowest prevalence of 30 (34.5%)
Table 1. Monthly prevalence of gastrointestinal helminthosis and parasite types of cattle in Gedebero Gutazer Wolene district, 2009.

<table>
<thead>
<tr>
<th>Month</th>
<th>Cattle examined</th>
<th>Strongyle</th>
<th>Toxocara spp.</th>
<th>Fasciola spp.</th>
<th>Trichuris spp.</th>
<th>Paramphistomum spp.</th>
<th>No. positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>November</td>
<td>58</td>
<td>6</td>
<td>5</td>
<td>8</td>
<td>1</td>
<td>1</td>
<td>21</td>
<td>36.2</td>
</tr>
<tr>
<td>December</td>
<td>58</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>20</td>
<td>35.1</td>
</tr>
<tr>
<td>January</td>
<td>58</td>
<td>4</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>17</td>
<td>29.3</td>
</tr>
<tr>
<td>February</td>
<td>58</td>
<td>4</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>12</td>
<td>20.7</td>
</tr>
<tr>
<td>March</td>
<td>58</td>
<td>20</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>3</td>
<td>38</td>
<td>65.5</td>
</tr>
<tr>
<td>April</td>
<td>58</td>
<td>12</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>29</td>
<td>50.0</td>
</tr>
<tr>
<td>May</td>
<td>58</td>
<td>10</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>24</td>
<td>41.4</td>
</tr>
<tr>
<td>Total (%)</td>
<td>406</td>
<td>61 (37.9)</td>
<td>36 (22.4)</td>
<td>26 (16.1)</td>
<td>22 (13.7)</td>
<td>16 (9.9)</td>
<td>161</td>
<td>39.6</td>
</tr>
</tbody>
</table>

Table 2. The prevalence of helminth parasites of cattle in different age group, sex and season, 2009.

<table>
<thead>
<tr>
<th>Parameter observed</th>
<th>No. of cattle examined</th>
<th>Strongyle</th>
<th>Toxocara spp.</th>
<th>Fasciola spp.</th>
<th>Trichuris spp.</th>
<th>Paramphistomum spp.</th>
<th>Total</th>
<th>Prevalence (%)</th>
<th>χ²</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>108</td>
<td>30</td>
<td>18</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>60</td>
<td>55.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3 year</td>
<td>152</td>
<td>26</td>
<td>15</td>
<td>9</td>
<td>10</td>
<td>3</td>
<td>63</td>
<td>41.4</td>
<td>59.32</td>
<td>0.000</td>
</tr>
<tr>
<td>&gt; 3 year</td>
<td>146</td>
<td>5</td>
<td>3</td>
<td>14</td>
<td>6</td>
<td>3</td>
<td>38</td>
<td>26.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>406</td>
<td>61</td>
<td>36</td>
<td>26</td>
<td>22</td>
<td>16</td>
<td>161</td>
<td>39.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry season</td>
<td>336</td>
<td>36</td>
<td>26</td>
<td>21</td>
<td>17</td>
<td>13</td>
<td>113</td>
<td>33.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wet season</td>
<td>70</td>
<td>25</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>48</td>
<td>68.5</td>
<td>39.18</td>
<td>0.000</td>
</tr>
<tr>
<td>Total</td>
<td>406</td>
<td>61</td>
<td>36</td>
<td>26</td>
<td>22</td>
<td>16</td>
<td>161</td>
<td>39.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>140</td>
<td>13</td>
<td>15</td>
<td>12</td>
<td>8</td>
<td>9</td>
<td>57</td>
<td>40.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>266</td>
<td>48</td>
<td>21</td>
<td>14</td>
<td>14</td>
<td>7</td>
<td>104</td>
<td>39.1</td>
<td>10.50</td>
<td>0.062</td>
</tr>
<tr>
<td>Total</td>
<td>406</td>
<td>61</td>
<td>36</td>
<td>26</td>
<td>22</td>
<td>16</td>
<td>161</td>
<td>39.6</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

was revealed from Enge with a significance difference (P < 0.05) (Table 3). The degree of Strongyle infection was determined from the total fecal egg count. From a total of 61 Strongyle positive fecal samples subjected to EPG count, 29.50% (EPG < 250) were lightly, 47.55% (EPG > 250 to 500) were moderately affected and 22.95% (EPG > 500) showed massive intensity of infection (Table 4).

DISCUSSION

The result of the current study demonstrates that helminth infections were highly prevalent in cattle of the study area. The higher prevalence of gastrointestinal poly parasites of this present study (39.6%) could be due to the fact that cattle could have frequent exposure to the same communal grazing land that causes contamination of pasture. The findings of this study agree with the results of other researchers who have...
reported a prevalence rate of 41.2% (Epherem, 2007) and 26.3% (Darsema, 2009) in Western Amhara region, Ethiopia. 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, a very high prevalence rate of 82.8% was reported by Etsehiwott (2004) in Holeta which could probably be due to the most favourable environmental condition for the development of larvae. Strongyles were the predominant (37.9%) helminthes followed by Toxocara (22.4%) and Fasciola (16.1%). The high level of multiple infections could be due to the inefficient methods of control including low attention given to the sub clinical forms, coupled with the prevailing chronic nutritional stress and suitability of the climate for survival and proliferation of the parasites (Biffa et al., 2007).

A significant variation was observed between different age groups in which young animals were higher number of eggs than adults particularly for Strongyle and Toxocara. This might be due to a limited previous exposure and immaturity of the immune system that resulted in higher development of the parasite. There are previous reports that concur with this result (Kloosterman et al., 1991; Ploeger et al., 1994; Nganga et al., 2004; Negatu, 2008), whereas a higher proportion of Fasciola and Paramphistomum were obtained in adults than young ones in this study. The lower number of calves infected with Fasciola and Paramphistomum could probably be due to the opportunity of exposure to the intermediate hosts (Vassilev, 1994, 1999; Pfuekenyi et al., 2005). It may also be due to the management system, whereby calves grazed around farms, whereas adults trekked long distances to valleys, flood plains or swampy areas during the dry season, so exposing adults to contaminated pastures. This higher proportion of flukes in adults than young animals confirms earlier observations of other workers (Maingi et al., 1993; Mbae et al., 2004; Pfuekenyi et al., 2005).

The present study revealed that sex of the studied animal did not show significant association with the prevalence of the parasite burden. This is more probably due to an equal opportunity for infection when they are exposed to the parasites in the communal grazing pasture. The absence of association between sex and prevalence agrees with that of Negatu (2008). However, the findings showed that females were more infected than their counterparts. Analysis of monthly parasite prevalence and levels of infestation confirmed that the wet season (March, April and May) when the rain begins presented more nematode parasite record than the dry season (November to February), whereas flukes were predominant at the end of rainy season which could be due to the more availability of the intermediate host. This result is in line with reports of Adrien et al. (2001).

The present study also compared and contrasted the status of gastrointestinal infestation of cattle against different PAs that a significant difference in prevalence rate was obtained. The prevalence value of parasite in Tilamo was higher than others. This is because of almost all small-scale farmers practiced a habit of keeping their animals for pasture grazing in groups for long period of time compared to the rest. This creates suitable environment for worm free animals in order to acquire high level of infective larvae from the infected pasture. Furthermore, according to Preston and Leng (1987) one would expect to find high worm burdens in cattle on overgrazed communal pastures.

Majority of infected animals (47.55%) had fecal egg count in the range of > 250 to 500 EPG and few proportions of animals (22.95%) had fecal egg count over 500. Statistically, no significant variation was observed in EPG among different age group and sex of animals though a higher EPG count was found in animals less than one year old. This is in line with the previous works done in Ethiopia (Fikru et al., 2006) and Kenya (Maichomo et al., 2004) that reported no association between

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### Table 3. Prevalence of gastrointestinal parasites of cattle by peasant association (PA’s), 2009.

<table>
<thead>
<tr>
<th>PAs*</th>
<th>Cattle examined</th>
<th>No. Positive</th>
<th>Type of parasite detected</th>
<th>Total</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilamo</td>
<td>124</td>
<td>57</td>
<td>Strongyle, Toxocara spp., Fasciola spp., Trichuris spp., Paramphistomum spp.</td>
<td>57</td>
<td>46.0</td>
</tr>
<tr>
<td>Enge</td>
<td>87</td>
<td>30</td>
<td>Strongyle, Toxocara spp., Fasciola spp., Trichuris spp., Paramphistomum spp.</td>
<td>30</td>
<td>34.5</td>
</tr>
<tr>
<td>Deneb</td>
<td>83</td>
<td>35</td>
<td>Strongyle, Toxocara spp., Fasciola spp., Trichuris spp., Paramphistomum spp.</td>
<td>35</td>
<td>42.2</td>
</tr>
<tr>
<td>Jimma</td>
<td>112</td>
<td>39</td>
<td>Strongyle, Toxocara spp., Fasciola spp., Trichuris spp., Paramphistomum spp.</td>
<td>39</td>
<td>34.8</td>
</tr>
<tr>
<td>Total</td>
<td>406</td>
<td>161</td>
<td>Strongyle, Toxocara spp., Fasciola spp., Trichuris spp., Paramphistomum spp.</td>
<td>161</td>
<td>39.6</td>
</tr>
</tbody>
</table>
Table 4. Degree of infestation of Strongyle egg type positive animals among different age group and sex.

<table>
<thead>
<tr>
<th>Season</th>
<th>No. of cattle examined</th>
<th>No. positive subjected to EPG</th>
<th>Degree of infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Light</td>
</tr>
<tr>
<td>&lt; 1 year</td>
<td>108</td>
<td>30</td>
<td>1 (33.33)</td>
</tr>
<tr>
<td>1-3 year</td>
<td>152</td>
<td>26</td>
<td>5 (19.23)</td>
</tr>
<tr>
<td>&gt; 3 year</td>
<td>146</td>
<td>61</td>
<td>3 (60.00)</td>
</tr>
<tr>
<td>Total</td>
<td>406</td>
<td>61</td>
<td>18 (29.50)</td>
</tr>
<tr>
<td>Male</td>
<td>336</td>
<td>13</td>
<td>4 (30.77)</td>
</tr>
<tr>
<td>Female</td>
<td>70</td>
<td>48</td>
<td>14 (29.17)</td>
</tr>
<tr>
<td>Total</td>
<td>406</td>
<td>61</td>
<td>18 (29.50)</td>
</tr>
</tbody>
</table>

EPG degree and age group.

CONCLUSION AND RECOMMENDATIONS

This study revealed a high prevalence of gastrointestinal parasitism. The study identified Strongyle, Toxocara, Trichuris, Fasciola and Paramphistomum. Under favorable climate and environmental factors, the potential of larval development, availability of infective larvae on grazing lands and the risk of acquiring the infective larvae especially that of Strongyle could be high. 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. Hence, to reduce the negative impacts of helminthosis on cattle production of the area and minimize pasture contamination and infection of susceptible hosts, cattle should be treated with effective broad spectrum anthelmintics at the beginning of rainy season. In addition, young cattle should receive great attention as they are most susceptible categories to helminthosis.

REFERENCES


UPCOMING CONFERENCES

11th International Congress on the Biology of Fish, Edinburgh, Scotland, 3 Aug 2014

International Conference on Coelenterate Biology, Eilat, Israel, 1 Dec 2013
Conferences and Advert

**December 2013**
International Conference on Virology and Infectious Diseases, Bangkok, Thailand, 24 Dec 2013

**November 2013**
4th International Conference on Agriculture and Animal Science, Phuket, Thailand, 23 Nov 2013
Related Journals Published by Academic Journals

- Journal of Infectious Diseases and Immunity
- Journal of Diabetes and Endocrinology
- Journal of Medicinal Plants Research
- Journal of Cell Biology and Genetics
- African Journal of Pharmacy and Pharmacology
- Journal of Dentistry and Oral Hygiene
- International Journal of Nursing and Midwifery
- Journal of Parasitology and Vector Biology
- Journal of Toxicology and Environmental Health Sciences
- Clinical Reviews and Opinions
- International Journal of Nutrition and Metabolism
- Journal of AIDS and HIV Research
- Journal of Cancer Research and Experimental Oncology
- Journal of Clinical Immunology and Immunopathology Research
- Journal of Clinical Medicine and Research
- Journal of Clinical Pathology and Forensic Medicine
- Journal of Medical Genetics and Genomics
- Journal of Medical Laboratory and Diagnosis
- Journal of Metabolomics and Systems Biology
- Journal of Neuroscience and Behavioral Health
- Journal of Physiology and Pathophysiology
- Journal of Public Health and Epidemiology
- Medical Case Studies
- Medical Practice and Reviews