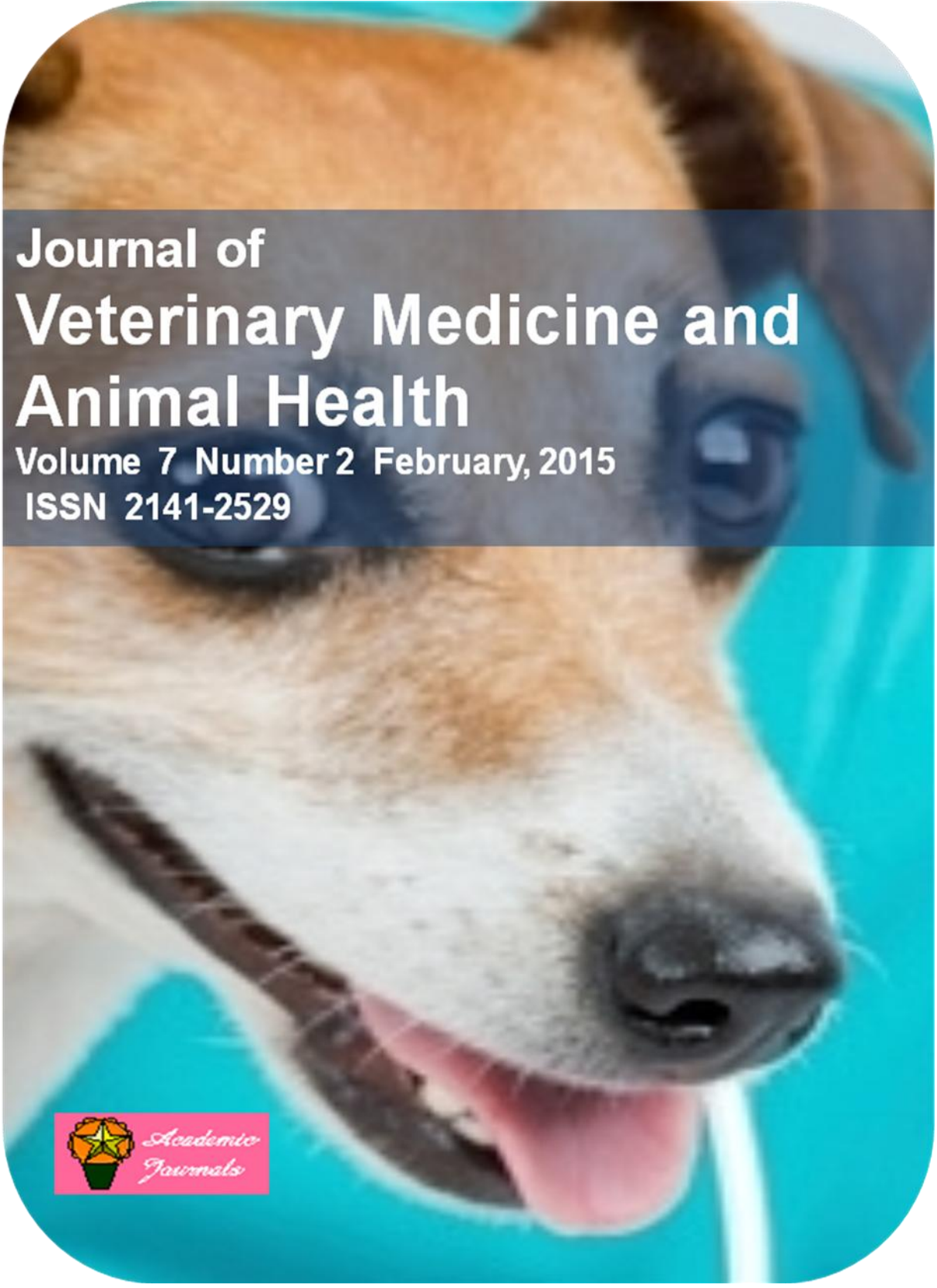

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Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Risk factors and level of awareness of canine brucellosis in Jos, Plateau state, Nigeria

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Jos has a large population of dogs because of its cultural acceptance as meat, good weather condition for exotic breeds, persistent security challenges and dog breeding activities which is very lucrative. This study was undertaken to determine the risk factors and level of awareness of canine brucellosis among dog owners in Jos by means of a structured questionnaire. Three hundred and fifty respondents were interviewed and 350 sera samples collected from dogs presented by these respondents were analysed using the Rose Bengal plate test (RBPT). The seropositivity of 113 (32.3%) obtained by the RBPT was compared with information provided by the respondents. Seropositivity of dogs managed indoors was 13.9% whereas it was 37.1% in those managed outdoors. A large proportion of 76.9% of the respondents lacked knowledge of canine brucellosis. Likewise, 81.4% of the respondents took no precaution before and after dog handling and a large proportion of 74.6% consumed undercooked dog meat. This study showed that there is a high risk of exposure of dog owners, handlers and Veterinarians to the disease and therefore enlightenment programmes on canine brucellosis as a zoonosis should be carried out in Jos.

Key words: Dog handling, structured questionnaire, RBPT, seropositivity, enlightenment, zoonoses.

INTRODUCTION

Brucellosis is a highly contagious bacterial zoonosis caused by members of the Brucella genus that can infect humans but primarily infects domestic animals and livestock (Wilkinson, 1993). Although, Brucella canis is the main cause of canine brucellosis (Wanke, 2004), Brucella abortus, Brucella melitensis and Brucella suis infections have also been reported in dogs (Baek et al., 2003; Hinic et al., 2010). It is a rough or mucoid small Gram-negative intracellular bacterium (Hollett, 2006) and it infects all breeds of dogs. Infections most commonly occur through contact with infected foetal tissues and post-parturient discharges (Godfroid et al., 2011). Household dogs are fed with dead foetuses from cows and remnants from slaughtered cattle with history of bovine brucellosis from abattoirs (Cadmus et al., 2010), and this is a significant means of transmission. In pregnant bitches, the infection localises in the reproductive tract where it causes placentitis with subsequent abortions

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and stillbirths (Lopes et al., 2010). Despite being infected, many dogs in most cases remain asymptomatic and appear to be healthy (Behzadi and Mogheiseh, 2011).

It has a global distribution and is one of the widespread zoonotic disease (Pappas et al., 2006). Infected animals usually serve as reservoirs of human infection (Namanda et al., 2009). Although, the overall proportion of B. canis causing human brucellosis is very low, its potential impact on population groups at the highest risk should not be underestimated as it poses a significant public health hazard since it is transmissible to humans especially those handling aborted foetuses (Cadmus et al., 2006). A prevalence rate of 7.6% occurred in 79 humans infected with canine brucellosis (Ofukwu et al., 2004).

Infection due to B. canis is endemic in the southern states of the USA and South America but sporadic in Europe and Asia (Corrente et al., 2010). Except in Nigeria (Cadmus et al., 2006), South Africa (Gous et al., 2005) and Zimbabwe (Gomo, 2013; Chinyoka et al., 2014), there is dearth of information on canine brucellosis in Africa. The increase in dog ownership in Nigeria is associated with some risk factors that render them vulnerable to brucellosis and many exotic breeds are imported that are not screened before entry into the country (Tafaderma, 2006; Ryhan et al., 2000). Serological examinations are often used to detect evidence of exposure to B. canis since they are relatively easy to perform and may provide a practical advantage of estimating prevalence in populations (Bae and Lee, 2009). Infection has been reported in persons in close contact with infected dogs and in laboratory scientists working with cultured B. canis (Lucero et al., 2010).

Overall, the presence and extent of canine brucellosis in the developing world has been poorly investigated in the past, and even presently, only few studies have revealed new data confirming the presence of B. canis in dogs, and moreover providing insights into the specific risk factors associated with brucellosis in dogs in different countries and regions. The increase in dog ownership and its associated risk factors coupled with the scanty information on canine brucellosis in the study area created the need for a research to determine the risk factors and level of awareness of canine brucellosis among dog owners in Jos so as to provide baseline information to help regulatory bodies and government agencies to make policies that will help control zoonotic canine brucellosis.

MATERIALS AND METHODS

The study area was Jos, Plateau State, Nigeria. Jos comprises two Local Government Areas namely Jos North which has three districts of Tudun Wada, Dong and Kabong and Jos South with four districts of Du, Gyel, Vwang and Kuru. It has an estimated population of about 900,000 residents based on the 2006 Nigerian census (National Population Commission, 2006). It lies between longitude 9° 56' North and latitude 8° 52' East. Cluster sampling method was used. A Local Government was considered as a cluster and three Veterinary clinics were randomly selected by ballot system from each local government area (LGA) from a list of all the Veterinary clinics in the two LGA. Clients (respondents) that came to the selected clinics with dogs were interviewed and the dogs sampled chronologically until the desired sample size of 350 was attained.

Questionnaire

The study was clearly explained to the clients/respondents and informed consent obtained before administering questionnaire and to ensure confidentiality, names of respondents were not recorded on the questionnaire. The structured questionnaire was administered by face to face interview to 350 respondents within the study area between April and June, 2013. Some of the questions had "yes" or "no" answer options and some had specific answers as options. The questionnaire had three sections A to C. The bio-data of the dog was contained in section A and had information on the dog's age, breed, sex, location, management system, obstetrical history (stillbirth, abortions) and number of conceptions. Section B contained the bio-data of clients/respondents and had information on educational qualification, occupation and knowledge on brucellosis while section C had information on risk factors for dogs and dog owners and contained questions on dog meat consumption, use of protective clothing and screening of dogs before breeding. The questionnaire was interpreted verbally in local language for those who could not understand English and their responses were written down.

Sampling

Dogs were properly restrained and five millilitres of venous blood was aseptically collected from the cephalic vein into a clean and well labelled sample bottle devoid of anticoagulant using sterile hypodermic needle and 10 ml syringe. The blood samples were allowed to clot by laying the sample bottles in a slanting position for an hour and the sera obtained by decantation into new well labelled sample bottles. Sera samples were stored at -20°C in a freezer and finally transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria in a Coleman box with ice packs for laboratory analysis.

Rose Bengal plate test (RBPT)

The antigen, negative and positive controls for the test were obtained from Animal Health Veterinary Laboratory Agency, Weybridge, United Kingdom. The procedure was performed as described by MacMillan (1990). Statistical analysis of data was done using GraphPad Prism 4 for Windows. Results are presented using tables and charts. Chi-square was used to test association between the prevalence of antibodies and relevant variables.

RESULTS

A prevalence rate of 113 (32.3%) of the 350 sera samples tested using the RBPT was obtained. Table 1 shows that canine brucellosis infection was higher among dogs kept outdoors (37.1%) than those kept indoors (13.9%) but the association was not statistically significant. Eighty seven of the 154 exotic breed of dogs presented by respondents to the selected clinics were

Table 1. Prevalence of canine brucellosis in Jos based on management system.

Management system	Number tested	RBPT positive (%)	χ^2	p-value
Indoor	72	10 (13.9)	0.449	0.503
Outdoor	278	103 (37.1)	-	-
Total	350	113	-	-

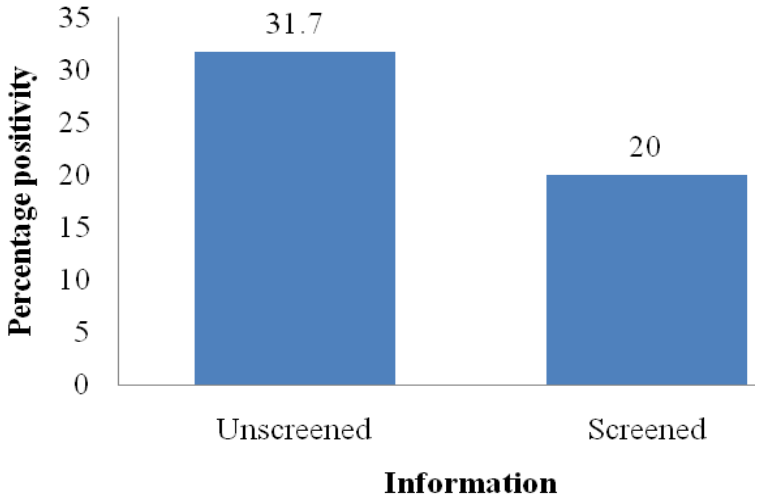

Figure 1. Percentage positivity in unscreened and screened exotic breed of dogs in Jos.

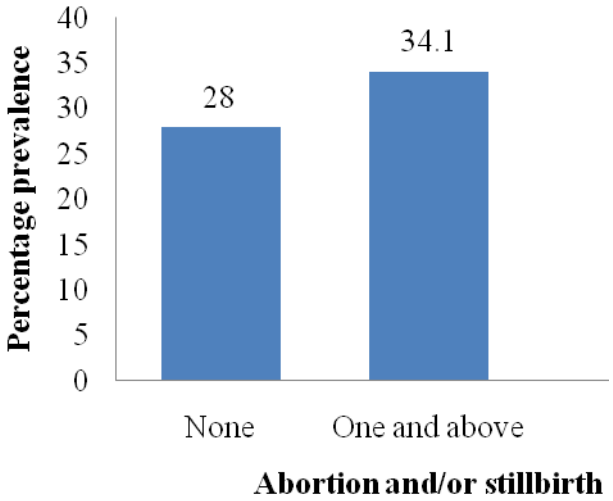

Figure 2. Prevalence of canine brucellosis in Jos based on history of abortion and/or stillbirth.

RBPT positive and of this, 82 were not screened before breeding while 5 were screened before breeding. Figure 1 shows that 26 (31.7%) of the 82 unscreened dogs were positive while 1 (20.0%) of the 5 screened dogs was positive and this was statistically significant (p=0.0169) when tested using the Chi square formula.

Eighty three (34.1%) of the 243 dogs that have had one or more abortion(s) and/or stillbirth(s) were positive for brucellosis while 30 (28.0%) of the 107 dogs that have had no abortion or stillbirth were positive. This is shown by Figure 2. Figure 3 shows the level of awareness of dog owners on canine brucellosis in Jos and 269 (76.9%) of the respondents had no knowledge on brucellosis while 81 (23.1%) knew about brucellosis. Based on the

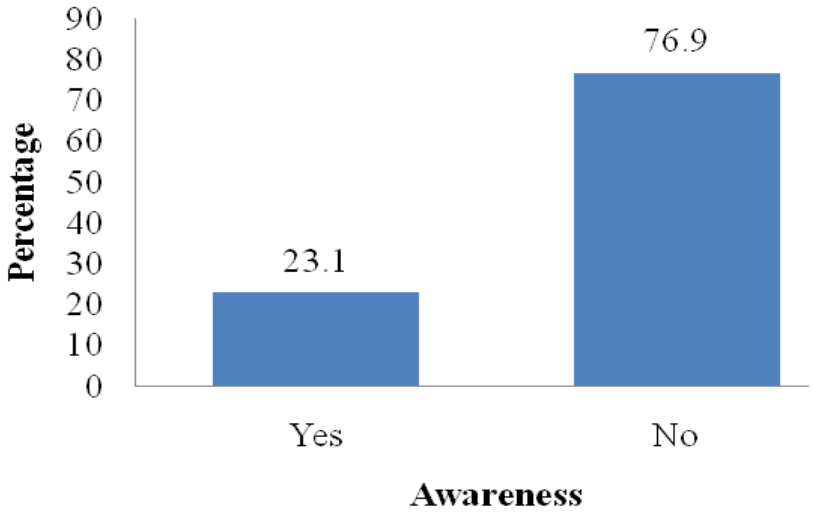

Figure 3. Level of awareness of dog owners on canine brucellosis in Jos.

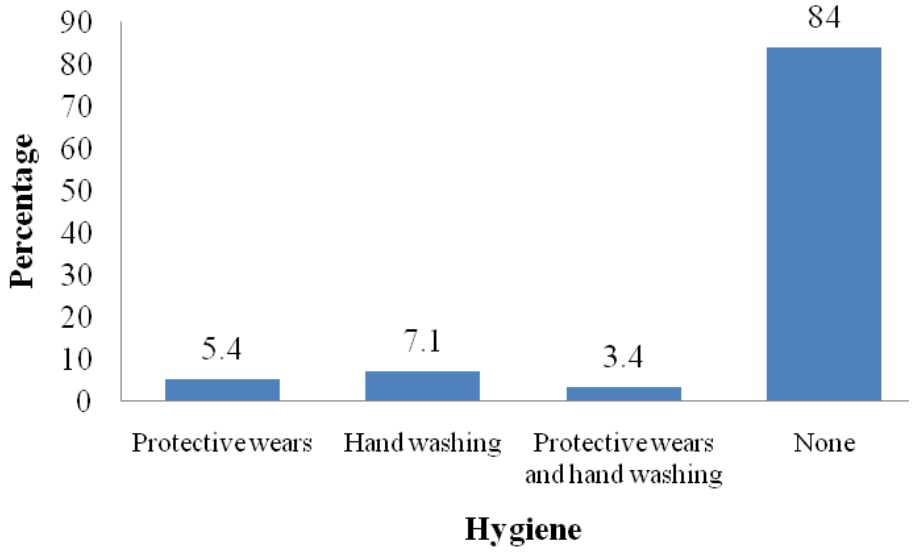

Figure 4. Level of hygiene of dog owners/handlers in Jos.

level of hygiene of the respondents, 294 (84.0%) took no precaution (neither used protective wears nor washed hands) before and after handling of dogs while 12 (3.4%) took precautions before and after handling of dogs. Nineteen (5.4%) used protective wears before handling dogs while 25 (7.1%) washed and disinfected hands after handling dogs. This is shown by Figure 4. The questionnaire revealed that 262 (75%) of the respondents consumed roasted (suya) dog meat, 74 (21%) consumed boiled dog meat and 14 (4%) consumed fried dog meat as shown by Figure 5.

DISCUSSION

Brucella infection in dogs in Jos was likely acquired through indiscriminate breeding and outdoor management system. Dogs kept by the outdoor management system could roam about freely and are at risk of picking up food materials contaminated with Brucella organism such as aborted or after-birth materials, they are also at risk of mating dogs infected with brucellosis. Godfroid et al. (2005) had stated that ingestion of tissues, foodstuff or fluid containing the organism is a major route of the disease transmission and that eating aborted foetuses can also lead to the disease. A previous study demonstrated a higher prevalence of infection in stray compared with non-stray dogs (Chikweto et al., 2013). Dogs have been shown to be mechanical and biological vectors of brucellosis and sexual transmission is also an important means of spread of the infection as males can

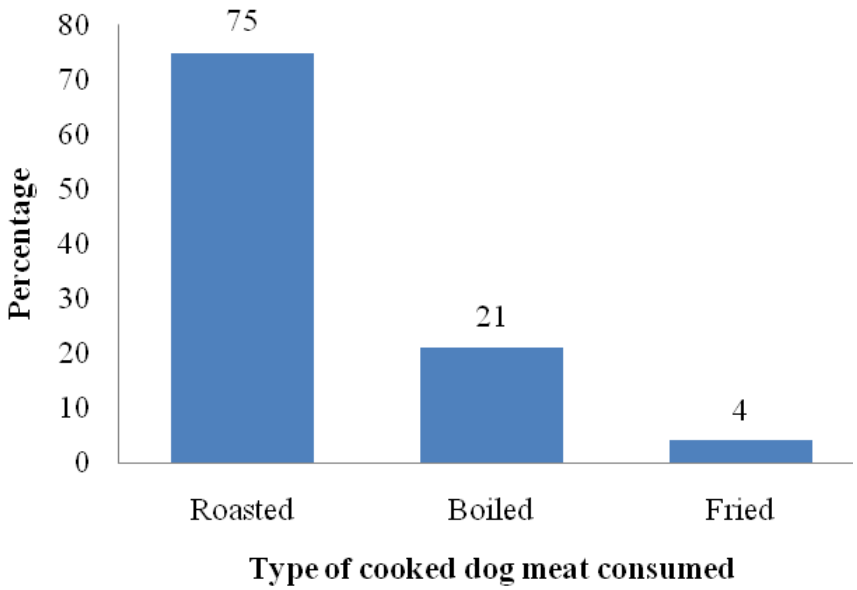

Figure 5. Type of cooked dog meat consumed by respondents.

excrete the organism in large numbers in their semen (WHO, 1986).

This study shows that seropositivity was higher in unscreened than screened exotic dogs and this was statistically significant as the p-value was less than 0.05 meaning that the occurrence of canine brucellosis is associated with the screening and non-screening of dogs. The importation of exotic breed of dogs into the country without screening could contribute to the prevalence of the infection in the study area and the country at large. Rhyan et al. (2000), stated that many exotic breed of dogs are imported that are not screened before entry into the country and that the introduction of dog breeding in Nigeria has contributed to the re-emergence of brucellosis as an international concern for both indigenous and foreign breeds of dogs, due to lack of pre-movement screening and an increase in the density of possibly infected foreign breeds of dogs.

The higher prevalence of brucellosis in dogs that have had one or more abortion(s) and/or stillbirth(s) suggests that the infection may be responsible for the abortions and stillbirths. Gyuranecz et al. (2011), stated that B. canis can cause abortion and stillbirth in pregnant dogs particularly at the 7^{th} to 9^{th} week of gestation. Based on the level of awareness of canine brucellosis among dog owners in Jos, more of the respondents were unaware of the disease compared to those who had knowledge on it and this could be responsible for the prevalence of the infection in the study area. Canine brucellosis continues to be a problem common in dogs simply because people lack enough information about it (Gail, 2013). A large proportion of dog owners used bare hands when handling dogs with no protective clothing such as coverall, laboratory coats, boots, hand gloves, or hand washing after handling of dogs. This is risky as Brucellae can enter through intact skin and abrasions (Mantur and Amarnath, 2008).

Most of the respondents consumed undercooked dog meat (roasted meat) and are at risk of contracting the disease because brucellosis can be transmitted through consumption of contaminated raw animal products, like improperly cooked meat (Seleem et al., 2010). Nicoletti (1989) also stated that the risk of infection is proportional to the degree of contact with Brucella infected edible bye products like dog's offal (Intestine, liver, kidneys, uterus and testicles).

Conclusion

This study has established risk factors such as outdoor management system, lack of screening of dogs, maintenance of low level of hygiene by dog handlers, consumption of undercooked dog meat among others. There is a low level of awareness of canine brucellosis among dog owners in Jos and therefore awareness and enlightenment programmes on canine brucellosis should be carried out with emphasis on its zoonotic importance. Prevention of stray dogs and indoor management system should be encouraged to reduce indiscriminate mating and consumption of infected food materials and if dog meat must be consumed, it should be properly cooked.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Journal of Veterinary Medicine and Animal Health

Review

Diagnostic approaches for tick-borne haemoparasitic diseases in livestock

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Tick-borne diseases (TBDs) are a major economic constraint to livestock production affecting the productivity of livestock worldwide. Identification of these haemoprotozan and rickettsial infections is essential in understanding the epidemiology and it is important to distinguish between species and subspecies involved. Conventional techniques including serological and microscopic examinations do not always meet these requirements. Clinical diagnostic and surveillance tools, such as the complement fixation test (CFT), the indirect fluorescent antibody test (IFAT) and the enzyme linked immunosorbent assay (ELISA) have been successfully used over decades. In addition, DNA-based tests for diagnosis, differentiation and characterisation of different haemoparasites have been developed. Molecular diagnostic techniques, such as DNA hybridization and polymerase chain reaction (PCR), allow detection of parasites in blood, tissue or ticks with high levels of sensitivity, specificity and reliability. In addition, some techniques can identify multiple pathogens in the same samples. Furthermore, these techniques can also be exploited to identify unambiguous species and subspecies. Under the precondition that these tests are correctly designed and validated, they provide a powerful tool for epidemiology, with greater advantages of affordability and amenability to standardization. The implementation of these techniques for studying TBDs worldwide will be invaluable. Thus, the aim of this study is to put together the details of the techniques in the form of small review consultation of the practitioners and researchers.

Key words: Diagnosis, livestock, molecular, haemoparasites, tick-borne diseases.

INTRODUCTION

'Diagnosis' is an art of knowing about the cause of a particular disease (Dia = through, gnosis = knowledge). Diagnosis of parasitic infections has changed remarkably as technical skills and knowledge have expanded. Ticks rank first as arthropod vectors of protozoa, rickettsiae, bacteria and viruses in nonhuman vertebrates and rank second only to mosquitoes as vectors of pathogens to humans (Zhou et al., 2009). Though tick borne diseases (TBDs) differ among ecological regions, their impact on animal production is more important in tropical and subtropical regions of the world as they pose major threat to the health and management of livestock in these regions

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(Jongejan and Uilenberg, 1994). These diseases are ranked very high in terms of their impact on the livelihood of resource poor farming communities in developing countries (Perry et al., 2002; Minjaw and McLeod, 2003; Bishop et al., 2008). The most important tick-borne haemoparasitic disease in veterinary medicine include the protozoan parasite of *Theileria* species (e.g. *Theileria annulata*, *Theileria parva*, *Theileria lestoquardi*), *Babesia* species (e.g. *Babesia bovis*, *Babesia bigemina, Babesia ovis*) and Rickettsial species (e.g. *Cowdria ruminantium, Anaplasma marginale, Anaplasma centrale*) (Uilenberg, 2006). In addition, several novel *Babesia* and *Theileria* (*Theileria uilenbergi* and *Theileria luwenshuni*) species were isolated from naturally infected sheep in China, where it cause severe and often lethal disease (Yin et al., 2007; Guan et al., 2010).

The direct impacts of these tick-borne pathogens are reflected in reduction of production, loss of weight and death of substantial proportion of the affected animals. By reducing the losses due to tick-borne diseases there would be a better chance to increase livestock production. The effective management of tick-borne haemoparasitic diseases requires rapid, reliable and highly sensitive diagnostic tests, which can also serve to monitor the effectiveness of the therapeutic and prophylactic measures. The common conventional method (history, blood or tissue smears examination, clinical signs, postmortem lesions and conventional serology) provide the basic needs for diagnosis, but have the disadvantage of sensitivity, specificity, involvement of expertise, labour intensiveness, etc., so do not meet complete requirements. The serodiagnosis is not different between current and past infection as the animal may already have cleared the pathogen, but remain seropositive. Nucleic acid based diagnostics, particularly, a wide range of DNA based techniques have been developed and validated for identification, characterization and pathogenic studies for various pathogens. The polymerase chain reaction (PCR) based assays permit identification of parasite at levels far below the detection limit of the commonly used parasitological techniques. Several techniques have been developed separately for detection for each species tick-borne haemoprotozoa (Figueroa and Buening, 1995) (Table 1). In this review, a detailed discussion on diagnostic procedures from classical to molecular approaches including clinical, parasitological, serological and molecular techniques is made.

CONVENTIONAL DIAGNOSTIC TECHNIQUES

The microscopic techniques for diagnosis of tick-borne diseases are still considered as the “gold standard” technique. Microscopic examination shows *Theileria* schizonts in the lymph node smears and piroplasms alone or along with schizonts in blood smears (Figure 1).

However, the detection of piroplasms in blood smears in the absence of clinical assessment and lymph node biopsy are difficult to interpret, since piroplasms of *T. annulata, T. parva, Theileria mutans* and *T. lestoquardi* can be found in the blood smears (Norval et al., 1992) which are difficult to differentiate based on the morphology. It is also quite difficult, if not impossible, to demonstrate parasites in carrier animals as the numbers of parasites in such animals fall below detectable levels soon after the acute stages of the disease (de Waal, 2012).

Babesiosis is diagnosed by examination of blood or organ smears stained with Romanowsky stain (Callow et al., 1993; Bose et al., 1995). The direct method involves identifying the parasite in the stained blood smears; however, this technique shows a low sensitivity in subclinical and chronic phase of the infection (Terkawi et al., 2011). Blood film examination requires very much expertise to differentiate between *Babesia* species from one or more animal species which look similar under stained preparation (Figure 2). Quantitative buffy coat (QBC), an acridine orange based improved technique, which concentrates and stains parasitized blood in one step (Levine et al., 1989) which may prove more valuable in low parasitaemic cases (Figure 3).

The most commonly used method for diagnosis of *Anaplasma* infection is the microscopic examination of Giemsa stained thin blood smears especially in clinical acute form of disease (Figure 4). The sensitivity of this method is 10^6 infected erythrocytes per milliliter of blood (Gale et al., 1996), but due to the low parasitemia in carrier cattle and difficulty to differentiate *Anaplasma* from other structures even by an experienced hand, this method is not recommended for the characterization of persistently infected cattle (Carelli et al., 2007). Sub-inoculation of *A. marginale* infected erythrocytes into susceptible splenectomized calves has been considered as the ’gold standard’ for detection of such cattle, but it is not practical for routine testing (Luther et al., 1980).

Owing to the rapid development of heartwater disease (Cowdriosis), the frequent lack of characteristic clinical signs, and sometimes even a total absence of lesions, the diagnosis of the disease in live animal is difficult (Camus et al., 1996). Tentative diagnosis relies on some clinical, epidemiological and macroscopic appearance of some organs. Differential diagnosis should consider diseases that cause central nervous system “CNS” manifestations. In dead animals, tentative diagnosis could be made by the presence of transudates in the pericardium and thorax at postmortem, but definitive diagnosis requires the demonstration of *C. ruminantium* in brain crush smears. This has been established much earlier and by the application of specific serological and molecular techniques in live animals.

The conventional microscopy for diagnosis is simple and does not require the purchase and maintenance of expensive equipments. However, limitations of conventional microscopy method include: (i) it is extremely

Table 1. Developmental progression of some of the molecular techniques commonly used in the diagnosis of tick-borne haematozoa.

Assay	Haematozoa	Reference
ELISA	*T. annulata; T. parva*; *C. ruminantium*	Kachani et al.(1996), Boulter et al. (1998), Ilhan et al. (1998), Bakheit et al. (2004), Renneker et al. (2008), Gray et al. (1980), Mboloi et al. (1999)
PCR	*T. annulata*, *T. parva*; *B. bovis*, *B. bigemina*	d'Oliveria et al.(1995), Bishop et al. (1993), Calder et al. (1996), Figueroa et al. (1992)
Multiplex PCR	*T. evansi, B. bigemina*	Figueroa et al. (1993),
Real-time PCR	*T. parva, A. marginale, Ehrlichia risticii, T. evansi*	Chaisi et al. (2013), Schotthoefer et al. (2013), Pusterla et al. (2000), Sharma et al. (2012)
DNA probe	*A. marginale, A. centrale*	Visser and Ambrosio (1987)
RLB	*Theileria* spp., *Babesia* spp., *Anaplasma, Ehrlichia*	Gubbels et al. (1999), Cornelis et al. (2002)
LAMP	*T. annulata, T. parva, T. lestoquardi, Babesia canis*	Salih et al. (2008), Lui et al. (2012), Thekisoe et al. (2010), Salih et al. (2012), Muller et al. (2010)

labour intensive particularly when a large number of samples are to be examined in a short period; (ii) trained technicians are required for accurate diagnosis; (iii) not of much use in cases, when haemoparasites are morphologically similar or too small to be missed or infection is very low; (iv) in some cases culture of the parasite is needed, which may require specialized media, which is again time consuming.

INDIRECT DIAGNOSTIC METHODS

When parasites occur at densities below the sensitivity of direct method employed or cannot be directly demonstrated in a biological sample due to the life cycle in the host, in those cases indirect methods of diagnosis are used, which include serological tests either used for detection of antibodies or antigens. Among the various serological tests, most important once include complement fixation test (CFT), indirect fluorescent antibody technique (IFAT) and enzyme-linked immunosorbent assay (ELISA).

CFT

CFT has been used for diagnosis of *Babesia*, *Theileria*, *Toxoplasma*, *Trypanosoma,* etc (Herr et al., 1985; Bose et al., 1995). Based on this test, commercial kit (COFEB Kit) has been developed for diagnosis of equine piroplasmosis (Sengupta, 2001).

IFAT

Indirect fluorescent antibody technique has been used since long for the diagnosis of parasites like *Babesia* spp. (Morzaria et al., 1977; Anderson et al., 1980), *Theileria (*Morzaria et al., 1977; Darghouth et al., 2004). IFA test has also been recommended by OIE as one of the diagnostic test for theileriosis.

ELISA

ELISA is increasingly being used for detection of parasite-specific antibodies, antigens and immune complexes (Kachani et al., 1992). ELISA based on schizont antigen functioned well in the case that cellular fraction was enriched from the soluble fraction (Manuja et al., 2000). On the other hand, ELISA for the diagnosis of *Theileria* spp. infection in sheep using piroplasm antigen obtained from experimentally infected sheep with parasitemia reaching 30% was developed (Gao et al., 2002).

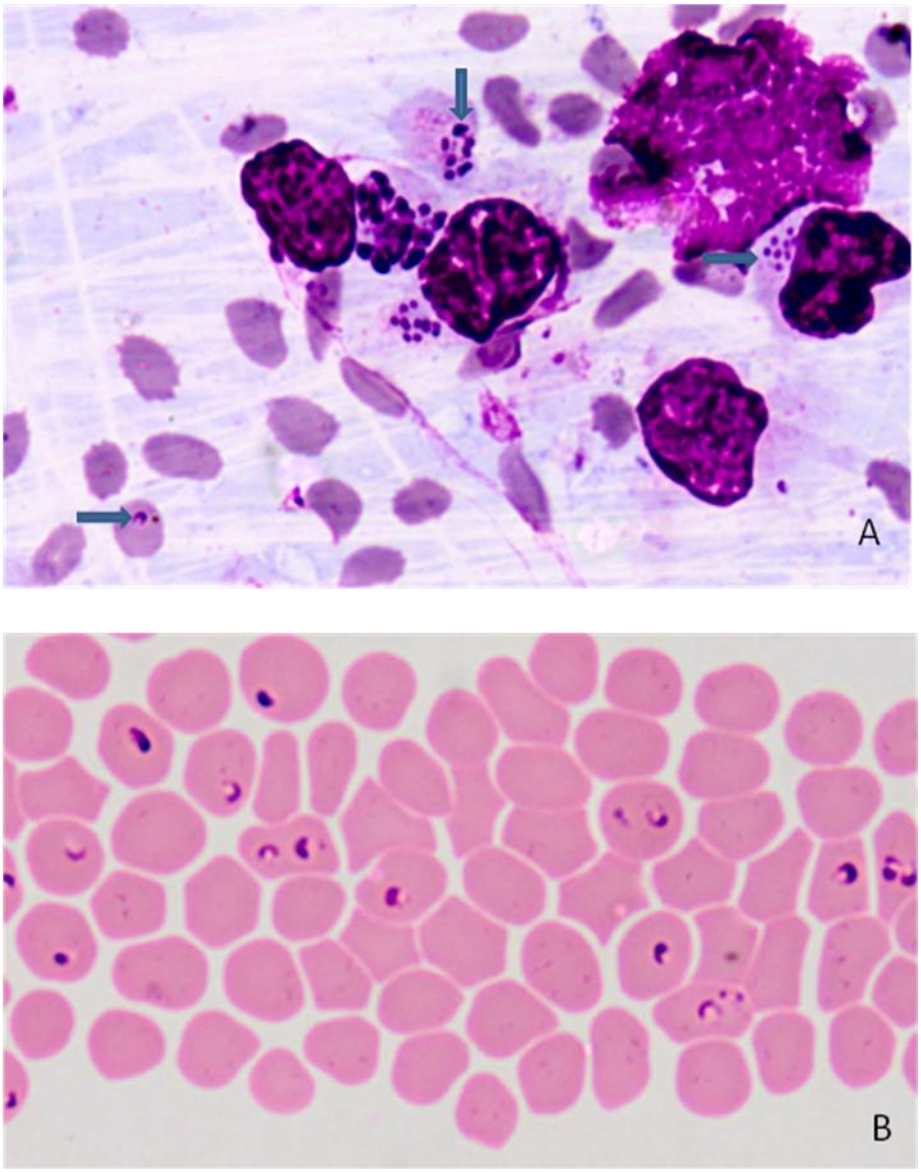

Figure 1. Photomicrograph of GSTBS revealing (A) Schizonts and piroplasm of *Theileria* spp., (B) Pleomorphic piroplasms of *Theileria* spp.

However, cross-reaction was found with *B. ovis*. This result may be questionable, because it could not be excluded that these sheep were already infected with both *Theileria* spp. and *B. ovis*.

In spite of relatively good performances of crude antigens, the disadvantages of this approach include the requirement for experimental animals for piroplasm antigen production and the batch-to-batch variation, beside the need to standardize protocols to obtain antigen from crude parasite material (Gubbels, 2000). These problems have been circumvented by use of several recombinant parasite antigens in ELISA. Two ELISAs based on recombinant proteins have been developed. Firstly, the sporozoite antigen (SPAG-1) has been demonstrated to detect exposure to *T. annulata*, but sensitivity and specificity of this ELISA has not been evaluated (Boulter et al., 1998). Secondly, a merozoite surface antigen, Tams-1 has been tested as a candidate antigen for a diagnostic ELISA (Ilhan et al., 1998). Moreover, an ELISA for detection of *T. annulata* infection was established and validated and applied for epidemiological studies in the field (Schnittger et al., 2002; Bakheit et al., 2004; Salih et al., 2005, 2007; Seitzer et al., 2007). An advance in serological diagnosis was achieved with the development of a competitive ELISA applying the TaSP antigen and using a monoclonal antibody (1C7) that was found to bind to TaSP antigen (Renneker et al., 2008). ELISA is widely used as the basis for epidemiological surveys

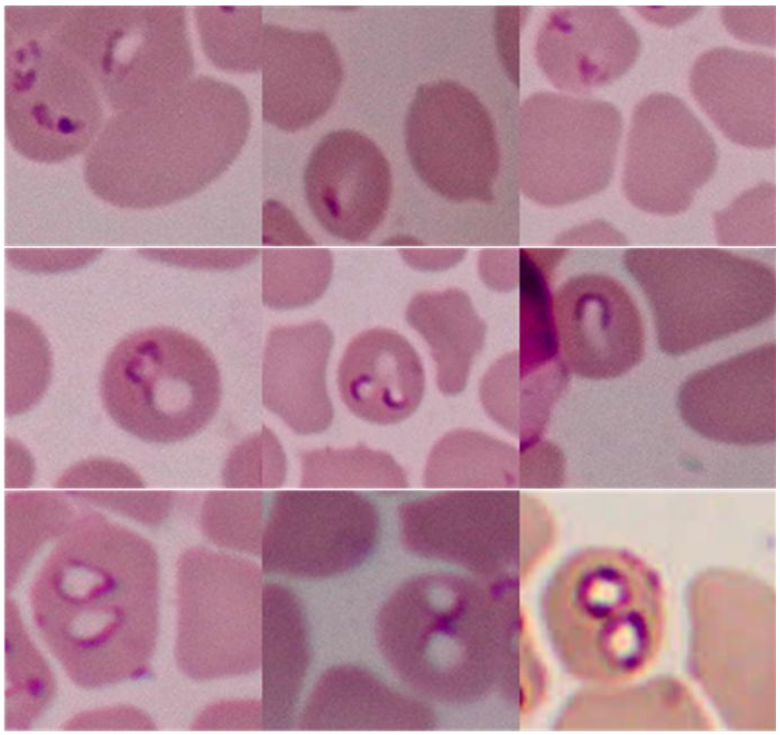

Figure 2. Photomicrograph of different shapes of *Babesia* spp. difficult to differentiate based on morphology.

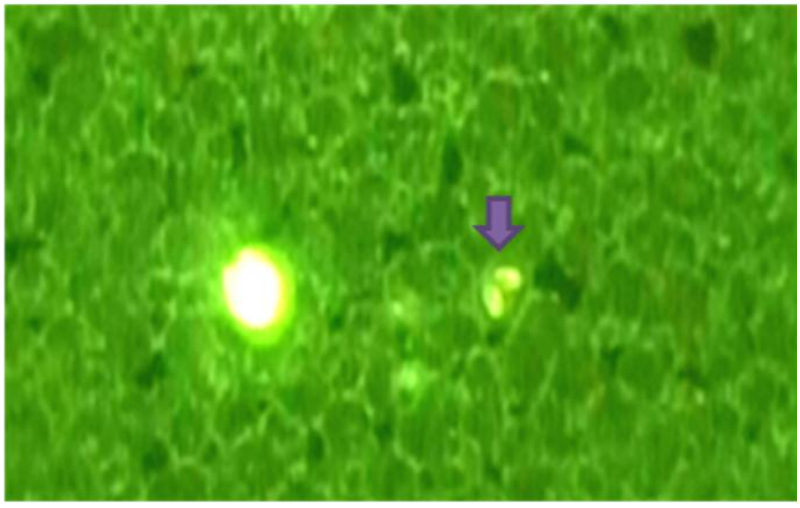

Figure 3. Photomicrograph of *Babesia* spp. in acridine orange stained blood smear showing pyriform bodies (arrow).

(Passos et al., 1998) and for evaluation of vaccination programme (Guglielmone et al., 1997). Serological tests have the disadvantage of relying on the presence of specific antibodies, which may take days or weeks to develop in an infected animal or may persist for months after the infection has been cleared (Mosqueda et al., 2012).

Mboloi et al. (1999) investigated the major antigenic protein 1 fragment B (MAP1-B) in ELISA for the diagnosis of *C. ruminantium* infection. They concluded that the MAP1-B ELISA is a useful test for the diagnosis of *C. ruminantium* infection in small ruminants. However, this test is known to be less sensitive in cattle than in small

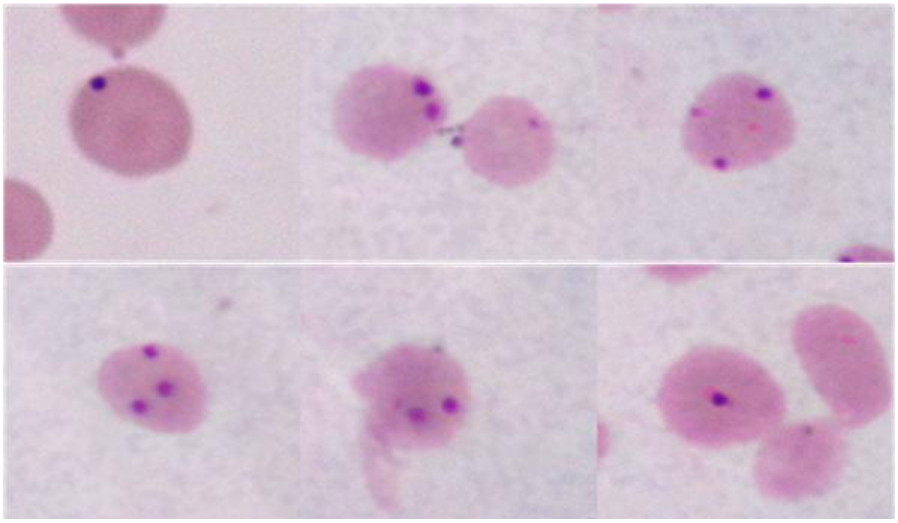

Figure 4. Photomicrograph of Giemsa stained blood smear showing *Anaplasma marginale*.

ruminant (Knopf et al., 2002).

NUCLEIC ACID-BASED TECHNIQUES

Molecular nucleic acid-based diagnostic techniques have been developed for a number of haemoparasite. They have been proved to be sensitive, easy to use, can analyze large number of samples and can detect the parasites directly in clinical and environmental samples without culture (Weiss, 1995; Dey and Singh, 2009). The first introduced diagnostics was nucleic acid hybridization technique, however, in the recent years, PCR and allied techniques along with genomic sequencing have over taken it, and have become a driving force for the development of rapid, sensitive and specific assays capable of genomic detection. They are widely used because of being highly sensitive and can be performed rapidly in a cost effective manner. Various molecular diagnostic techniques that have been developed for diagnosis of parasites include conventional PCR, RAPD-PCR, RFLP-PCR, multiplex-PCR, real-time PCR, reverse transcriptase PCR, PCR-ELISA, micro-arrays, loop-mediated isothermal amplification (LAMP), etc.

PCR

Many authors discussed the use of molecular tools for the study of *Theileria* parasites, which affect ruminants, mostly bovines. Tanaka et al. (1993) utilized a probe derived from a gene encoding a 32 kDa intra-erythrocytic piroplasm surface protein of *Theileria sergenti* (*Theileria orientalis*). Their method was sufficiently sensitive to detect four parasites per microlitre of blood with a 10 µl sample. Detection of *T. annulata* in blood samples of carrier cattle using polymerase chain reaction (PCR) was reported (d'Oliveria et al., 1995). The assay employed primers specific for the gene encoding the 30 kDa major merozoite surface antigen of this species. This PCR assay was found to be highly specific and sensitive (three parasites per microlitre of blood). Allsop et al. (1993) developed another technique based on PCR using oligonucleotide probes, which detected small subunit ribosomal RNA sequences (srRNA). These probes were efficient in discriminating between six different tick-borne parasites harboured by cattle. Bishop et al. (1993) were able to further increase the accuracy of the identification of isolates or strains by using random amplified polymorphic deoxyribonucleic acid 'DNA' (RAPD). Moreover, several real-time PCR assay has been developed for diagnosis and quantitation of many tick-bone parasites (Dong et al., 2013; Schotthoefer et al., 2013; Bloch et al., 2013).

The sensitivity and specificity of molecular methods is very high and over the years a number of different approaches have been developed to detect *Babesia* spp. in the hosts and vectors. Deoxyribonucleic acid (DNA) probing was the first developed method, which was used to detect babesial DNA from parasitized blood (Buening et al., 1990). Figueroa et al. (1994) set up a PCR-based diagnostic assay to detect *B. bovis* in chronically infected cattle. The target sequence was a gene encoding a 60 kDa merozoite surface protein. The level of sensitivity was high as the PCR product was detected in blood samples containing approximately 20 µl of packed cell with a parasitemia of 0.000001%. Nested PCR (nPCR) has been effective for the detection of carrier animals infected with *B. bigemina*; the sensitivity was reported to be as low as one infected erythrocyte in 108 cells (Figueroa et al., 1992). Carson et al. (1994) used RAPD

to reveal markers of species and isolates for *B. bovis* and *B. bigemina.* As mentioned earlier, RLB technique has proved highly sensitive and specific for the detection of these parasites (Gubbels et al., 1999). A hot-start PCR (semi-nested) to detect *B. bovis, B. bigemina* based on aspartic proteinase babesipsin gene was developed and successfully applied (Martin et al., 2008; Awad et al., 2011). Multiplex PCR (Figueroa et al., 1993), real-time PCR (Buling et al., 2007) and multiplex LAMP (Iseki et al., 2007) are in process of development and validation for identification and characterization of *B*. *bigemina*.

Probes specific for *A. marginale* and *A. centrale* have been isolated (Visser and Ambrosio, 1987). These probes could detect 127 and 8 ng DNA of *A. centrale* and *A. marginale*, respectively. This probe could also be used on blood from field samples to detect parasiteamia in cattle. On the other hand, the *A. marginale* probes described by Goff et al. (1988) could detect 0.01 ng DNA, which is equivalent to a parasiteamia of 0.000025% (Eriks et al., 1989). This probe could also detect the presence of the parasite in the infected tick vector. Presently, knowledge about carrier state of anaplasmosis is so difficult to know the epidemiological status in enzootic regions. PCR based on msp4 gene for *A. marginale* and *Anaplasma ovis* (de la Fuente et al., 2001, 2003) have also been developed. Sequencing of gltA and ompA genes, identification of *Rickettsia* species based on the sizes of highly variable intergenic spacers, namely, dksA-xerC, mppA-purC, and rpmE-tRNAfMet was carried out. Application of multiplex PCR for simultaneous amplification of 3 spacers combined with capillary electrophoresis separation technique is simple, accurate, and high-throughput fragment sizing with considerable time and cost savings (Nakaoa et al., 2013). PCR base tests including PCR ELISA and duplex PCR have been developed and applied successfully with high sensitivity and specificity to differentiate tick borne haematozoan diseases (Galle et al., 1996; Ala and Wayne, 2005; Torina et al., 2008; Ashuma et al., 2013; Sharma et al 2013). Further, nested PCR devised for detection of *A. marginale* in cattle shows increased specificity and sensitivity (Ybanez et al., 2013). Recently, semi-quantitative multiplexed-tandem PCR for the detection and differentiation of four *T. orientalis* genotypes in cattle has been applied (Pereraa et al., 2014).

There are certain reservations to the routine use of such technique. First of all, contamination of the laboratory environment has to be rigorously controlled and this implies numerous controls for quality diagnosis. Suggestions for avoiding contamination have been presented by Altwegg (1995) and Carino and Lee (1995). The first suggestion relates to the organization of the laboratory space so as different stages of diagnosis are carried out in separate areas. The second suggestion relates to the use of Uracil DNA Glycosylase (UDG) for preamplification sterilization of the PCR product (Longo et al., 1990). For the detection of contamination, negative control must be realized at each step of the PCR preparation (Comes et al., 1995). PCR may also fail due to the inhibition of specific amplification (false negative). Thus, the use of positive control is necessary to increase confidence in negative PCR results. The cost of diagnosis is the second drawback of PCR. Although PCR identification costs are being progressively reduced, they remain higher than those of parasitological and immunological techniques. The third drawback is the absence of quantification. Only limited attempt of using PCR has been conducted to date to estimate the intensity of parasitemia due tick-borne parasitic diseases.

RAPD-PCR

Random Amplification of Polymorphic DNA-PCR also known as AP-PCR (arbitrary primed PCR), in which primers of arbitrary sequences are used to amplify fragments of the genome. This technique is very simple, fast and does not require either prior knowledge of the DNA sequence or DNA hybridization. This technique has been employed to differentiate species of *Leishmania* and also to study polymorphisms of *Plasmodium*, *Trypanosoma*, etc (Hajjaran et al., 2004).

PCR-RFLP

PCR-Restriction Fragment Length Polymorphism is used for diagnosis of species and genotypes of parasites. It is performed by digesting the PCR products obtained from parasitic gene amplification, by restriction enzymes or endonucleases. These enzymes cut DNA into fragments of certain sizes, whose analysis on agarose or poly-acrylamide gel results in different patterns of fragment sizes, enabling the identification. Zaeemi et al. (2011) were able to differentiate among *T. lestoquardi*, *Theileria ovis*, and *T. annulata* in case of sheep. Recently, semi nested PCR-RFLP was used for detection of persistent anaplasmosis in tick infested cattle (Jaswal et al., 2014).

Multiplex PCR

Multiplex polymerase chain reaction is a modification of conventional polymerase chain reaction in order to rapidly detect deletions or duplications in a large gene in a single reaction. It is a variant of PCR which offers a significant advantage over single-plex PCR as this two or more target loci from one or more organisms are amplified using mixture of locus-specific primer pairs in a single reaction (Edwards and Gibbs, 1994; Markoulatos et al., 2002). Multiplex PCR had been employed in detection of concurrent infections in field for the detection of haemoprotozoans (Figueroa et al., 1998). Various duplex PCR for the detection of concurrent infections of

economically important haemoprotozoans have also already been standardized in our laboratory with convenient large scale field application (Sharma et al, 2013; Kaur et al., 2012).

Real-time PCR (RT-PCR)

This technique involves the analysis of genome using fluorogenic probes that release fluorescent signals during amplification. The advantages of real time PCR assay over conventional PCR are that it is relatively rapid and convenient because there is no need to perform gel electrophoresis to visualize the PCR products. Real-time PCR is a simple, fast, closed and automatized amplification system responsible for decreasing the risk of cross contamination. This technique has been used for the detection of a number of parasites including *Leishmania*, *Plasmodium, Trypanosoma* (Bell and Ranford-Cartwright, 2002; Gasser, 2006; Sharma et al., 2012) in various regions of the world. Real-time PCR has engendered wider acceptance of PCR due to its improved rapidity, sensitivity, reproducibility and the reduced risk of carryover contamination (Mackay, 2004). Jeong et al. (2003) applied real-time PCR for diagnosis and quantification of *T. sergenti* using specific primer for 33 kDa gene. A pan-*Theileria* FRET-qPCR that can detect all recognized *Theileria* spp. of ruminants in a single reaction has also been developed (Yang et al., 2014).

PCR-ELISA

By PCR ELISA, the sensitivity of ELISA and specificity of PCR are combined and used for the detection of parasitic genome. The PCR products are hybridized to an immobilized capture probe. The assay thus measures sequences internal to the PCR product and is a less expensive assay and can be an alternative to real time PCR. This ELISA is useful for detecting and differentiating between multiple targets. This technique has been used in detection and quantification of *Trypanosoma evansi* in animals and vectors (Chansiri et al., 2002). The sensitivity limit of PCR-ELISA was 0.01 pg, which corresponded to one parasite/ml of blood. No cross-reactivity of the assay was observed against *B. bovis, B. bigemina, A. marginale, Theileria* spp. and host DNA (Chansiri et al., 2002).

RLB

Two integrated approaches were developed to detect several *Theileria* or *Babesia* spp. in one assay (Figueroa et al., 1993; Allsop et al., 1993). Using these approaches, multiple species can be detected in one assay without performing independent PCR reactions for each parasite (Gubbels et al., 1999). One of such techniques, reverse line blot (RLB) hybridization, combines a genus specific PCR with hybridization to membrane bound type/species-specific oligonucleotide for differential detection. This technique can differentiate all known *Theileria* and *Babesia* spp. of importance in cattle in the sub-tropics on the basis of their differences in 18S subunit rRNA gene sequences (Gubbels et al., 1999). The specificity of the techniques result from the fact that amplified conserved domains of the 18 srRNA genes of the parasites are hybridized to species specific oligonucleotide immobilized on a solid membrane.

LAMP

Recently, a rapid, simple, and sensitive technique, loop mediated isothermal amplification (LAMP), was developed (Notomi et al., 2000). This is a novel strategy for gene amplification which relies on the auto-cycling strand displacement synthesis of target deoxyribonucleic acid (DNA) by *Bst* DNA polymerase under isothermal conditions. Further improvement of the technique has been achieved by the use of additional loop primers, which increased its efficiency and rapidity (Nagamine et al., 2002). The LAMP technique allows visual detection of amplified products through the addition of fluorescent dyes such as SYBR Green (Poon et al., 2006) and measurement of turbidity (Mori et al., 2001). Unlike PCR, LAMP is carried out at a temperature range of 60 to 65°C eliminating the need of a thermal cycler. In addition, the reaction can be carried out without the need of DNA extraction. The method has been successfully developed for the detection of several TBDs (Salih et al., 2008; Liu et al., 2008; Muller et al., 2010; Thekisoe et al., 2010;Salih et al., 2012).

DNA Microarrays

The technology commonly known as gene chip, DNA chip, or biochip was originally developed for mapping of genes being used to detect a wide variety of pathogens through multi-gene detection. The microarrays consist of solid supports like glass slide or silicon chip or nylon membrane, onto which the nucleic acid sequences from thousands of different genes are attached at fixed locations. The main advantage of this technique is that it combines DNA amplification strategies with subsequent hybridization to oligonucleotide probes specific for multiple target sequences. Secondly, it allows for the simultaneous analysis of a larger number of genetic features in a single trial. However, the technique is costly. This technique has been used in detection and genotyping of vector-borne parasites of medical importance like *Leishmania*, *Plasmodium*, *Toxoplasma*, and *Trypanosoma* (Duncan, 2004).

CONCLUSION

Diagnosis of haemo-parasitic infection has been largely based on clinical symptoms and identifying/ demonstrating the causative agent by parasitological techniques. Although, microscopy is still considered as a gold standard in the diagnosis of many parasitic diseases, it cannot be applied to all situations particularly where the diagnostic requirements demand defining the carrier status. Although, the use of various serological methods provide definite clues about the parasitic infection in general, but these tests have some limitations. In this direction, the recently introduced molecular biological techniques will certainly be of help, though presently their use on large scale is mainly limited to large parasitology laboratories. The use of DNA hybridization probes, although developed several years ago, has never been developed to suite regular diagnostic laboratories and has now been super ceded by polymerase chain reaction. The ability of PCR to detect very small quantities of a target material and the absence of the need to use radioactive elements are two of the advantages of PCR compared with hybridization techniques. However, more accurate identification of a PCR product may require the use of specific nucleic acid probes. But, it is not evident, with exception of RLB which is now being commercially produced, that the use of the technique will spread as a routine diagnostic tool in the laboratories. The use of molecular biology tools based on nucleic acid for tick-borne diseases will therefore, for sometime continue to be used in research activities rather than for day-to-day diagnosis in the laboratories. However, recombinant antigens based ELISAs may be available for routine diagnosis in the field.

Conflict of Interest

Authors have no conflict of interest.

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Full Length Research Paper

Internal and external parasites of camels (*Camelusdromedarius*) slaughtered at Addis Ababa Abattoir, Ethiopia

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A cross-sectional study was undertakento estimate the prevalence of internal and external parasites of camels slaughtered at Addis Ababa abattoir, Ethiopia. A total of 384 of camels originating from Borena and Metehara areas were examined during the study period and all (100%) of them were found to harbor at least two parasite species. In this study,the prevalence of tick, gastrointestinal parasites, *Cephalopinatitillator*,Hydatid cyst, and *Sarcoptesscabiei var. cameli*were 100, 95.6, 68.2, 65 and 35.4%, respectively. The gastrointestinal parasite's ova/oocyte identified include*Strongylus* species, *Trichuris*species, *Strongyloides*species and coccidiaat prevalence of 78.1, 47.1, 44.5 and 25.3%, respectively. Of the total 1347 pooled samples of tick collected from 40 randomly selected camels.*Rhipicephaluspulchelis*, *Rhipicephalusevertsievertsi*, *Hyalomma dromedary, Amblyommagemma*,*Amblyommavariegatum* and *Boophilusdecolaratus*were identified at a proportion of 53.90, 21.01, 13.66, 7.5, 3.19 and 0.74%, respectively. The average tick burden from half body region of camels was 33.7 ± 6.24 (range 26 to 53).In general, this study indicates that parasites are still the major problems hindering the productivity and health of camels, hence implementation of strategic control measures and further studies are recommended to reduce the effect of parasites on camel health and productivity.

Key words: Abattoir, Addis Ababa,Ethiopia, camel,*Cephalopinatitillator*,gastrointestinal parasite,*Hydatid cysts*,*Sarcoptesscabiei*var.*cameli*,tick.

INTRODUCTION

Camels are an important source of milk, meat and their dung is used for fires. They are also used for riding and transport purpose. In Ethiopia, camels are exported mainly to Egypt and Sudan, and are also slaughtered for meat consumption duringritual occasions (Dirie and Abdurahman, 2003).Despite the fact that, camels provide lots of socio-economic advantagesand are the preferred domestic animal species in the ever-changing climate, so far it was neglected by researchers and development planners(Bekele, 2010).

Severalendo and ectoparasites have been identified as the major problems affecting the health, productivity

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andperformance of camels (Anwar and Khan, 1998; Parsaniet al., 2008; Bekele, 2010). Among ectoparasites of camel, mange mites caused by *Sarcoptesscabiei var. cameli* (Parsaniet al., 2008; Dinkaet al., 2010;Awolet al., 2014) and various species of ticks (Richard, 1979; Melaku and Fisseha, 2001; Lawalet al., 2007; Parsaniet al., 2008; Dinkaet al., 2010; Kiros et al., 2014) have been reported. In addition, camel is also known to be infected with various helminthes and protozoan parasites like coccidia (Rechard, 1979; Anwar and Khan, 1998; Melaku and Fisseha, 2001; Parsaniet al., 2008; Bekele, 2010). Nasopharyngeal myiasisand hydatidosis are also major problems of camels (Zumpt, 1965; Burgemeisteret al., 1975; Hussein et al., 1982; Higgins, 1985; Pandevet al., 1986; Wubet, 1987; Musa et al., 1989; Njorogeet al., 2002).Most of the studies conducted in Ethiopia on camels are limited to the eastern part of the country (Wubet, 1987; Zelalem, 1994; Abebe, 2001; Zeleke and Bekele, 2004; Dinkaet al., 2010) and do not cover the whole country. Therefore, this study was carried outto estimate the prevalence and identify the genus level or species diversity of internal and external parasites of camels of Borena and Kereyu origins slaughtered inAddis Ababa abattoir, Ethiopia (Figure 1).

MATERIALS AND METHODS

Study area

This study was conducted at Addis Ababa abattoir enterprise, Akaki branch, Ethiopia. All camels slaughtered were originated from the Borana(semi-arid) and Kereyu(arid) areas of Ethiopia. Borana is located at approximately 600 km South of Addis Ababa at an altitude of 500 to 2500 m above sea level. It has an annual rainfall of 450 to 650mm in bimodal pattern with long rains expected between March and May and the short rains between October and November. Kereyu is located at about 250 km East of Addis Ababa at 80° 54 E longitude and altitude of 930 m above sea level.It has an average annual rainfall of 504mm. The mean annual maximum and minimum temperature are 32.40 and 18.5°C, respectively (NMSA, 1999).

Studymethodology

Studytype and animals

Cross-sectional study was undertaken, from November to April, on 384 camels to assess the prevalence and species/genus level composition ofinternal and external parasites of camels slaughtered at Addis Ababa abattoir. The number of camels slaughtered varied from 7 to 11 each day. The abattoir was visited two days a week. All camels slaughtered during the time of visitwere examined and sampled without discrimination of their age, sex, body condition and origin.

Data collection

General physical examination was conducted on each camel in the lairage. All data regarding the age (based on dentition), sex, body condition (hump structure) and origin of camels were recorded appropriately(Schwartz and Dioli, 1992; CACIA, 1995).

Fecalsample collection and examination

Fresh fecal samples were collected directly from the rectum of slaughtered camels. Each samplewas placed and codedappropriately in universal bottles, and transported using ice box into parasitology laboratory of College of Agricultural and Veterinary Medicine, Addis Ababa University. The collected samples wereexamined using simple sedimentation method for trematodes eggs and floatation method for eggs of nematodes, cestodes and coccidianoocysts(Soulsby, 1982; Urquhart et al., 1996).

Sample collection and identification of tick and mange mite

All visible adult ticks were collected from half-body regions (on right side) of camels. Ticks were collected in labeled plastic bottles containing 70% ethanolfrom 40 randomly selected camels. Skin of suspected camels affected by mange was scraped until capillary bleeding and the scraping waspreserved in a labeled bottle containing 10% formalin. Both tick and skin scraping samples were taken into parasitology laboratories of College of Agricultural and Veterinary Medicineof Addis Ababa Universityand College of Veterinary Medicine of Mekelle University.Identification of tickswas performed using the keys of Okello-Onenet al. (1999) and Walker et al. (2003). Identification of mites was carried out with the help of morphological characteristics after processing with 10% KOHsolution (Soulsby, 1982;Urquhart et al., 1996).

Examination of *Cephalopinatitillator* and hydatidcyst

After slaughtering, camel heads were dissected and gross examination was performedon the nasal cavity, frontal sinuses, turbinate bones and nasopharynx for the presence of *Cephalopinatitillater*larvae. Liver, lungs and heart of slaughtered camels were grossly examined and palpated for the presence of hydatidcyst. The contents of hydatid cysts were examinedafter aspiration using syringe and incision.

Data analysis

The data was entered into Microsoft excel spreadsheet and coded appropriately. For data analysis, SPSS version 16 was used. In this data analysis, descriptive statistics was used to determine the prevalence of parasites in camel. The chi-square test was also used to determine the existence of any association between the infection and the risk factors like age, origin, body condition score and sex. In all cases, 95% confidence intervals and$P<0.05$were set for significance.

RESULTS

Out of the 384 camels examined,all (100%) of them were found to harbor two or more of the parasite species. In this study, four species of the gastrointestinal (GIT) parasite, six species of tick, one species of mite, hydatid cyst and *C.titillator*larvae were identified.The preva-lence of tick,GIT parasite, *C.titillator,*hydatic cystand mange mite infestation were 100,95.6, 68.2, 65 and 35.4%, respectively (Table 1).

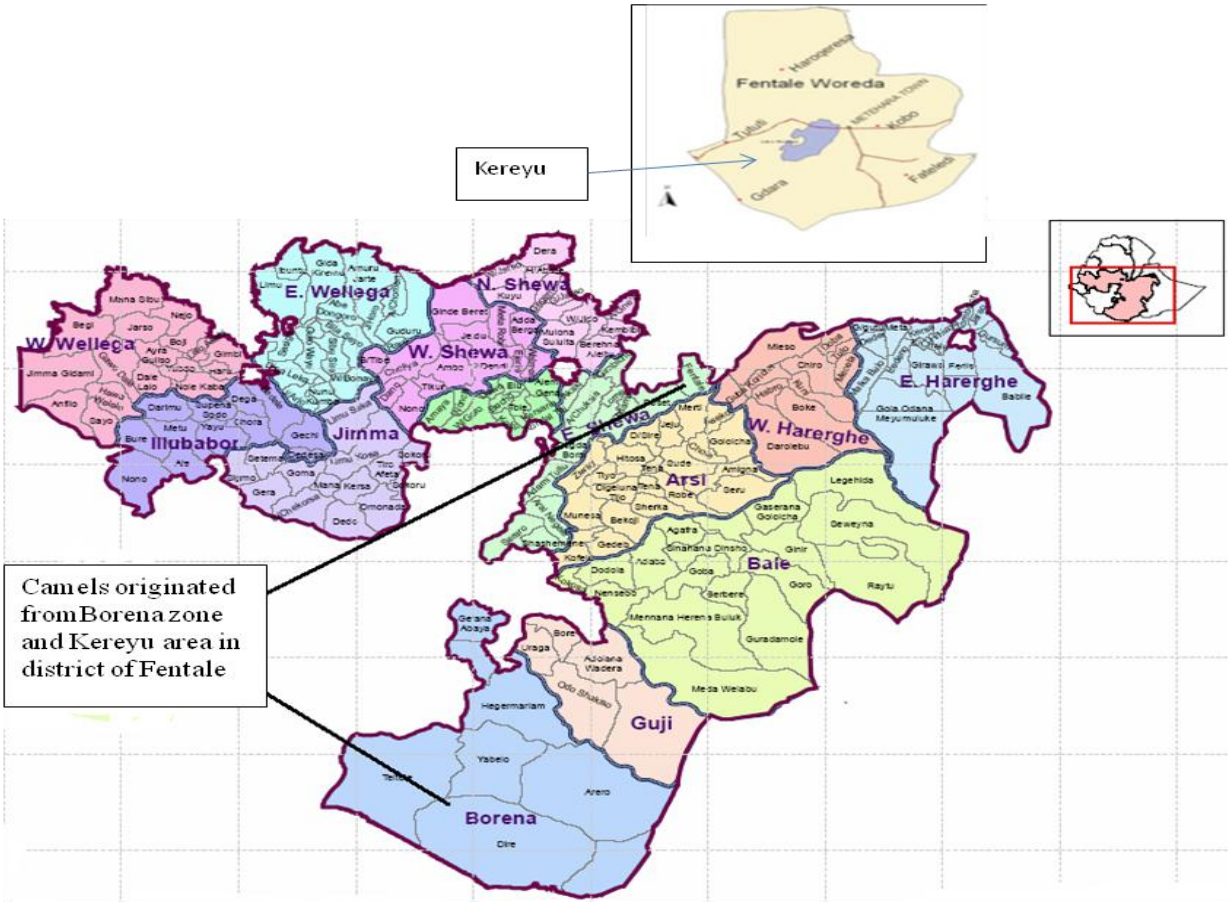

Figure 1. Zones of Ethiopia Map, showing the origin of camels (Borena and Kereyu) slaughtered at Addis Ababa abattoir.(Pantuliano and Wekesa, 2008; Kasa et al., 2011;Kasaye et al., 2013).

Table 1. The prevalence of tick, *Cephalopinatitillator,* hydatid cysts*, Sarcoptesscabiei var. cameli* and GIT parasites in camels slaughtered at Addis Ababa abattoir, Ethiopia.

Parasite	Number of infected camels	Prevalence (%)
Tick	384	100.0
Cephalopinatitillator	262	68.2
Hydatid cysts	250	65.0
Sarcoptesscabiei var. cameli	136	35.4
GIT parasites	367	95.6
Coccidia	97	25.3
Strongyloides spp.	171	44.5
Strongylus spp.	300	78.1
Trichuris spp.	181	47.1

GIT: Gastrointestinal.

Ticks were observed on all (100%) of the examined camels (Table 1).Of the total 1347 pooled hard ticks collected from the half body region of 40 camels, *Rhipicephalus pulchelis*, *Rhipicephalus evert sievesi*, *Hyalomma dromedary, Amblyomma gemma*,*Amblyomma variegatum* and *Boophilus decolaratus* were identified at a proportion of 53.90, 21.01, 13.66, 7.5, 3.19 and 0.74%, respectively (Table 4). The average tick burden from half body region of camels was 33.7±6.24 (range 26 to 53). In addition to tick infestation, 136 (35.4%) of the examined

Table 2. The distribution of tick, *Cephalopinatitillator,* hydatid cysts*, Sarcoptesscabiei var. cameli* and GIT parasites infestation among age, sex, origin and body condition score in camels slaughtered at Addis Ababa abattoir, Ethiopia.

Risk factor	Category level	No.	Number of infected camels (%)							
			Ticks	*Cephalopina titillator*(%)	Haydatid cysts (%)	*Sarcoptesscabei var. cameli*	*Coccidiaspp* (%)	*Strongylodies* spp.	*Strongylus*s pp.	*Trichuris*spp.
Body condition score	Fatty	28	28 (100)	17 (60.7)	17 (60.7)	13 (46.4)	12 (42.9)	11 (39.3)	17 (60.7)	17 (60.7)
	Good	14	14 (100)	97 (66.9)	94 (64.8)	54 (37.2)	35 (24.1)	70 (48.3)	109 (75.2)	65 (44.8)
	Thin	95	95 (100)	72 (75.8)	58 (61.1)	26 (27.4)	18 (18.9)	39 (41.1)	78 (82.1)	37 (38.9)
	Moderate	116	116 (100)	76 (65.5)	81 (69.8)	43 (37.1)	32 (27.6)	51 (44.0)	96 (82.8)	62 (53.4)
	P-value		-	0.29	0.538	0.210	0.71	0.656	0.45	0.79
Origin	Borena	363	363 (100)	249 (68.6)	237 (65.3)	128 (35.3)	91 (25.1)	161 (44.4)	285 (78.5)	171 (47.1)
	Metehara	21	21 (100)	13 (61.9)	13 (61.9)	8 (38.1)	6 (28.6)	10 (47.6)	15 (71.4)	10 (47.6)
	P-value		-	0.522	0.752	0.792	0.719	0.77	0.445	0.964
Sex	Male	61	62 (100)	45 (72.6)	38 (61.3)	20 (32.3)	14 (22.6)	25 (40.3)	48 (77.4)	31 (50.0)
	Female	322	322 (100)	217 (67.4)	212 (65.8)	116 (36.0)	83 (25.8)	146 (45.3)	252 (78.3)	150 (46.6)
	P-value		-	0.422	0.491	0.570	0.596	0.467	0.883	0.622
Age (year)	5-8	85	85 (100.0)	48 (56.5)	54 (63.5)	39 (45.9)	28 (32.9)	50 (58.8)	60 (70.6)	35(41.2)
	9-12	132	132 (100.0)	98 (74.2)	93 (70.5)	41 (31.1)	30 (22.7)	48 (36.4)	106 (80.3)	70 (53.0)
	>12	167	167 (100.0)	116 (69.5)	103 (61.7)	56 (33.5)	39 (23.4)	73 (43.7)	134 (80.2)	76 (45.5)
	P-value		-	0.21	0.7	0.66	1.88	0.05	0.163	0.199

At 95% confidence interval.

camelshad mange mite infestation and only *Sarcoptesscabiei var. cameli* was identified from all of the collected skin scraping samples (Table 1).

The GIT parasites ova/oocyte identified during the study period include *Strongylus*species, *Trichuris* species, *Strongyloides*species and coccidia at prevalence of 78.1, 47.1, 44.5 and 25.3%, respectively (Table 1). No trematode and cestode ova were identified. In general, there was no significant difference in the prevalence of parasites between/among the different risk factors (Table 2).

C. titillator larvae were found in the nasal cavity, pharynx, turbinates and sinuses of 68.2% (n=262) camels (Table 1). Hydatid cysts were encountered in 65% (n=250) of camels (Table 1). Hydatid cysts of variable sizes (2 to 8 cm in diameter) were found in the lung, liver, and in both organs of the same animals at a proportion of 59.6% (n=149), 9.6% (n=24) and 30.8% (n=77), respectively. There was significant difference (P-value < 0.0001) in the localization of hydatid cysts between lungs and liver (Table 3). They were also varied in number from 2 to 7 on single organ. 62% of infected camels harbored only cysts that had calcified or yellowish material inside the capsule, but the rest (38%) harbored at least one cyst that had clear water like fluid inside the capsule.

DISCUSSION

The present study assesses the prevalence of internal and external parasites encountered on camels slaughtered at Addis Ababa Abattoir, Ethiopia. All (100%) had two or more of the parasite species. Similar studies conducted by Al-Ani etal.(1998)andSharrifetal.(1998)inJordan,Anwar

Table 3. Organ distribution of hydatid cysts in camels slaughtered at Addis Ababa abattoir, Ethiopia.

Organs affected	No. of affected camels	%
Lung only	149	59.6
Liver only	24	9.6
Lung and liver	77	30.8
Total	250	100

P-value < 0.0001.

Table 4. The proportion of tick species collected from 40 randomly selected camels slaughtered at Addis Ababa abattoir, Ethiopia.

Tick species	No. of ticks collected	Proportion (%)
Rhipicephaluspulchelis	726	53.90
Rhipicephalusevertsi-evertsi	283	21.01
Hyalomma dromedary	184	13.66
Amblyommagemma	101	7.50
Amblyommavariegatum	43	3.19
Boophilusdecolaratus	10	0.74
Total	1347	100.00

and Khan (1998) in Pakistan, Dia (2006) in Burkina Faso and Bekele (2010) in Southern Ethiopia also reported a higher prevalence of parasites in camel. This high prevalence of parasites could be related to rearing of camels in marginal areas where veterinary services are not available or very limited (Tefera, 2004).

In this study, tick infestation was detected in all (100%) of the examined camels. This result was supplementary to the findings of Al-Ani et al. (1998),Melaku and Fesseha (2001), Dia (2006), Bekele (2010) and Kiros et al. (2014). Of the total 1347 pooled samples of ticks collected from half body region of 40 randomly selected camels,*R.pulchelis*, *R.evertsievertsi*, *H. dromedary, A.gemma*, *A.variegatum* and *B.decolaratus* were identified at a proportion of 53.90, 21.01, 13.66, 7.5, 3.19 and 0.74%, respectively. The average tick burden in half body region of camels was 33.7±6.24 (range 26 to 53). Similar species of ticks and greater tick load per camel were also reported by Zeleke and Bekele (2004), Bekele (2010), Nazifi et al. (2011) and Kiros et al. (2014). In addition to feeding on animal blood, ticks also act as vector for diseases, causing tick paralysis, and direct damage to tissue, so that providing entry for opportunistic micro-organisms and fly larvae. Tick infestation also causes loss of appetite, leading to a reduction in growth rate and decreased productivity, and results in increased calf mortality (Schwartz et al., 1983; Hart, 1990; Nelson et al., 1977; Jabbar et al., 2007).

Gastrointestinal parasites weredetected in 95.6% (n=367) of the examined camels. The GIT parasites ova/oocyst identified during the study period were *Strongylus*spp., *Trichuris* spp., *Strongyloides*spp. and *coccidia* at prevalence of 78.1, 47.1, 44.5and 25.3%, respectively. These results agree with the findings of Richard (1979), Hussein et al. (1987), Al-Ani et al.(1998), Anwar and Khar (1998), Sharif et al.(1998), Agab and Abbas (1999), Bekele (2010), Bamaiyi and Kalu (2011) and Swai et al. (2011). The ova of cestode and trematode were not found in this study, even though Richard (1979), Anwar and Khar (1998), Sharif et al. (1998), Bekele (2010), Bamaiyi and Kalu (2011) and Swai et al.(2011) have reported these parasites from camels. Gastrointestinal parasites reduce the productivity and performance of camels, and also predispose them to other infection diseases. Gastrointestinal parasitism is generally associated with diarrhea, weakness, constipation and emaciation (Richard, 1979)

*C.titillator*wasfound on 68.2% of camels. This finding was in agreement with the result of Al-Aniet al. (1998) (74%) and Morsyet al. (1998) (71.7%), but higher than Al-Aniet al. (1998) (33%),Sharrifet al.(1998) (33%) and Bekele (2001) (52%).This variation in the prevalence of *C.titillator* infestation might be attributed to the different management systems and environmental condition that exist among those areas.*C.titillatar*has several impacts on respiratory function, feeding, health and productivity of the animals. Infested camels lose their appetite and show respiratory problem and abnormal behavior resembling cranial coenuriasis (Zumpt, 1965). Pathological lesions of the nasal sinuses and death of camels associated with secondary pathogenic bacteria and viral infections were also reported previously (Burgemeisteret al., 1975; Hussein et al., 1982; Musa et al., 1989; Al-Aniet al., 1991).

The prevalence of hydatidcyst recorded in this study was 65%. This resultwas higher than the findings of

Wubet (1987), Abdul-Salam (1988), Woldemeskel (2001), Ahmadi (2005), Bitsat (2009) and Mohammed (2010). The high prevalence in the present study could be due to the presence of high population of dogs which are closely associated with livestock in the field and barn as well as due to high population of wild carnivores in the area of the majority camels origin (Borena) (Balako, 1999) and due to lack of proper condemnation of organs infected with hydatidcyst in pastoral areas (Bekele, 2008). These facilitate easy access of infected organs to dogs and wild carnivores which are the principal definitive hosts and maintain the life cycle of the parasite. Hydatid cysts varied in number from 2 to 7 on single organ. They were also of variable sizes (2 to 8 cm in diameter) and found at the proportion of 59.6% in the lung, 9.6% in liver and 30.8% both in lung and liver of infected animals. However, the findings of Abdl-hafez et al. (1986), Kamhawi et al. (1995), Ibrahim and Craig (1998.) and Haridy et al. (2006) indicated a higher rate of liver infection than lung. 62% of infected camels harbored only calcified cysts but the rest (38%) harbored at least one non calcified cyst. This was in accordance with Abdl-hafez et al. (1986), Kamhawi et al.(1995), Chai et al.(1998), Sharrif et al. (1998), Ahmadi (2005) and Bitsat (2009).

Mange mite infestation was detected in 35.4% (n=136) of the examined camels. This result was higher than the report of Anwar and Khan (1998), Dinka et al. (2010) and Awolet al. (2014) who reported a prevalence of 13.4, 10.68 and 16.70%, respectively. Only *S.scabiei var. cameli* was identified. This species of mange was also reported by Al-Ani et al. (1998), Agab and Abbas (1999), Lawal et al. (2007), Bekele (2010), Dinka et al. (2010) and Awol et al. (2014). Sarcoptic mange caused by *S.scabiei var. cameli* is extremely contagious and serious problem in camels (Nayel and Abu-Samra, 1986; Pegram and Higgins, 1992; Parsani et al., 2008). It is also the second most important camel disease after trypanosomiasis (surra) in terms of its effect on production in camel herds across the world (Mochabo et al., 2005; Nayel and Abu-Samra, 1986). Sarcoptic mange is also of zoonotic nature. Camel owners are the main sufferers due to close association with camels (Parsani et al., 2008).

In general, this and others studies indicated that parasites are among the major constraints of camel health and production. Considering the existence of limited veterinary service in camel rearing areas, well integrated studies and appropriate control measure should be implemented to improve the health and productivity of camels. Furthermore, due to the zoonotic importance of hydatidosis and *S.scabiei var. cameli,*public awareness should be created to control these parasites.

Conflict of Interest

Authors have no conflict of interest.

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Major gastrointestinal helminth parasites of grazing small ruminants in and around Ambo town of Central Oromia, Ethiopia

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A cross-sectional survey was conducted to estimate the prevalence of small ruminant helminthosis and associated risk factors in and around Ambo town of central Oromia, Ethiopia between February and May, 2013. A total of 120 small ruminants were examined using standard parasitological procedures. The study showed that 49.2% of the study animals were found to harbor eggs of one or more gastrointestinal helminth parasites. Both sheep and goats have been shown to harbor strongyle species, *Fasciola* and mixed infections. The dominant helminth parasites observed in infected animals were strongyle species (81.4%). In addition, lower infection rate of *Fasciola* of 10.2% and mixed infections of 8.3% were found. The species level prevalence of the parasites was 47.8% (43/90) and 53.3% (16/30) in sheep and goats, respectively. Though the infection rate of gastrointestinal tract (GIT) parasites was higher in goats than sheep, the difference was statistically insignificant (P>0.05). Moreover, statistically insignificant association (P>0.05) was observed between animal species and infection with strongyles species, *Fasciola* or mixed infections. In this survey, no statistically significant effect (P>0.05) of animal sex, age, location and management system on prevalence of the helminth parasites was observed. However, prevalence significantly varied (χ^2=15.16; P=0.000) among different body condition scores. Animals with thin (OR=9.24, 95% CI: 2.70-31.57) and moderate (OR=5.10, 95% CI: 1.70-15.26) body condition scores were associated with high relative risk of infection with GIT helminth parasites than fat animals. In conclusion, body condition score was found to be the potential risk factor and should be considered during designing control measures against helminthosis of small ruminants in the study area.

Key words: Helminth parasites, prevalence, coprological examination, small ruminants, Ambo, Oromia.

INTRODUCTION

Small ruminant population of Ethiopia is about 48 million of which 26 million is sheep and 22 million is goat (CSA,

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2008). Small ruminants provide about 46% of the national meat consumption and 58% of the value of hide and skin production (Awgichew et al., 1991). They have many advantages over large ruminants for most smallholder farmers, including among others: less feed costs, quicker turnover, easy management and appropriate size at slaughter (Abegaz, 2002; Donkin, 2005). Nevertheless, small ruminant productivity is still low compared to the population due to poor nutrition, diseases and 'poor' genetic make-up of the indigenous stock (Tibbo et al., 2004).

Among diseases, helminthosis constitutes one of the most important constraints to small ruminant production in Ethiopia (Tibbo et al., 2004; Zeryehun, 2012). Studies conducted on ruminant helminthosis of various regions of Ethiopia have revealed a prevalence range from 47.67 to 84.1% (Demelash et al., 2004; Regassa et al., 2006; Dagnachew et al., 2011). The pervasive occurrence of parasitic infections in grazing animals, the associated loss of production, the cost of anthelmintics, death of infected animals and increasing frequency of drug resistance are all major concerns (Singla, 1995; Tibbo et al., 2004; Odoi et al., 2007).

In Ethiopia, helminthosis is responsible for 25% mortality and 3.8% weight loss in highland sheep and causes an estimated annual loss of about 700 million Ethiopian birr (Ngategize et al., 1993). Helminthosis is associated with enormous losses due to condemnation of affected organs at slaughter (Kumsa and Wossene, 2006). Several previous studies conducted in different parts of Ethiopia have revealed that the most common genera of parasitic helminths of small ruminants are *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*, *Bunostomum*, *Strongyloides*, *Fasciola* and *Trichuris* (Regassa et al., 2006; Kumsa and Wossene, 2006; Kumsa and Bekele, 2008).

The incidence of helminth parasite infections varies greatly area to area depending on the relative importance of many factors like nutrition status, pasture management, climatic condition, animal immunity and host preference (Singla 1995; Radostits et al., 2006). To better identify appropriate control strategies for helminth control of small ruminants in the smallholder systems, it is important to investigate the burden of small ruminant helminthosis and identify specific risk factors that are unique to this area and farming system. To the knowledge of the authors, no information published in refereed scientific journals on the burden of gastrointestinal tract (GIT) helminth infections of small ruminants in and around Ambo town is available. This study was, therefore, designed to investigate the magnitude, and composition of helminths of small ruminants in and around Ambo town in central Oromia Regional State of Ethiopia. In addition, an attempt was made to identify the possible risk factors associated with the occurrence of infections in small ruminants that may help to devise effective control measures against the parasites in the study area.

MATERIALS AND METHODS

Study area

This study was conducted between February and May, 2013 in Ambo town of West Showa zone in Oromia Regional State, Ethiopia. Ambo town is the capital of West Showa administrative zone of the Oromia Regional State. The town is located at 114 km away from Ethiopian capital, Addis Ababa, to the west of the country. The town is located at altitude of 2,185 meter above sea level (masl). The geographical location of Ambo town is approximately between 8°56'30"N and 8°59'30"N latitude and between 37°47'30"E and 37°55'15"E longitude. Based on 20 year meteorological data, the mean annual temperature, the annual maximum and the annual minimum temperatures of the area were about 18.8, 26 and 10.76°C, respectively (Nemomsa, 2013). The mean annual rainfall is about 1,143 mm and the highest rainfall occurs from June to September. The town and its surrounding areas are dominated by Eucalyptus trees. Major soils of the area are vertisols consisting of 67% clay, 18% silt, 15% sand and 1.5% organic matter (Nemomsa, 2013). According to the National Population and Housing census carried out in 2007, the population of the town was 67,514, out of which 34,276 (50.8%) were males and 33,238 (49.2%) were females (CSA, 2007). During the study period, there were approximately 112,236 heads of cattle, 24,966 heads of sheep and 16,399 heads of goats in Ambo district. In the study area, ruminants are managed by communal holding of all species such as cattle, sheep, goats and equines together. The urban agricultural activities are dominated by livestock production like medium (>5 dairy animals) and smallholder (<5 dairy animals) dairy farming (Lemma et al., 2001), animal fattening, and sheep and goat farming.

Study animals and their management

The study animals were two populations of small ruminants managed under extensive smallholder and semi-intensive husbandry systems. On one hand, small ruminants belonging to smallholder farmers found in and around Ambo town that were kept under traditional extensive management system were used. In the study area, ruminants were allowed to graze on communal or private owned pasture land without provision of supplementary feeds except some leftover foods. The major feed resources in the area were natural pasture, hay, crop residues and crop-aftermath and tree/shrub fodders. The major crop residues fed to animals by majority of the farmers were teff, maize, and sorghum. Planting forages was not common. The animals were housed in houses with muddy grounds roofed with either hay or corrugated iron. Strategic de-worming was not practiced by the farmers of the area, but animals were often treated with chemicals when clinical helminthosis was evident.

On the other hand, all small ruminant animals kept at Ambo University farm at the time of the study were also included as study animals. ~~study~~ The animals ~~included from Ambo University livestock farm~~ were managed under semi-intensive management system in which they ~~animals~~ were kept on grazing pasture during the day and housed during the night in sheltered pens. They were vaccinated against ovine pPasteurellosis and sheep pox annually and were de-wormed with albendazole bi-annually before and after the main rainy season. The last drenching of the animals was performed in October of the previous year. Individual animals were treated against any infectious diseases. To determine the body conditions of the study animals, body condition scoring system developed by Ethiopian Sheep and Goat Productivity Improvement Program was used (ESGPIP, 2008). Age groups were categorized

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into young (<1 year), adult (1-4 years) and old (>4 years) based on farmers response and observations made during

Table 1. Prevalence of small ruminant gastrointestinal tract parasites in sheep and goats in Ambo town during the study period.

Species	No. exam	No. positive (%)	Type of parasites eggs		
			Strongyles (%)	*Fasciola* (%)	Mixed infection (%)
Sheep	90	43 (47.8%)	36 (40.0%)	4 (4.4)	3(3.3)
Goats	30	16 (53.3%))	12 (40.0%)	2 (6.7)	2 (6.7 3.3%)
Total	120	59 (49.2%)	48 (40.0)	6 (5.0%)	5 (4.2 8.3%)

sampling. The number of goats included in the present study was low compared to sheep because of the low population of goats reared in the study area.

Study design and sampling

A cross-sectional study design was used to collect random samples from the study animals to address the objectives of the study. Simple random sampling was used to select study locations in and aroundwithin the town, while Ambo University livestock farm was included purposively. Households owning the small ruminants study animals were identified based on data obtained from district office of agriculture and 24 households (farms) who were willing to participate in the survey were selected and every animal in the selected farms were included as study animals. Accordingly, A total of 120 animals (98 animals were sampled from animal populations owned by smallholder farmers (the average number of small ruminants per farm was 4.0) and the remaining 22 animals were sampled from university farm.

Parasitological examination of specimens

Random fecal samples were collected directly from the rectum of the study animals using disposable plastic gloves and placed in plastic fecal bags that were then labeled. The collected samples were preserved in 10% formalin and dispatched to Veterinary Laboratory of Ambo University for coprological investigations. Parasitological examination was done by direct smear and flotation techniques following the standard procedures for nematode parasites (Hansen and Perry, 1994). In addition, for eggs of liver flukes, coproscopic examination was performed according to the sedimentation technique described by Hansen and Perry (1994). As *in vivo* identification of infections relies on the microscopic detection of parasite eggs in host faeces (Gareth, 2009), the collected fecal samples were processed and examined under the 10x magnification. Parasite eggs were identified using keys given by Soulsby (1982). Speciation of the parasites was not carried out due to laboratory capacity reasons.

Data management and analysis

All the data obtained from the study were entered into MS Excel data sheets and coded. The coded data were imported and analyzed using SPSS version 16.0 (SPSS, Inc. Chicago). Point prevalence was used in this study. Percentages (%) were used to measure prevalence of the parasites as described by Hansen and Perry (1994) and chi-square (χ^2) was used to measure associations between prevalence and the various independent variables including species of the animals, age, sex, location, management system and body condition scores. Fisher's Exact Test was used to measure associations between prevalence and variables that have less than 5 numbers of observations. Univariate logistic regression analysis was conducted to examine the relationship between the outcome variable and the different explanatory variables. Logistic rRegression coefficients were used to estimate odds ratios for each of the independent variables. in the model. Odds ratios (OR) with 95% confidence intervals (CI) were used to assess the level of association of the dependent variable and independent variables. In all the analyses, a 95% confidence interval and P-value of less than 0.05 (P<0.05) was set for significance of statistical associations between the dependent and independent variables.

RESULTS

Overall prevalence of helminthosis

The present study showed that 49.2% (n=59) were found to harbor one or more GIT parasite species eggsparasite eggs. About 81.4% of these infected animals were found to harbor strongyle species and 10.2% were harboring only *Fasciola* species. In addition, 8.5% of the study animals were found to harbor mixed infections of strongyle and *Fasciola* parasites.

Prevalence of helminth parasites by animal host and parasite species

Coprological examination in both sheep and goats have shown the presence of strongyle species, *Fasciola* and mixed infections with the two types of parasites. The species level prevalence of the parasites werewas 47.835.8% (43/12090) and 53.313.3% (16/1230) in sheep and goats, respectively (Table 1). Though the infection rate of gastrointestinal helminths was higher in goats than sheep sheepthan goats; the difference was not statistically significant (P>0.05) between the two small ruminant animalspecies. In addition, no statistical association (P>0.05) was observed between animal species and infection with eitherstrongyle species, or *Fasciola* and mixed infections.

Prevalence of helminth parasites by animal small ruminantsex and age groups

Prevalence of helminth parasites of 250.0 and 248.32% were found in male and female animals, respectively. No

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statistically significant difference (P>0.05) in prevalence of helminths was observed between different sexes. In addition, no effect of sex on prevalence of infection was detected

Table 2. Overall and specific helminth parasite prevalence in different age groups of small ruminants in the study area.

Age	No. exam	No. positive (%)	Type of parasites		
			Strongyles (%)	*Fasciola* (%)	Mixed infections (%)
Young	62	30 (48.4)	26 (41.2)	2 (3.2)	2 (3.3.2)
Adult	42	17 (40.5)	13 (30.9)	1 (2.4)	3 (7.1)
Old	16	12 (75.0)	9 (56.2)	3 (18.7)	0 (0.0%)
Total	120	59 (49.2)	48 (40.0)	6 (5.0%)	5 (84.2)

Table 3. Prevalence and risk factors of GIT helminth parasites in small ruminants at Ambo town during the study period.

Factor	Categories	No. exam	No. positive	Prevalence (%)	χ^2	P value	OR	95.0% CI (OR)
Location	Kebele 01	34	18	53.3	0.872	0.832	-	-
	Kebele 02	34	15	44.1		-		
	AU farm	22	10	45.4		-		
	Teltele	30	16	53.3		-		
Body condition score	Thin	26	21	80.7	15.75	0.000	9.24	2.70-31.57
	Moderate	62	28	45.2		-	5.10	1.70-15.26
	Fat	32	10	31.2		-		
Management type	Extensive	98	49	50.0	0.149	0.700	-	-
	Semi-intensive	22	10	45.4		-		

*AU: Ambo University

though the overall prevalence of helminth infection was isfound to be higher in old young animals (725.0%) compared to adult (140.25%) and young old (48.410.0%) animals; no statistically significant differences (P>0.05) were observed between the age categories (Table 2). Besides, the present study did not find statistically significant association (P>0.05) between the different age groups of the study animalsand the prevalenceand prevalence of the different parasites considered.

Prevalence of helminth parasites by body condition, location and management type

Concerning the prevalence of helminth infections in different body conditioned animals, higher prevalence was observed in thin (80.7%) than moderate (45.2%) and fat (31.2%) animals. Thus, a significant association (χ^2=15.75; P=0.000) in prevalence was shown among animals with different body condition scores. Consequently, animals with thin and moderate body condition scores were associated with a high relative risk of being infected with GIT helminth parasites than fat animals. On the other hand, no statistically significant association was observed between parasite infection rate and different locations (P>0.05). Moreover, in the present study, type of management system did not significantly influence (P>0.05) the prevalence of infections in study animals (Table 3).

DISCUSSION

The present study revealed the overall prevalence of GIT helminth parasites of small remnants to be 49.2%. The overall prevalence found in the current study is consistent with the findings of Kumsa and Wossene (2006) and Dagnachew et al. (2011) who reported similar prevalence of small ruminant helminthosis from East and North Ethiopia, respectively. Nevertheless, the overall prevalence in the present study is lower than reports of Regassa et al. (2006), Fufa et al. (2009), Bitew et al. (2011), Kumsa et al. (2011), Zeryehun (2012) and Ibrahim et al. (2014) from different regions of Ethiopia. The difference, among others, could be due to

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differences in agro-ecology, management of the animals, and breed of the animals.

Coprological examination in both sheep and goats have shown the presence of strongyle species, *Fasciola* and mixed infections in both species of animals. The dominant helminth parasites found during the study period were strongyle species (81.4%). This finding is consistent with other reports from different parts of Ethiopia

(Regassa et al., 2006; Dagnachew et al., 2011; Kumsa et al., 2011; Ibrahim et al., 2014) and elsewhere (Agyei, 2003; Waruiru et al., 2005; Githigi et al., 2005; Odoi et al., 2007; Ntonifor et al., 2013). The high prevalence of strongyles may be due to the suitability of the climatic condition of Ambo for survival and transmission of the parasites. In addition, the poor management practices including the poor hygienic practices employed by the farmers may be the other contributing factor as the epidemiology of nematodosis is determined by environmental factors (Thamsborg et al., 1996; Ng'ang'a et al., 2004). The aggregate prevalence of *Fasciola* spp. for sheep and goats was 10.2%. The low coprological prevalence of fasciolosis could be due to the low number of metacercariae intake by the animals owing to low ambient temperature which is not favorable for the snail intermediate host (Andrews, 1999). In addition, 8.5% of the study animals were found to harbor mixed infections of strongyle and *Fasciola* species Mixed infections characterized by the presence of two or more helminth genera in both sheep and goats in this study is in agreement with the findings of other researchers in the country (Abebe et al, 2010; Regassa et al., 2006; Tefera et al., 2011; Kumsa et al., 2011; Ibrahim et al., 2014) and elsewhere (Asif et al., 2008; Agyei, 2003; Githigia et al., 2005; Waruriu et al., 2005). Polyparasitism has been suggested to be an important cause of morbidity and loss of production in small ruminants (Kumsa et al., 2010; Ibrahim et al., 2014). Moreover, the presence of interaction and compromization of the immune system of the host by polyparasitism has been described to increase their susceptibility to other diseases or parasites (Wang et al., 2006).

On the other hand, the present study has shown ed the prevalence of GITgastrointestinal parasites to be 437.83% (439/90) and 53.346.7% (164/30) in sheep and goats, respectively. A relatively similar prevalence ratewas reported by Abebe et al, 2010; Kumsa and Wossene (2006) and Dagnachew et al. (2011) from different areas of Ethiopia. Even though the infection rate was higher in goats than sheep, the difference was not statistically significant (P>0.05). While some studies have reported that goats are more susceptible than sheep to a similar challenge (Ntonifor et al., 2013), others have reported that sheep usually suffer heavier worm burdens because of the difference in their grazing habits (Baxendell, 1984; Tembely and Hansen, 1996). Nevertheless, in the present study, though sheep and goats differ in their feeding habits, both species were kept together on common grazing land. Mixed crop livestock production predominates in the area where farm animals including small ruminants are kept together on confined grazing land which may expose goats to acquire more susceptibility for the same species of parasite infection. In consequence, the condition could be due to less or slow development of immunity in goats to GIT parasites compared with the situation in sheep. Goats do not build up an effective immune response against helminth infections and so remain susceptible to disease throughout their lives. The risk is enhanced if they are forced to graze rather than browse (Urquhart et al., 1996; Radostits et al., 2006). Sheep faced prolonged challenge over generations and had developed good resistance (Urquhart et al., 1996).

In our study, male and female animals were found to be equally susceptible to infection with gastrointestinal helminth parasites. The absence of statistical association between sex and prevalence of GIT parasites is in agreement with that of Keyyu et al. (2003) and Regassa et al. (2006). Nevertheless, it is in disagreement with other reports including Maqsood et al. (1996) and Urquhart et al. (1996) who found higher infections in female animals than males with a significant difference between them. It is assumed that sex is a determinant factor influencing prevalence of parasitism (Maqsood et al., 1996) and females are more prone to parasitism during pregnancy and per-parturient period due to stress and decreased immune status (Urquhart et al., 1996). In addition, Dagnachew et al. (2011) reported a higher prevalence of helminth infection in female animals.

In the present study, both species of small ruminants recruited from found in different locations were infected with GIT parasites, though no statistically significant association (P>0.05) was observed between prevalence and locations. Previous studies indicate that different climatic conditions in different locations are important factors for development, multiplication and survival of nematode parasites (Woldemariam, 2005) and these could be translated to differences in the risk of acquiring the parasites between animals managed under different locations. In our study, absence of association between location and prevalence in small ruminants could be due to relative similarity similarity in agro-ecology between study locations and a relatively similar management systems practiced by farming communities. Likewise, in this study, old animals were found seem to have higher prevalence of GIT parasites than young and adult animals though it was not statistically insignificant. The higher prevalence of GIT parasite infections in older animals is in agreement with Garedaghi et al. (2013). This finding is in contrast with the hypothesis that older animals can acquire immunity against GIT parasites which has been supported experimentally by different studies (Gamble and Zajac 1992; Knox, 2000). Similarly, a number of authors have demonstrated an increased prevalence in young age than old age (Gupta et al., 1976;

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Raza et al., 2007). The higher prevalence in old animals may be due to the waning of immunity as animals get older coupled with the poor management of the animals (Radostits et al. 2006).

In this study, a significant difference was observed in prevalence of helminth infection in relation to body condition score where a higher prevalence of gastro-intestinal parasites were recorded in thin and moderate body conditioned animals compared to other animals. This finding agrees with Keyyu et al. (2006), and Negasi et al.

(2012), and Gonfa et al. (2013). In addition, Radostits et al. (2006) and Odoi et al. (2007) indicated that animals with poor condition are highly susceptible to infection and may be clinically affected by worm burdens too small to harm an otherwise well-fed healthy animal. Moreover, Knox et al. (2006) observed that a well-fed animal was not in trouble with worms, and usually a poor diet resulted in more helminth infections. Furthermore, helminths also led to a loss of appetite and poor utilization of food, which results in a loss of body weight. Hawkins and Morris (1978) demonstrated that weekly growth rates of wool and live weight decreased with increasing fluke burdens in sheep.

Conclusions

This study revealed the importance of helminthosis in small ruminant populations in the study area. Among the potential risk factors, body condition score was found to be the important potential risk factor for infection of small ruminants with gastrointestinal helminth parasites. Therefore, body condition score should be considered during designing control measures against helminthosis of small ruminants in the study area. In addition, further studies with large sample size, and wide geographical coverage should be conducted in different seasons of the year so as to establish the epidemiology of the infections and to implement holistic helminthosis control in the study area.

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Conflict of interest

The authors have no conflict of interest.

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Ultrasonographic assessment of uterine involution and ovarian activity in West Africa Sahelian goats

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This study aimed to characterize uterine involution and early ovarian postpartum activity in Sahelian goats. A total of 21 goats were allowed to nurse their kids and were examined every other day for complete uterine involution or daily for follicular development using a 5 MHz transrectal linear transducer. The obtained results indicated three phases of uterine regression, a fast involution phase (rates of 0.71 cm/day), a second moderate involution phase (0.17 cm/day), and a low rate of 0.14 cm/day that ended by day 22.44 ± 1.54. During the experiment, two goats showed an abnormal uterine involution and were excluded from the general profile. The 19 other goats exhibited a very similar profile of uterine involution described by the logarithmic equation $y = -2.33\ln(x) + 9.43$ ($R^2 = 0.98$, $P < 0.05$) where y = uterine diameter (cm) and x = days postpartum. Ovarian activity started within the first week postpartum and was characterized by appearance of one to three follicles ranged in mean size [4.95 ± 1.45 to 8.56 ± 0.66] on both ovaries. These findings could assist practitioners to decide when goats should be bred following parturition.

Key words: Goat, ovary, postpartum, ultrasonography, uterus.

INTRODUCTION

During the postpartum period (PP), the functionality of the reproductive system and preparation for a new pregnancy should be re-established. This includes uterine involution and resumption of cyclic ovarian activity (Kandiel et al., 2012; Elsheikh et al., 2013). Complete uterine involution is a prerequisite to the maintenance of pregnancy. The time estimated for the completion of uterine involution in sheep varies between 17 and 40 days (Ungerfeld and Sanchez-Davila, 2012; Rubianes et al., 1996; Hauser and Bostedt, 2002). In goat, completion of uterine involution and resumption of sexual activity following parturition depend on several factors, such as nutrition, parity, breed, nursing of offspring and season of parturition (Badawi et al., 2014; Delgadillo et al., 1998;

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Greyling, 2000). Different research reports have shown different intervals to complete uterine involution. While Degefa et al. (2006) demonstrated complete macroscopic uterine involution by day 19 PP, Greyling and van Niekerk (1991) reported day 28 PP as the day of complete uterine involution. Moreover, histochemical study of caprine endometrium indicated complete regression of endometrium and re-epithelialization by day 16 PP (Sanchez et al., 2002).

It is difficult to judge the time of uterine involution in the goat, because the uterus cannot be examined by rectal or abdominal palpation. In most studies, uterine involution was investigated after slaughter (Van Wyk et al., 1972; Rubianes and Ungerfeld, 1993) or by laparotomy (Rubianes et al., 1996) or using radio-opaque markers (Tian and Noakes, 1991).

Ultrasonography provided a non-invasive technique to image directly in the reproductive tract. In goat, ultrasonography is routinely used for pregnancy diagnosis (Hesselink and Taverne, 1994; Buckrell, 1988). Recently, it was shown that transrectal ultrasonography is a useful and reliable method to observe the uterine involution and follicular dynamics in goats (Kandiel et al., 2012; Badawi et al., 2014).

The Sahelian goat is one of the most common breed in West Africa, which is used for meat production. Its rapid reproductive rate is its most important advantage. It shows estrous activity throughout the year (Traoré et al., 2006). However, there is still little information concerning the course of postpartum period in goat of this breed. Moreover, the ultrasonic characterization of reproductive function in goat is very scanty in the literature.

The objectives of this study were to characterize uterine involution and early ovarian activity in West African Sahelian goats.

MATERIALS AND METHODS

This study was undertaken at the station of Ouagadougou University from November 2011 to May 2013. A total of 21 nulliparous West African Sahelian goats were used in this study. Their ages and weights at the beginning of the experiment ranged from 12 to 15 months and 25 to 35 kg, respectively. They were clinically free from any infectious, parasitic or genital diseases. During the study period, all goats were kept under uniform standard management practice and housed in the Agriculture Farm of Ouagadougou University. They were kept in a shed attached with an open yard. Each animal was given daily 1 kg concentrated ration, in addition to a green fodder. The goats were synchronized using a 12-days chronogest® CR treatment with intravaginal sponges containing 20 mg of flurogestone acetate (FGA, Intervet International B.V., Intervet Ireland Ltd, Iternet productions S.A., Rue de Lyons, France) (Baril et al., 1993).

They were mated with a fertile buck and examined for pregnancy one month later using a linear array ultrasound scanner. All does were confirmed to be pregnant and gave birth in October after normal gestation period and normal parturition. They were allowed to nurse their kids.

The ultrasonographic inspection was performed transrectally by a single operator employing the Chison Ultrasonic Scanner (Chison Medical Imagin Co. Ltd, 8300) equipped with a 5-MHz transducer. The doe was lightly restrained by one person against railing in standing position. One of the hind legs was folded up at the time of scanning for proper placement of the probe. An ultrasound coupling gel was applied each time to the probe to develop good contact and to remove air between probe and animal skin. Thereafter, the rectum was evacuated from feces and air with the aid of the lubricated fingers of the operator. The ultrasound probe fixed to an extension rod was inserted into the rectum. For scanning of the uterus and ovaries, the probe was moved approximately 60° to each side around its longitudinal axis. Uterine horns were scanned once every other day starting from day one PP until there was no further reduction in the uterine diameter for two successive weeks. Ovarian structures were scanned daily for the first PP week (Hayder and Ali, 2008). Parameters for the determination of the endpoint of uterine involution were the transversal diameter of uterine horns of ≤2 cm and the lack of contents in the uterine cavity (Hauser and Bosted, 2002).

All follicles with a diameter greater than 2 mm were sketched and the video image recorded in external disk to allow individual structure to be monitored. Parameters for the present investigation were the transversal cross-sectional diameter and number and size of follicles of the right and left ovaries. The interval from parturition to caruncules disappearance and their mean size were recorded by determining the diameters of three to five caruncles in each uterine horn. Data were presented as mean ± standard deviation and were analyzed using repeated-measures of ANOVA. Differences were considered to be statistically significant at $P \leq 0.05$

RESULTS AND DISCUSSION

This is the first study to provide baseline information on the use of real-time ultrasonography for the assessment of PP uterine involution and ovarian activity in West African Sahelian goats. All included does showed normal gestation period ranging from 145 to 157 days. In all cases, the delivery was uneventful, and placenta was expelled within 10 h after kidding. The kids started suckling within 1 h after kidding.

At the term of the gestation period, a total number of 26 kids were born with about 38.46% of twinning kids (5 animals delivered twin's kids). Average birth weights of male (n = 17) and female kids (n = 9) were 2.26 ± 0.7 and 2.32 ± 0.7 kg, respectively. Non-significance difference was found between male and female birth weights.

During transrectal scanning, the does showed no signs of distress apart from short avoidance behavior when the scanner passes through the anal sphincter.

The average PP uterine and caruncules diameters, as estimated ultrasonographicaly, at the different postpartum periods are shown in Table 1. The uterine diameter could not be estimated by day 0 in most of does, as it was too large to fit effectively on the screen. The readings were taken from day 1 onwards (Figure 1A).

The does showed a physiological regression of the uterus and caruncules with transversal diameters of 9.40 ± 0.71 and 1.78 ± 0.42 cm on day 1 PP decreasing to 1.72 ± 0.27 and 0.12 ± 0.13 cm on days 31 and 9 PP, respectively (Figure 1; A, B, C and D).

The mean time required for uterine complete involution was 22.44 ± 1.54 days (range 18 - 25 days) and was characterized by a small cross-sectional diameter (<2 cm)

Table 1. Uterine and caruncules mean (± SD) and range diameters (cm) of Sahelian goats on different days postpartum.

Days postpartum	Uterine horn diameter (cm)		Caruncules diameter (cm)	
	Mean ± SD	Range	Mean ± SD	Range
1	9.40 ± 0.71	8.50-11.0	1.78 ± 0.42	1.14-2.38
3	6.71 ± 1.53	5.67-8.47	1.34 ± 0.38	1.15-1.82
5	6.60 ± 1.40	4.6-8.10	0.86 ± 0.68	0-1.04
7	4.41 ± 2.40	2.70-6.10	0.59 ± 0.47	0-1.05
9	4.29 ± 1.26	2.11-6.27	0.12 ± 0.13	0-0.25
11	3.55 ± 0.86	2.27-4.39	-	-
13	3.18 ± 0.64	2.24-3.90	-	-
15	3.09 ± 0.36	2.60-3.40	-	-
17	2.91 ± 0.10	2.78-3.04	-	-
19	2.71 ± 0.12	2.53-2.81	-	-
21	2.21 ± 0.26	1.84-2.6	-	-
23	1.92 ± 0.15	1.66-2.05	-	-
25	1.87 ± 0.05	1.81-1.93	-	-
27	1.66 ± 0.07	1.61-1.72	-	-
29	1.71 ± 0.06	1.60-1.80	-	-
31	1.72 ± 0.27	1.50-1.90	-	-

Table 2. Early postpartum ovarian structure in West African Sahelian goat: Characteristics of follicular development during the first week postpartum.

Days postpartum	Mean number of follicles		Mean diameter (mm) ± SD	
	Right ovary	Left ovary	Right ovary	Left ovary
1	2	3	5.77 ± 1.51	7.05 ± 2.62
2	3	1	6.97 ± 1.7	5.88 ±1.04
3	3	2	7.56 ±1.97	6.69 ±1.85
4	3	3	4.95 ± 1.45	7.38 ±1.54
5	2	1	7.68 ± 0.59	8.25 ± 2.05
6	1	1	5.51 ± 0.93	5.44 ±1.13
7	2	1	8.57 ± 1.79	8.56 ± 0.66

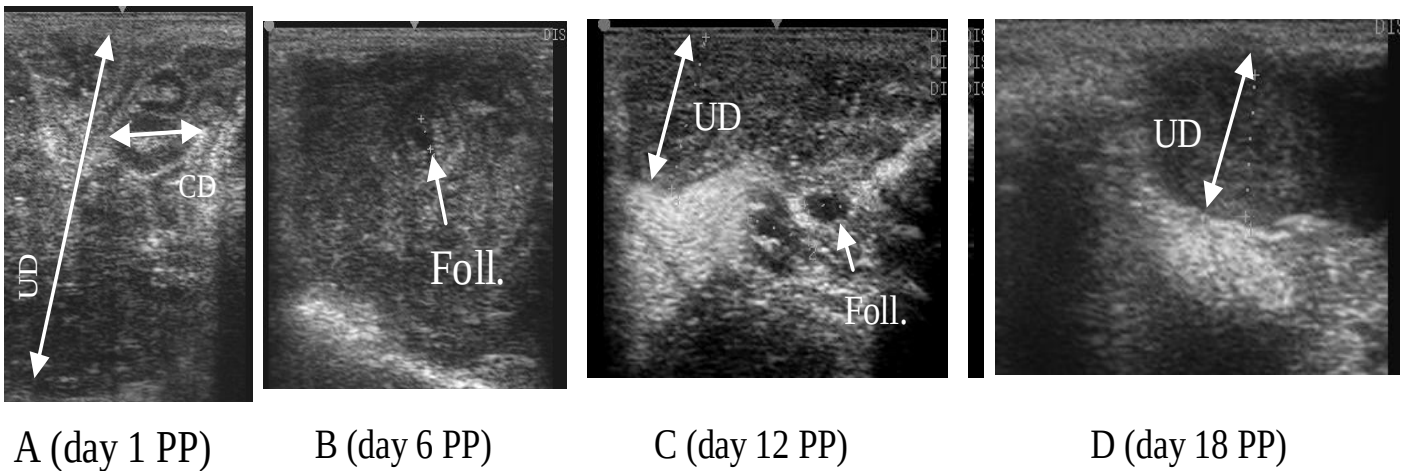

Figure 1. Ultrasonic images of uterine horns at different days postpartum. UD: Uterine diameter, CD: caruncule diameter, Foll.: follicle.

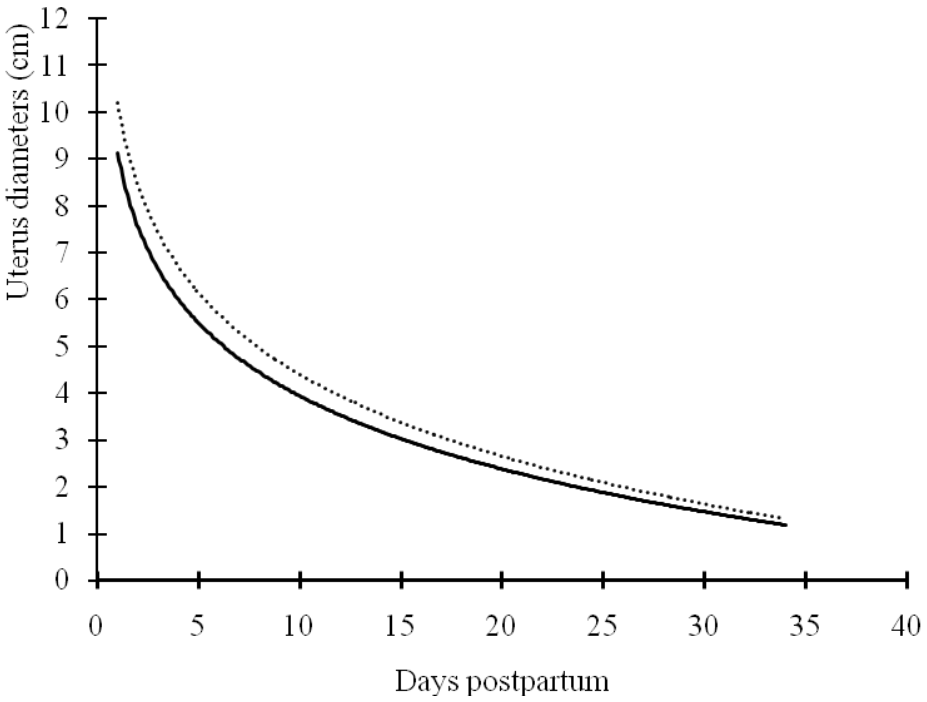

Figure 2. Profiles of uterine involution in does bearing single. (—, R^2 = 0.933) and twins (……, R^2 = 0.931) fetuses.

of uterine horns and absence of lochia in uterus (Figure1D).

These findings were in close agreement with previous reports on goats (Takayama et al., 2010; Ababneh and Degefa, 2005; Baru et al., 1983; Sanchez et al., 2002). In Jordan local balady goats, microscopic involution and complete regeneration of the caruncular epithelium was also evident by the same period (Degefa et al., 2006). However, the present findings were earlier compared to the reports of 28 days in Boer goats (Greyling, 2000; Greyling and van Niekerk, 1991).

In comparison with sheep, most studies reported slower involution and found that the uterine regression in ewes finished approximately after 30 days PP (Hayder and Ali, 2008; Rubianes and Ungerfeld, 1993), in contrast to other authors which observed the end of the uterine regression in sheep by day 17 PP (Hauser and Bostedt, 2002).

The variability regarding the time required for a complete uterine involution may result from differences in breed, reproductive status and methods. During the experiment, one goat (F96) lost her kid on day 1 PP and ended uterus involution 10 days later. She was mated on day 18 PP and was diagnosed pregnant one month later.

Another goat (F85) showed an abnormally higher uterine diameter on day 13 PP onward up to days 20 PP than the previous diameter with accumulated fluid within the lumen. These two goats were excluded from the general profile. The 19 other goats exhibited a very similar profile of uterine involution.

9/19 and 10/19 females completed uterine involution by the third (ranging from days 18 to 21) and fourth (ranging from days 22 to 25) weeks postpartum, respectively. The calculated rates of involution during the first, second and third PP weeks were 0.71, 0.17 and 0.14 cm/day, respectively. Furthermore, regression analysis showed a high positive correlation between diameters of the uteri in both goats and days PP (r = 0.98). The course of uterine regression was established on these data and was described by the logarithmic equation:

$y = -2.33\ln(x) + 9.43$, ($R^2 = 0.98$, $P < 0.05$)

where y = uterine cross sectional diameters (cm), x = postpartum period in days.

In ewes, Hauser and Bostedt (2002) described the course of uterine regression by exponential function as:

$Y = E + A*e^{-b*t}$.

The data revealed significantly faster reduction between the first and second weeks ($p < 0.01$) than between the second and third weeks ($P < 0.01$). This was evidenced by 67.43% of the total reduction in diameter recorded on day 7 postpartum. After day 21 PP, the reduction in size was not significant. At this point of time,

97.16% of the hypertrophy as a result of pregnancy was reduced.

The faster reduction of uterine diameter during the early postpartum has been reviewed previously and seem to be a response of myometrial contractility that plays a major role in clearing lochial debris from the uterus after parturition (Ababneh and Degefa, 2005; Hauser and Bostedt, 2002).

The statistical analysis showed significant (P = 0.02) higher values of the uterine diameter during the first week PP in females bearing twins fetuses than the females bearing single fetus. However, no significant difference was observed in uterine diameter during the second, third and fourth weeks between females bearing twins and females bearing single fetuses.

The females bearing single fetus (n = 14) showed a physiological regression of the uterus with a transversal diameter of 9.16 ± 0.48 cm on day 1 postpartum, decreasing to 2.16 ± 0.31 cm on day 21, while the does bearing twins fetuses showed a transversal diameter of 9.76 ± 0.79 cm on day 1 postpartum, decreased to 2.25 ± 0.08 cm on day 21 postpartum, and complete involution was delayed as the diameter of the uterine horn was reduced to 1.97 ± 0.06 cm on day 24 postpartum. The typical ultrasonographic patterns of uterine regression in does bearing single and twin fetuses were established as shown in Figure 2.

The uterine wall covered with caruncules and uterine lumen was readily identified by different ultrasonographic echotextures (Figure 1A). In does bearing single fetus, the caruncules were completely degenerated on an average by days 5 postpartum, whereas in the twins, they were delayed until day 9 postpartum. At those points of time, their echotextures appeared similar to the endometrium and ultrasonography differentiation and measurements were impossible to be carried out.

The regression of the caruncules in the first week PP was in good agreement with different studies (Hauser and Bosted, 2002; Rubianes and Ungerfeld, 1993; van Wyk et al., 1972). However, in Balady goat, complete regression of the caruncules was not completed until day 19 PP (Degefa et al., 2006). In addition, lochia was cleared as early as day 7 PP in primiparous goats.

Ovarian follicular dynamic of the experimental does during the early postpartum period (days 1 to 7 PP) are summarized in Table 2. In all does (n = 19), ovarian anatomical events during the early post-partum period (days 1 to 7) were similar. At least one follicle was examined on each ovary examined within 7 days after kidding (Figure 1C). The mean size of follicles ranged from 4.95 ± 1.45 to 8.56 ± 0.66 mm on both ovaries.

These findings were fairly in close corroboration with the result reported in Serrana goat (Simoes et al., 2006). In Jennies, Dadarwal et al. (2004) found three to seven follicles of size 10 to 15 mm diameter on one or both ovaries examined within 8 to 24 h after foaling. Those follicles reached to >25 mm in diameter on days 5 to 12 PP. In non-nursing Shiba goats, the first postpartum ovulation was observed between days 7 and 13 following parturition (Takayama et al., 2010). The early return to active follicular development in West African Sahelian goats demonstrated the ability of the ovary to resume activity early after kidding. In Shiba Goats, three to six follicles, whose diameters ranged from 1 to 7 mm, were observed throughout the cycle; a few follicles grew to more than 5 mm in diameter and most of them atrophied during the luteal phase (Orita et al., 2000). No significant differences (P ≥ 0.05) were detected between number and size of follicles between does bearing single and twin fetuses.

It could be concluded that, complete uterine involution in West African Sahelian goats occurred during the 3rd and fourth weeks post kidding, while the ovarian follicular dynamics started on the first week PP. The early return to active follicular development suggests that ovarian responsiveness may not be the major reason for the variable duration of the post-partum periods commonly observed in tropical goats. However, the period of ovarian observations may extend until standing estrus period to permit definitive conclusions about quality of follicular dynamic and ovulation in this breed. These findings should assist practitioners and Sahelians goat breeders to decide when goats should be bred following parturition.

In a future study, factors responsible for the shorter involution period in West Africa Sahelian goats should be investigated.

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Conflict of Interest

Authors have no conflict of interest.

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