Journal of

Veterinary Medicine and Animal Health

Volume 9 Number 5 May 2017
ISSN 2141-2529

Academic Journals

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Journal of Veterinary Medicine and Animal Health

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academicJournals

Vol. 9(5), pp. 78-82, May 2017
DOI: 10.5897/JVMAH11.011
Article Number: 554812A63860
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Tsetse density and bovine trypanosomosis in selected sites of Konta, Ethiopia

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Received 11 March, 2011: Accepted 26 September, 2016

A cross-sectional study was conducted in selected sites of Southern Nation, Nationalities and People Regional State (SNNPRS) of Konta, Ethiopia. The purposes of study were to determine the apparent density of tsetse flies and prevalence of bovine trypanosomosis. Ngu traps for entomological survey were deployed at grazing and watering points of animals in the village of Dolba (Kerara Peasants Association, PAS) and near Gojeb River. Assessment of tsetse indicated the presence of *Glossina pallidipes* with the apparent density 8.45% flies/trap/day. Other biting flies (tabanids) were also caught along with tsetse that transmits trypanosomes mechanically. For parasitological study, a total of 400 blood samples were collected from randomly selected animals and examined for the presence of trypanosomes and indicate overall prevalence of trypanosomosis in study cattle as 12%. The dominant trypanosome species were found *Trypanosoma conglonse* 29 (60.4%) followed by *Trypanosoma vivax* 14 (29.2%), *Trypanosoma bruci* 3 (6.25%) and mixed infection (*T. congolonse* and *T. vivax*) of 2 (4.2%). Based on these results it is concluded that trypanosomosis is a major constraint of livestock production in the study area.

Key words: Konta, Southern Nation, Nationalities and People Regional State (SNNPRS), Tsetse, Trypanosomosis, trypanosome, prevalence.

INTRODUCTION

Trypanosomosis is serious constraints to livestock production and agricultural development in Ethiopia. A total of 14.8 million cattle, 6.12 million sheep and goats, 1 million camels and 1.23 million equine are at risk of contracting trypanosomes (MoA, 1995). Trypanosomosis is a wide spread and economically important disease in human and animals (Sumbria and Singla, 2016). It is caused by protozoan parasites belonging to the family trypanosomatidae genus *Trypanosoma*, which inhibits the blood plasma, various bodies' fluids and tissue of the host (Singh and Singla, 2012). The species of *Trypanosoma* known to exist in Ethiopia, which are pathogenic to cattle and small ruminants, are: *Trypanosoma vivax*, *Trypanosoma congolense* and *Trypanosoma bruci*. They are distributed mainly in tsetse belt of the country (south west and southern parts) between 33˚and 38˚E and 5˚ to 12˚N. *T. vivax*, also found in area outside of the belt, where it can possibly

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transmitted by mechanical vectors of biting flies like tabanus and stomoxy (Eneyew, 1997).

The most important trypanosome species affecting cattle are: *T. congolense, T. vivax* and *T. brucei*, which are generally termed as Nagana (Jordan, 1986; Langridge, 1976) and *T. eqiperdum* for equines (Dagnachew and Shafo, 1981). In 1962, the cattle survey in southern Ethiopia, by the livestock division, established that bovine trypanosomosis was a major cattle disease in the Omo valley. It was stated that the problem of trypanosomosis is the main cause of decline in the number of cattle and particularly draught oxen (Abebe and Jobere, 1996).

Trypanosomosis depends on the distribution of the vectors, the virulence of parasite and the response of the host (Langridge, 1976). Tsetse flies are the major and true cyclical vectors. They are blood feeding insects that belongs to phylum arthropoda, class insecta family muscidae sub family glossinidae and genus *Glossina*. Today five species of glossina have been identified in Ethiopia: *Glossina submorsitans* and *Glossina palldipus* (savanna groups), *Glossina fuscipes* and *Glossina tacnoidus* (Riverine groups) and *Glossina longipennis* (Langridge, 1976). In the area of tsetse transmitted trypanosomosis which affects cattle production, trypanocidal drugs, both prophylactic and curative drugs and other tsetse control methods such as insecticide application on the back of animals are the most widely used methods of trypanosomosis control.

Trypanosomosis is the most serious disease of cattle, which cause great socio-economic losses in SNNPR. The socio-economic impact of trypanosomosis is reflected on direct losses such as mortality, morbidity, reduction in milk and meat production, and still birth (Leak et al., 1993). Thus, trypanosomosis is one of the causes for food in security in the Omo-Ghibe belt as well as the tributaries of Gojeb Rivers, where wide grazing land which favors animal production is located. Therefore, the objectives of the current study were: To assess the apparent density and distribution of tsetse and other biting flies and prevalence of bovine trypanosomosis Konta special districts.

MATERIALS AND METHODS

Study area

The present study was conducted in 5 selected peasant association (PAs) namely Biteti, Chida, Kecharoba, Kerara, and Mareka in Konta special woreda of SNNPRS which is located 498 km south of Addis Ababa. The area has humid, sub-humid climate with a moderately hot temperature and reliable annual average rain fall of 1200 mm. The annual temperature and altitude ranges from 12 to 24°C, 1062 (Gojeb river) to 1542 (Kerara PAs) m.a.s.l. respectively. Livestock species are cattle, sheep, goats, mule and donkey. The predominant species in the area is cattle which are estimated at 20,985 in the study area 5 PAs. Livestock management system is mixed farming system. The animals in the area mainly depend on communal grazing fields and watering points are at Gojeb River and its tributaries (Konta Woreda Agricultural Office).

Study population and design

Cross-sectional survey was conducted from January 2009 to March 2010 on indigenous cattle. They were kept under traditional extensive husbandry system with communal herding and watering in small and big rivers.

Sampling method and sample size determination

The sampling method applied in the present study was a simple random sampling and the sample size was approximated by using formula given in Thrusfield (1995) using 95% confidence interval and expected prevalence of 50%.

$$n = \frac{1.96^2 \text{ (Pex (1-Pex))}}{d^2}$$

Where, n = sample size; Pexp = expected prevalence; d^2 = desired absolute precision.

Accordingly, the estimated sample size was 384 animals; however, to increase the precision 16 cattle were added and a total of 400 cattle were sampled.

Entomological survey

Tsetse population and other flies were sampled using NGU traps deployed for 24 h at two sites and baited with acetone and 3 weeks old cow urine (Brightwell et al., 1992). All odors were placed on the ground about 15 cm up wind of the traps. The traps polls were greased to avoid the entry of insect predators like ants. Fly challenge has been taken as the product of the relative density of tsetse flies, their trypanosome infection rate and the proportion of feed that they have taken from domestic livestock. Site selection was done to include suitable tsetse habitats like savannah area, river valley, livestock grazing area and watering points and vicinity to assumed wild game reserve areas.

Parasitological survey

Blood samples were collected randomly from ear vein by using sterile blood lancet and capillary tubes. A capillary tube were filled with blood from animals to ¾ of their height and sealed at one end with crystal seal. The capillary tubes were loaded on the microhaematocrit centrifuge symmetrically and centrifuge at 1200 rpm for 5 min (Murray et al., 1977). Packed cell volume (PCV) was determined using haematocrit reader (Woo, 1969). After the PCV was read, capillary tubes were broken 1 mm below the buffy coat to include the RBC layer and the content were expressed on microscopic slide and mixed and cover with a 22 × 22 mm cover slip and examined under 40x objectives (Murray et al., 1977). From positive samples thin blood smears were made, fixed with methanol for 5 min and stained with geimsa solution for 30 min and examined using oil emersion under 100 x objectives to identify the species of trypanosomes.

Data management and analysis

Data collected from vector fly and trypanosomosis infection survey were entered into Microsoft excel spread sheet and analyzed using

Table 1. Tsetse flies and tabanides catches during the study period.

Altitude (m.a.s.l)	Traps deployed site description	No. of traps	Tsetse spp. flies/trap/day	Biting flies spp. flies/trap/day		
			G. pallideps	Tabanus	Stomoxys	Haematopota
1062	Drainage lines of the Gojeb river*	8	9.5	0.5	1.875	0.375
1546	bush**, acacia and short grass	8	7.4	0.125	0.875	0.25

*, Trap positioned at watering; **, trap positioned at grazing area.

Table 2. Species of trypanosomosis involved in disease process in each PAs.

Pas	Animals		Trypanosomosis species				Prevalence (%)
	Examined	Infected	T.c	T.v	T.b	Mixed	
Biteti	84	10	5	5	0	0	11.9
Kecharoba	76	11	5 (45.5%)	3 (27.3%)	2 (18.2%)	1 (9.0%)	14.5
Kerara	80	7	3 (42.8%)	2 (28.6%)	1 (14.3%)	1 (14.3%)	8.75
Chida	81	12	9 (75.0%)	3 (25.0%)	0	0	14.8
Mareka	79	8	6(75.0%)	2 (25.0%)	0	0	10.1

T.c, Trypanosoma congolense; T.v, Trypanosoma vivax; T.b, Trypanosoma brucei.

statically software program SPSS. Descriptive statics like percentage can be used to determine prevalence of trypanosomosis and chi- square (χ^2) used to evaluate the associations with different risk factors. In the analysis, confidence level was held at 95% and p<0.05 was set for significance.

RESULTS

Hematological findings

Considering anemia as one of the major signs of a herd infected with trypanosomosis, the anemic status of sampled animals was assessed by measuring the mean packed cell volume. The range of PCV value in parasitaemic was 12 to 30% and aparasitaemic 18 to 40%. Out of total 48 trypanosome positive cattle, 87.5% had PCV value was less than 26 and 12.5% of positive were found having normal PCV (Table 4).

Entomological survey

Tsetse flies found during the survey were only *G. pallidipes*. This species were registered at suspected fly habitat of kerara PAs and around Gojeb River which is watering points for farmers near and away from the river. Biting flies like tabanides also caught along with tsetse flies. The mean catch of *G. pallidipes* at kerara PAs was 7.4 flies/trap/day whereas around Gojeb River was 9.5 flies/trap/day and an overall apparent density was 8.45 flies /trap/day. A total of 167 flies were caught out of which *G. pallidipes* accounted 80.8% and tabanide accounted 13.2%. *G. pallidipes* were abundant at high gallery forest and valley flanks along the drainage lines of the river which is 59.3% whereas 43.7% flies were caught at Kerara PAs which tsetse are more suspected habitat were the vegetation dominated by thorny bush, short grass and dispersed acacia species (Table 1).

Parasitological survey

Overall trypanosome prevalence cattle in the 5 PAs were 12%. The dominant trypanosome species was *T. congolense* (60.8%) followed by *T. bruci* 3 (6.25%), *T. vivax* (29.17%) and mixed infection (*T. bruci* and *T. vivax*) 4.2% (Table 2). Almost similar prevalence of trypanosomosis was registered at the different study sites Table 3.

DISCUSSION

The disease trypanosomosis is the main important livestock constraint impeding the agricultural activity and livestock productivity. It was stated that trypanosomosis is the main cause of decline in the number of cattle and particularly draught oxen (Abebe and Jobre, 1996). The overall trypanosome prevalence in study area was 12% (Table 3). This result was comparable with works different scholars: Twelde (2001) who reports (15%) at Keto settlement area south western Ethiopia; Habtewold (1993 and 1995) at Humbo larena of Wolaita zone (9.3%) and at Konso wereda (11.5%). But, slightly lower than the finding of Afework (1998) at Pawe, North-west Ethiopia (17.2%), Abebe and Jobere (1996) for tsetse infested

Table 3. Prevalence of trypanosomosis in selected PAs of Konta special Woreda.

Name of surveyed PAS	No. of samples	Total positive	Prevalence (%)
Biteti	84	10	11.9
Kecharoba	76	11	14.4
Kerara	80	7	8.75
Chida	81	12	14.8
Mareka	79	8	10.12
Total	400	48	12

Table 4. Hematological findings.

PAs	Sample size	Mean PCV value (%)	% Cattle with PCV<26	% Cattle with PCV ≥ 26	Range
Biteti	84	24.96	48.81	51.19	14-36
Kecharoba	76	24.75	56.58	43.42	12-38
Kerara	80	24.33	62.50	37.50	17-40
Chida	81	25.90	29.63	70.37	13-37
Mareka	79	27.97	26.57	73.42	18-38

area of Ethiopia (17.67%). The study result also revealed that majority of infection (60.4%) were due to *T. congolense* a comparable results was reported by different researches who reported *T. congolense* in different parts of Ethiopia: Muturi (1999) at Merab Abaya, south west Ethiopia (66.1%), Afework et al., (2001) at Pawe, North west Ethiopia (60.9%) and Abebe and Jobre (1996) for tsetse infested area of Ethiopia (58.5%).

The results of present study showed an overall apparent density of *G. pllidipes* 8.45 flies/trap/day (Table 1) which was greater density than 1.9 and 1.0 in the late and dry season, respectively, reported by Masangi (1999) in the south rift valley of Ethiopia. This could suggest an absolute increase in the number of tsetse flies due to favorable environment has enough moisture, vegetation growth and suitable habitat or spread of flies from the rivers where they usually inhibit during day season to more open areas there by increasing relative density.

The present findings indicated that the tsetse fly distribution along the two altitudinal level (1062 m.a.s.l) was different from another (1546 m.a.s.l) irrespective of the areas indicating that the catch was decreasing with the increasing altitude (Table 1). This was in agreement with the previous work done in Ethiopia by Verysen et al*.,* (1999) who found a significant high catch (over 93%) in altitude between 1100 and 1400 m.a.s.l level in the southern rift valley of Ethiopia.

Present finding reported 1.5% of the parasitaemic and 53.75% of the aparasitaemic animals PCV values was greater than or equal to 26% this result was in agree well with the result obtained by Rowland's et al. (2001) at Ghibe valley in the south western Ethiopia, in which he indicated that as the proportion of samples detected parasitaemic increased, PCV value decreased.

CONCLUSION AND RECOMMENDATIONS

The results of the present study revealed that trypanosomosis is the most important problem for agricultural activity and animal production at study area of Konta special wereda in SNNPRS. One species tsetse flies *G. pallidipes* and biting flies such as tabanids were caught in the study area. Based on the conclusions made above the following recommendations are forwarded:

1. Designing and implementing of control strategies of trypanosomosis focusing on applying integrated approach (vector control and chemotherapy).
2. Further studies on the epidemiological aspects and development of drug resistance in pathogenic trypanosome are required.
3. Awareness creation about the disease and its transmission.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 9(5), pp. 83-87, May 2017
DOI: 10.5897/JVMAH2012.028
Article Number: D72FCB563862
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Gastrointestinal nematodes of small ruminants in Guto Gida District, East Wolloega, Ethiopia

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Received 31 May, 2012; Accepted 15 August, 2016

A study by qualitative fecal examination of 384 fecal samples (201 sheep and 183 goats) was conducted from November 2011 to April 2012 with the objective to determine the major gastrointestinal (GIT) nematodes of small ruminants and their prevalence in sheep and goats in Guto Gida District. The study showed that 186 (92.5%) sheep and 150 (81.97%) goats were found to harbor eggs of GIT nematodes. Both sheep and goats were infected with identical parasites species, but with different level of infection. The six genera of nematodes were identified with prevalence of 21.87, 14.87, 12.5, 10.67, 11.19 and 7.29% for *Haemonchus, Trichostrongylus, Trichuris, Oesophagostomum, Bunostumum* and *Strongloides*, respectively. There was a significant difference (p<0.05) in the prevalence of GIT nematodes between sex and species of animals but not for different age group. The study showed that GIT nematodes are major problems of small ruminants in the study area. Therefore, comprehensive study on GIT nematodes, cost effective control strategy and awareness creation to the farmers should be instituted in the area.

Key words: Sheep, goats gastrointestinal nematodes, Guto Gida District, Nekemte.

INTRODUCTION

In Ethiopia, helminth infections in ruminants are characteristically chronic and insidious in nature. The parasites attract very little attention, including research funds, when compared with viral, bacterial and some protozoan diseases. This is despite of the fact that they undoubtedly exert a heavy toll on the health and productivity of a vitally important livestock resource with obvious implications for the rural and national economy of the country. Gastro intestinal parasites are a worldwide problem for small and large scale farmers, and their impact is major for sub-Saharan Africa in general and Ethiopia in particular. This is due to the range of agro-ecological factors suitable for diversified host and parasite species (Regassa et al., 2006). Endoparasites are responsible for the deaths of one third of calves, lambs and goat kids, and considerable production losses due to parts of carcasses, being condemned during meat inspection. It is well recognized that in a resource poor country, helminth infections of sheep and goats are factor responsible for economic losses through reductions in productivity (Abunna et al., 2009, Abouzeid et al., 2010). Although helminth parasites of small ruminants are ubiquitous in the climatic zones of Ethiopia where prevailing weather provides favorable conditions for their

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survival and development, their presence does not mean that they necessarily cause overt disease (Abunna et al., 2009).

Among the diseases that constrain the survival and productivity of sheep and goats, gastrointestinal nematode infection rank highest on the global scales, with *Haemonchus contortus* recognized as a major parasite for both small and large scale small ruminant production (Abunna et al., 2009). These disease have major impact on morbidity and mortality rates with annual losses as high as 30 to 50% of the total value of livestock production of Ethiopia (Abunna et al., 2009). With little inputs, sheep and goats play an important role in rural economy through the provision of meat, milk, blood and cash, accumulating capital, fulfilling cultural obligations, manure and contribution to the national economy through export of live animals meat and skins (Abunna et al., 2009).

The prevalence of gastro intestinal parasite, the genera of helminthes parasites involved species and the severity of infection also vary considerably depending on local environmental and managements practices (Singla 1995; Taylor et al., 2007). Therefore, the distribution and prevalence of the disease should be represented by geographical areas that could roughly correspond to climatic conditions (Regassa et al., 2006). These, information are important in the formulation of parasites control strategies since the degree of infection varies according to the parasites involved and other factors (Urquhart et al., 1996; Wadhawa et al., 2011). Thus, the study was designed to determine the prevalence the major GIT nematodes of sheep and goat in Guto Gida Woreda to recommend the most effective control strategies.

MATERIALS AND METHODS

The study was conducted from November 2011 to April 2012 in Guto Gida District, East Wollega zone, Oromia regional state. It is located in western part of Oromia at latitude of 09° 04′; 957″N, longitude of 36°, 32′, 928″ E and altitude of 2124 m above sea level. It is located at 331 km far from our capital city Addis Ababa. It is annual rainfall range from 1800 to 2200 mm. The maximum temperature is 25°C and the minimum temperature of the area is about 20°C. The area receives long rainy season from June to September and short rainy season from March to May. The area is rich in natural vegetation that comprised of tropical rain forest trees, all grasses and brushes (data obtained from agricultural office).

Study animals and design

A cross-sectional study was conducted on small ruminants of all age and sex category to determine the prevalence of nematodes parasites by collecting fecal samples from individual grazing animals.

Sample size determination

Simple random sampling strategy was followed to collect feces from individual animals. The sample size was decided based on the formula described by Thrusfield (2005) with 95% confidence interval at 5% desired absolute precision and by assuming the expected prevalence of 50%. The estimated sample size was calculated by the formula:

$$N = \frac{1.96^2 \, Pexp\ (1\text{-}pexp)}{d^2} = 384$$

Where N = required sample size; pexp = expected prevalence; d = desired absolute precision.

Parasitological examination

Laboratory diagnosis using flotation, fecal culture and Baermann technique conducted for the identification of the parasite.

Collection of eggs

Faecal pellets collected from the rectum of sheep and goats were placed in small vial. Warm water was slowly added to the faeces and the pellets stirred until a relatively uniform homogenate was obtained and liquid suspension was obtained. The suspension was filtered through sieve with 3 mm aperture. The resulting suspension was again made to pass through a sieve of 150 µm pore size. The suspension was then poured into 15 ml test tubes and centrifuged for 2 min at 377 g and the supernatant was decanted. The tube was agitated by vortex mixer to loosen the sediment. Saturated sodium chloride was then added to the test tube until the meniscus forms above the test tube on which the cover slip was placed. After 3 to 5 min, the cover slip was carefully taken off the tube and put into microscope slide for observation (Bowman, 1999).

Fecal culturing

The method of incubating fecal samples at room temperature to hatch egg to larvae and development to L3 was followed, so that the larvae hatched can be indentified according to the criteria listed as follows: Taking certain amount of feces in a tray; incubating it under suitable moisture content for 14 to 21 days with continuous moistening at an interval of 3 days. The recovery larvae (L3) were studied and identified and the criteria used for identification were based on shape of larvae, head, number and shapes of gut cells, presence or absence of retractile bodies, larvae sheath coverage and length of sheath tail. Then L3 were harvested using Baerman apparatus after 14 days of incubation and were identified (Annon, 2005).

Data management and analysis

The collected sample was entered into Microsoft excel and analyzed using statistical software packages for social science (SPSS). Descriptive statistics like percentage can be used to determine prevalence of GIT nematode and Chi-square (χ^2) used to check the association between prevalence of GIT nematodes and risk factors. In the analysis, confidence level was held at 95% and $p<0.05$ was set for significance.

RESULTS

The overall prevalence of gastro intestinal nematodes

Table 1. Overall prevalence of gastro intestinal nematodes of sheep and goats.

Species	No. of examined animals	No. of positives	Prevalence (%)
Sheep	201	186	92.5
Caprine	183	150	81.79
Total	384	336	87.5

Chi(χ^2) =9.789; Pr = 0.02.

Table 2. Prevalence of gastro intestinal nematodes in sex.

Sex	No. of examined animals	Sex		No. of positive male and female sheep and goats	
		Male	Female	Male	Female
Sheep	201	103	98		
Caprine	183	89	94	161 (83.86%)	175 (91.14)
Total	384	192	192		

Pearson Chi^2(χ^2) = 4.6667; Pr = 0.031.

infection was 87.5% and the species wise is 92.5 and 81.97% in sheep and goats, respectively, which was a significant difference in infection rate between the two animal species (Table 1). Prevalence of gastro intestinal nematodes of infection of sheep and goats on the basis of sex is shown in Table 2. There is statistical significant difference (P<0.05) in prevalence of gastro intestinal nematodes which was 175 (91.15%) and 161 (83.85%) in female and male, respectively. In this study species, sex and age were considered as a risk factors and revealed significant difference (p<0.05) with variation in species, and sex of animals while in the case of age, there is no significant difference (p>0.05) in sheep and goats but there is higher number of young sheep and goats infected than old age group.

In this study, 6 types of gastro intestinal nematodes were identified during the study period based on their morphology described (Urquart et al., 1996). During the study period, fecal samples were cultured to determine genera prevalent as shown in Table 4. *Haemonchus* (21.87%) were the most frequently identified helminthes followed by *Trichostrongylus, Trichuris* with *Strongloides* been the least (7.29%) identified in the study.

DISCUSSION

In the present study, the prevalence of gastro intestinal nematodes was 87.5% (Table 1) in sheep and goats. This result coincides with the result of Gebreyesus (1986) (96.38%) at Ogaden range lands; Esayas (1988) (90.41 and 82.13%) in sheep and goats in and around Wolayita soddo; Tesfalem (1989) (88.1 and 84.32%) in sheep and goats in and around Mekelle; Melkamu (1991) (91.435%) in sheep in and around kombolcha; Bayou (1992) (90.94 and 94.855%) in sheep and goats of Gonder; Yoseph (1993) (92.23 and 94.1%) in sheep and goats in Mendayo district of Bale; Genene (1994) (93.22 and 92.24%) in sheep and goats of four Awarajas of Eastern shoa; Getachew (1998) (90.23 and 88.3%) in sheep and goats of Buno province of Iluababor; Tefera et al. (2009) (91.32 and 93.295%) in sheep and goats in and around Bedelle, respectively.

Statistically significant difference (p<0.05) (Table 1) was recorded between sheep and goats with relative lower number of goats infected than sheep which may be due to different habits of grazing by these two species of animals. This study also indicates significant different was observed between sex of animals and females are more infected than male (Table 2). Thus, pregnant or lactating ewes/does become the major source of infection for the new born. In some manner, other studies in Africa have shown that the age and immune status of the host animals have significant influences on the GIT eggs pus (Magona and Musis, 2002). On the contrary, there was no statistical significant difference (p>0.05) recorded in this study between young and adult but relatively higher number of young animals are infected than older once (Table 3). This could be due to equal exposure of both age groups of animals and they are from similar agro-ecological area.

The prevalence of *Haemonchus* species was 21. 87% (Table 4) in sheep and goats in the study area which disagreed with the work of different scholars: Ahmed (1988) (88.23% prevalence in East Wollega zone); Kumsa and Wossene (2006) (95.1% in ogaden region); Naod et al. (2006) (81.18% in Awassa in Hawassa in sheep and goats). The prevalence of *Trichostrongylus* species was 14.8% in sheep and goats. This result is disagreed with Abunna et al. (2009) who reported prevalence of 90.45% in sheep and goats; Tefera et al. (2009) (43.5 and 55% in sheep and goats in and around

Table 3. Prevalence of gastro intestinal nematodes in different age groups.

Age	Results	
	Positive (%)	Negative (%)
Adult	157 (86.74)	24 (13.26)
Young	179 (88.18)	24 (11.82)

Pearson Chi^2(χ^2) = 0.1807; Pr = 0.671.

Table 4. Relative percentage of parasite identified in sheep and goats.

Species of parasites	Frequency	Percentage
Haemonchus species	84	21.87
Trichuris species	48	412.5
*Trichostrongylu*s species	57	14.8
Oesophagostomum species	41	10.67
Bunostomum species	32	8.33
Strongloides species	28	7.29

Bedelle). But, this study is consistent with the finding reported by Sissay et al. (2007), where *Haemonchus contrortus* was the most prevalent parasites of GIN; this could be associated partly with breed susceptibility, biological plasticity and high environmental adaptability.

The prevalence of *Strongloides* species was detected to be 7.49% in sheep and goats in the study area and this is in line with the work of Tefera et al. (2009) (13.04%) in sheep and 20% in goats in Bedelle and the prevalence of *Bunostomum* species was detected to be 8.33% in sheep and goats in the study area and this was in disagreement Tefera et al. (2009) (26.1 and 35% in sheep and goats in and around Bedelle). This difference in the prevalence of parasite in different area might be attributed to the difference in breed of sheep and goats and different agro-ecological zones where the animals are kept.

Conclusion and recommendation

The result of present study showed the high prevalence rate of gastro intestinal nematodes infection in small ruminants in Guto Gida District, East Wolloega. There was highly significant difference in infection level on the basis of sex and species. This shows that the general scientific facts on those bases can be recognized in the study area and the traditional husbandry and animal management have also valuable contribution in the detection of gastro intestinal nematodes. The existence of these parasites has an impact on the productivity and can hamper the sustainability of the revenue generated from small ruminant production by small holder society of East Wollega zone. Therefore, based on the result obtained the following recommendation are forwarded:

1. To get clear epidemiological picture of GIN parasites, comprehensive study should be launched in the area.
2. Effective utilization of available of feed resource such as agricultural by products, natural pasture and appropriate nutritional supplements program for young and lactating animals.
3. Detail study should be conducted to identify the parasite using more sensitive and specific methods such as postmortem examination.
4. Strategic nematode control practice should be implemented.
5. Field veterinarian should be aware of the importance and burden of GIN in sheep and goats.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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academicJournals

Vol. 9(5), pp. 88-91, May 2017
DOI: 10.5897/JVMAH2012.030
Article Number: 899D1C963864
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Gastrointestinal nematodes of donkeys and horses in Gondar town northwest, Ethiopia

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Received 30 December, 2012: Accepted 29 August, 2016

A study on the prevalence of gastrointestinal (GI) nematodes of equines was conducted from November 2011 to April 2012 in Gondar town to determine the prevalence and associated risk factors. A total of 103 horses and 281 donkeys were examined coprologicaly for the presence of GI nematode eggs using flotation techniques. Coprological result indicated horses and donkey harbor one or more nematodes infection in the total prevalence rate of 89.32% (92) and 98.22% (276) respectively. The two genera of parasite commonly encounter during the study period were strongyle type of egg (84.47 and 98.22%) and *Parascaris equorum* (43.69 and 23.35%) of horses and donkeys, respectively. Mixed infections were detected with prevalence of 76.9 and 40.8% in horses and donkeys, respectively. There was significant (P<0.05) difference in prevalence of strongyle type of eggs and *P. equorum* between species. Body condition is one factor which shows significant different in the numbers of nematodes eggs both in donkeys and horses as both the species with poor body condition harbor more parasitic egg than good body condition. Because of their importance and impact of disease on the use of equine a compressive study for strategic parasitic control measures should be implemented.

Key words: Gastrointestinal (GIT), nematodes, donkey, horse, Gondar, Ethiopia.

INTRODUCTION

Ethiopia has the largest livestock population of Africa country with estimated 35 million tropical livestock unit. This includes 2.03 million horses, and 7.43 million donkeys (CSA, 2015). According to CTA (1997) and Tegegne et al. (1999), 50% equine population of Africa is still found in Ethiopia. Equine play an important role in rural communities providing power and transport at low cost they can be used for various agricultural operations and also provide the much needed transport in rural areas for activities such as carrying water, building materials, agricultural products and people. Horses and mules are faster and more powerful animals for work but, on which is more costly to buy and maintain than a donkey (Pearson et al., 2003).

Despite its huge population size, equine remains marginal due to high prevalence of malnutrition, management constraints and disease like parasite. Parasitism represents a major obstacle to development of the livestock farming system in the country (Jobre et al., 1991) and characterized by high morbidity and mortality rates are to be mentioned.

Gastrointestinal nematodes are serious health hazards, contributing to poor body condition, reduced power output, poor productive performance and short life span

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(Pandey et al., 1994). Similarly, horses, donkeys and other equines is host for large numbers of internal parasites including blood protozoan parasites (Sumbria et al., 2014; Sumbria et al., 2015a, b; Sumbria et al., 2016). The vitality and other well-being of all ages are threaded by variety of internal parasites and the use of control measures ensures vigor and the best performance. The most common internal parasites are strongyles, *Parascaris*, pin worms (*Oxyuris equi*), and bots. Additionally, less important parasitic infection belongs to cestodes, lung worms, trematodes and intestinal thread worms (*Strongyloides*) (Powel and David, 1992; Hendrix and Charles, 2006). Despite the huge numbers and the increasing importance of equines in the Ethiopian economy, knowledge about the health problems affecting their welfare is limited for most parts of the country more over no previous study done on equine nematodes in and around Gondar town. Hence, it is necessary to examine the status and impact of these diseases and existing control measures (William and Masiga, 1998). Therefore, the objective of this study was to determine the prevalence of horses and donkeys gastrointestinal nematodes in the study area.

MATERIALS AND METHODS

Study area

The study was conducted in Gondar town and located 727 km North West of Addis Ababa. North Gondar zone located at an altitude ranging 1500 to 3500 m.a.s.l.. Numerous mountains, plateaus, hilly and sloppy areas, rivers, streams and lakes mark the topography of the zone. This zone is divided in to sub humid (“Wurch and Dega”), semi-arid (“Woynadega”) and arid (“Kola”). The Gondar town is located at latitude of 12° 40’N and longitude of 27° 25’E with “Weyenadega” climate zone. The average minimum and maximum daily temperature of the area varies between 22, 30.7 and 12.3°C, respectively. The region receives a bimodal rain fall, the average annual precipitation rate being 1000mm. the short rains occur during the months of March, April and May while the long rains extend from June to September. The production system observed in the area combines mixed crop livestock production type. This zone had a livestock population of, 2.03 million cattle, 0.6 million sheep, 0.54 million goats, 0.25 million equine species and 1.9 million poultry (CSA, 2015).

Study population

The study animals were equines of two species (donkeys and horses) in Gondar town. All donkeys and horses were considered irrespective of age, sex and body condition score. These age classes were based on age of first work, productive age and the life span of Ethiopian donkeys (Svendsen, 1997). Donkeys were also grouped into different body condition score according to Svendsen (1997).

Study design

A cross-sectional study was carried out from November, 2011 to April, 2012 by collecting data on events associated with gastrointestinal nematodes of equines that are found in Gondar town.

Sample size determination

Simple random sampling strategy was followed to collect feces from individual animals. The sample size was decided based on the formula described by Thrusfield (2005) with 95% confidence interval at 5% desired absolute precision and by assuming the expected prevalence of 50%. The estimated sample size was calculated by the formula.:

$$N = \frac{1.96^2 \, Pexp\ (1\text{-}pexp)}{d^2} = 384$$

Where N = required sample size; Pexp = expected prevalence; d = desired absolute precision.

Methodology

Fecal sample was collected directly from the rectum of each animal using glove/freshly defecated feces with strict sanitation for each species, age and sex group and the sample placed in air and water tight vials and taken to the University of Gondar Veterinary Parasitology Laboratory. In the laboratory the sample was subjected to flotation technique and for identification of GIT nematodes egg of equines.

Parasitological examination

Saturated saline flotation technique

From collected sample, 3 g of fecal sample weighted and put into universal bottle and 42 ml of floatation fluid (NaCl) was added then mixed thoroughly. The presence of egg was appreciated using cover slip and test tube method (Urquhart et al., 1996).

Data management and analysis

The collected sample was entered into Microsoft excel and was analyzed using statistical software packages for social science (SPSS). Descriptive statics like percentage was used to determine the prevalence of GIT nematode and chi- square (χ^2) was used to look into the association of between prevalence of GIT nematodes and risk factors. In the analysis, confidence level was held at 95% and p<0.05 was set for significance.

RESULTS

The overall prevalence of GI nematodes was found as 368 (95.83%) as shown in Table 1. As indicated in Table 2 the prevalence of gastrointestinal nematodes on the basis of species shows 92(89.32%) and 276(98.22%) in horses and donkeys respectively. Out of the recorded eggs, 363(94.53%) was strongyle-type and 105(27.34%) of *P. equorum* eggs was recorded nematode eggs. The study also shows the prevalence rate of parasitic infection in different body condition of the animals. The result indicated the highest infection rate was recorded in poor body condition animals which was 99.07% but

Table 1. The overall prevalence of gastrointestinal nematode parasites.

No. of animals examined	No. % of positive animals	Prevalence
384	368	95.83%

Table 2. The prevalence of nematode parasites eggs on the basis of species and the type of nematode eggs.

Species	No. examined	No. of positive animals	Strongly type egg	*Parascaris equorum* egg
Horse	103	92(89.32%)	87(84.47%)	45(43.69%)
Donkey	281	276(98.22%)	276(98.22%)	60(21.35%)
Total	384	368(95.83%)	363(94.53%)	105(27.34%)

Pearson chi^2 (1) =14.9524; Pr=0.001.

Table 3. Prevalence of gastrointestinal nematode parasites on the basis of body condition.

Body condition score	No. of examined animas	No. of positive animals
Good	67	54(80.6%)
Medium	210	208(99.05%)
Poor	107	106(99.07%)
Total	384	368(95.83%)

Pearson chi^2(2) =47.1848; Pr=0.001.

medium and good body condition animals were relatively resistant for the nematode infection and the result was statically significant P>0.05 (Table 3)

The study indicate horses and donkeys were harboring one or mixed infection for strongyle and *P. equorum* parasite egg with the most frequently identified parasitic egg of strongyle type followed by *P. equorum* and there was significant difference in the prevalence rates of *P. equorum* and strongyle type of egg. Out of the total 103 donkey examined, 42(40.8%) were infected with strongyle type and *P. equorum* while out of the 281 horse examined, 216(76.9%) harbor both strongyle type and *P. equorum* egg type as described in Table 4.

DISCUSSION

Gastrointestinal nematode infection is the main disease condition affecting donkeys and horses in tropical and sub-tropical areas of the world (Svendsen, 1991). In the present study, microscopic fecal examination showed that the GI nematode parasite was an important health problem in the study area with over all prevalence rates of 95.83% which was 89.32 and 98.22%% in horse and donkey, respectively (Table 2). This is not much less than with the report by Tegegne et al. (1999) who indicated that no animal was found free out of 1340 examined from Adami Tulu, Ethiopia.

In the present study, the prevalence of strongyle species was 84.47 and 98.22% in horses and donkeys, respectively (Table 2) which was lower than those reported by Ayele et al. (2005) and Mulate (2005) who reported 100% prevalence in donkeys at Dugda Bora District and high land of Wollo provinces, respectively and Getachew et al. (2010) who reported 99% prevalence in working donkeys of Ethiopia. The prevalence was higher in the present study than the report of Saeed et al. (2010) and Seid (2011) who reported 65.51% on horses and 65.1% on both horses and donkeys, respectively. This difference may be due to ecological difference of those areas and deworming of animals when they went to the clinic for other bacterial diseases in the study areas.

In the present study, the prevalence of *P. equorum* was 43.69 and 21.35% in horses and donkeys respectively (Table 2). Similar report was generated by Ayele et al. (2005) at Dugda Bora District, Ethiopia. This is in agreement with the 34% prevalence reported by Tegegne et al. (1999) at Adami Tulu on donkey and also 32.5% prevalence reported by Ayele and Dinka. This might be due to the fact that horses were more frequently used in cart; also, they do not have enough time to graze on field only often in evening for one to two hours whereas donkeys have more time to graze on field. In this study age, and sex wise prevalence was not assessed because of the horses and donkeys were all adults and males that

Table 4. Prevalence of mix infection of GIT nematode based on egg type.

Species	No. of examined animals	No. of positive	Strongyle and *Parascaris equorum* mix found
Donkey	103	92(89.32%)	42 (40.8%)
Horse	281	276(98.22%)	216 (76.9%)

found in Gondar town working on cart and carrying of sand and stone besides the number of horses and donkeys are much vary because of few number of cart horses in the area.

CONCLUSION AND RECOMMENDATION

Besides the managerial problems from the owners there is also lack of knowledge on periodic deworming and treatment equines by taking to veterinary clinics when they get sick. Because of this reason almost all horse and donkey were positive for strongyles and moderately infected with *P. equorumin.* In line with above conclusion, the following recommendations are recommended:

1. Periodic deworming program shall be implemented for some animals by government for showing the importance until the community adapts.
2. Awareness should be created by teaching peoples in the meeting, by dramas on radio or television (mass media) about managerial systems and on the importance of GIT nematodes of equine.
3. Mixing of horses and donkey grazing should be avoided.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 9(5), pp. 92-96, May 2017
DOI: 10.5897/JVMAH11.043
Article Number: E724B8264020
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Bovine dermatophytosis in Holeta agricultural research center, Ethiopia

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Received 2 December, 2011; Accepted 1 September, 2016

A cross sectional study was conducted at Holeta agricultural research center to determine the prevalence of dermatophytosis. The overall prevalence of dermatophytosis in a total of 384 animals was 167 (43.49%), as determined by using direct microscopic examination and culture isolation. There was difference in dermatophytosis infection rates among the cattle breeds examined, the highest being 86.49% in 25% Boran (BO) X 75% Holstein Frisian (HF) followed by 56.95% in 50% BO X 50% HF and 25% in 100% Jersey animals. The study also revealed a significant difference (χ^2= 24.7359, P = 0.001) in infection rates among different age groups, the highest being in calves (62.28%) and lowest in old animals (25%). A significant difference was also observed between the season of the year and infection rates, the highest being in the wet (64.12%) season, when compared with the dry season (27.10%). However there was no significant difference in dermatophytosis infection rates in the different categories of body condition scores and with sex of the animals. The study presents the highest prevalence of dermatophytosis in the study area and warrants immediate action accordingly.

Key words: Dermatophytosis, cattle, Holeta, dairy farm.

INTRODUCTION

Dermatophyte are pathogenic fungi that have a high affinity for keratinized structures like nails, skin or hair, causing superficial infections known as dermatophytosis in both humans and animals (Luciene et al., 2008). The Etiologic agents of the dermatophytosis are classified in three anamorphic (asexual or imperfect) genera, *Epidermophyton*, *Microsporum*, and *Trichophyton*, of anamorphic class *Hyphomycetes* of the *Deuteromycota* (Fungi imperfecti) (Irene and Richard, 1995). On the basis of anamorph morphology, two species of *Epidermophyton*, approximately 18 species of *Microsporum* and 25 species of *Trichophyton,* are considered valid members of these genera (Mucoma, 2000).

Trichophyton verucosum is usually the cause of ring worm in cattle, but *Trichophyton metagrophytes*, *Trichophyton equinum*, *Microsporum gypsum*, *Microsporum nanum*, *Microsporum canise*, and other have been isolated too (Mcgavin and Zarchary, 2007). Animals can acquire infection with geophilic dermatophite

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from soil or from contact with infected animals. Zoophilic and anthrophilic dermatophite are obligate pathogens which are unable to replicate in the soil.

Dermatophite growing on keratinized structures rarely produce macrocondia and consequently rely on the production of arthrospores for transmission. Each zoophilic species tends to parasitize a particular animal species (Weitzman and Summerbel, 1995) and disseminated by direct contact including fomite and premises (Hirsh et al., 2004).

T. verrucosum is the usual cause of ringworm in cattle. Calves are most commonly affected and often develop characteristic lesions on the face and around the eyes. In heifers, cows' lesions occur on the neck and limbs. Oval areas of affected skin are alopecic with grayish white crust. Infection is most common in winter months (Quinn et al., 2002). Ringworm fungi chiefly attack keratinized tissues, particularly the stratum corneum and hair fibers, which result in autolysis of the fiber structure, breaking off the hair, and alopecia. Exudation from invaded epithelial layers, epithelial debris and fungal hyphae produce the dry crusts which are characteristic of the disease.

The lesions progress if suitable environmental conditions for mycelial growth exist, including a warm humid atmosphere, and a slightly alkaline pH of the skin. Ringworm fungi are all strict aerobes and the fungi die out under the crust in the center of most lesions, leaving only the periphery active. It is in this mode of growth it produces the centrifugal progression and the characteristic ring form of the lesions (Radostitis et al., 2007). Diagnosis of dermatophytosis is based on demonstration of consistent clinical sign, examination of affected hair with a wood lamp, microscopic examination of hair or skin spacemen, and fungal culture (Songer and Post, 2005).

Animal in many cases, dermatophytosis is theorized with self limiting disease, with the duration of infection ranging from one to four month. The spontaneous regression is partly related to the development of a strong cell mediated response, correlates with the onset of a delayed type hyper sensitivity, which usually result in elimination of the dermatophite, resolution of the lesion and local resistance to re-infection. Immunity to dermatophytosis is transient and re-infection may occur (Moriello and Deboer, 1995; Smith, 2009). Other mechanism which is associated with the elimination of infection includes an increased rate of desquamation from the stratum corneum and an increase in the permeability of the epidermis allowing penetration of inflammatory fluids (Wagner and Sohnle, 1995).

Isolation and treatment of infected animals, the provision of separate grooming tools, blankets and feeding utensils and disinfection of these items after use on affected animals, are necessary to controlled disease. Cleaning and disinfection of stables with a commercial detergent or a strong solution (2.5 to 5%) of phenolic disinfectant, 5% lime sulfur, 5% formalin, 3% captan or 5% sodium hypochlorite is advisable where practicable. Good results are also claimed for the disinfection of buildings with a spray containing 2.0% formaldehyde and 1.0% caustic soda (Radostitis et al., 2007). Therefore, the objective of this study was to determine the prevalence of dermatophytosis in Holeta agricultural research center dairy farm.

MATERIALS AND METHODS

Study area

This study was conducted in Holeta agricultural research center (HARC - Holeta and Adaberga dairy farm), Oromia Region, in Central Ethiopia, from November 2010 to April 2011. The HARC has two farms in Holeta and Adaberga (around Enchine). 50% Boran cross with 50% Holstein Frisian breed and 25% Boran cross with 75% Holstein Frisian breed live in Holeta and 100% Jersey breed, live in Adaberga.

Geographically the area is located 32 km North West of Addis Ababa with 09°02 N latitude and 38°03 E longitudes. The climatic condition of the area is predominantly temperate and receives a mean annual rain fall ranging from 84.5 to 89.7 mm. The altitude is 2400 meter above sea level.

According to the data documented by Holeta agriculture resource center Metrology's (1999), the minimum and maximum temperature of the district is 4.8 to 22.4°C, respectively. The farm system of production is semi intensive. The farm had calving pens and individual calf pens form 0 to 6 months old animals and other animals, which had their own houses according to their breed. Calves were isolated from their dams' immediately after birth, taken to calf hatch for some time and then to calf pen, where they were housed and managed for about six months.

Study design and population

A cross sectional study design was used to determine the prevalence of dermatophytosis in Holeta agricultural center by simple random sampling method. The study population consist of both cross breed of 50% Holstein Frisian × 50% Boran and 75% Holstein Frisian × 25% Boran, of 100% Jersey.

Sample size determination

The sample size was calculated based on the formula given below as described by Thrusfield, 2005.

$$n= \frac{(1.96)^2 \times P\,exp\,(1-Pexp)}{d^2}$$

Where n= number of sample size
P exp= expected prevalence (50%)
d^2= absolute precision (5%)
CI= confidence interval (95%)
Therefore the total sample size is = 384

Sample collection

For the prevalence study of dermatophytosis, skin scabs were collected directly into petridish plates from the clinical lesions of the

Table 1. Prevalence of dermatophytosis in three breeds of cattle.

Breed	No of samples examined	No of positive samples	Prevalence (%)
50BO x 50HF	151	86	56.95
25BOx 75HF	37	32	86.49
100% Jersey	196	49	25.00
Total	384	167	43.49

χ^2= 66.2358 P-value = 0.001, HF- Holstein Frisian, BO- Borena

Table 2. Comparison of direct microscopic examination (KOH) with fungal culture identification.

KOH (direct microscopic examination)	Culture positive	Culture negative	Total
KOH negative	0	217	217
KOH positive	167	0	167
Total	167	217	384

animals by using gloves and scalpel blade. After sampling, the plates were labeled and transported to the laboratory immediately.

Microscopic examination and fungal culture

Direct microscopic examination was undertaken by placing the scrapings directly onto a microscope slide and covering them with 10% potassium hydroxide (KOH). The KOH positive cases were subjected to culture study, cleaned aseptically with 70% ethanol and the scabs were collected in a sterile slide with the help of sterile scalpel blades.

The cultures were performed in Sabouraud dextrose agar (SDA) media, and the mycological identifications were based on macroscopic and microscopic examination of the culture isolates. The macroscopic examinations of dermatophytes were characterized by duration of growth, surface morphology and pigment.

Data analysis

The collected sample was entered into Microsoft excel and was analyzed using statistical software packages for social science (SPSS). Descriptive statistic like percentage can be used to determine prevalence and chi- square (χ^2) used to look the association of between prevalence of dermatophytosis and risk factors. In the analysis, confidence level was held at 95% and $p<0.05$ was set for significance.

RESULTS

The overall prevalence of bovine dermatophytosis in Holeta Agricultural Research Center during the study period was 43.5%. All samples were positive to direct microscopic examination using KOH, which were also positive to culture determination (Table 2). There was difference in the prevalence of dermatophytosis infection rates between the different cattle breeds examined. The highest being in 25BO X 75HF (86.49%) followed by 50BO X 50 HF and 25BO X 75HF were 56.95 and 25%, respectively (Table 1).

The study revealed differences in the prevalence of dermatophytosis infection rates among different age groups, the highest being in calves (62.28%) and lowest in old animals (25%) (Table 3). No significant difference between the two sexes was found even though the proportion is relatively higher in males (46.75%) than in females (42.67%), as described in Table 4.

The study also revealed a significant difference (χ^2 =52.8165, P = 0.001) in infection rates between the wet (64.12%) and the dry season (27.10%), where wet environment cause higher prevalence of dermatophytosis as described in Table 5. Different in dermatophytosis infection rates were recorded among the three body condition categories of the animals, higher in the medium body condition animals (56%) and lowest in the poor body condition animals, as described in Table 6.

DISCUSSION

From the total of 384 animals selected randomly, 167 (43.39%) (Table 1) animals were positive for dermatophytosis. The present study is consistence with the study conducted outside of Ethiopia in Central Anatolia, Kirikkale province, Turkey, in which 38% prevalence of dermatophytosis in cattle was reported by Yildirim et al. (2010), and lower than other two studies; one reported by Ghafarokhis, (2009) in Iran showing a prevalence of 99% of *T. verrucosum* and another by kojovoii et al. (2011) showing a prevalence of 62.3% in Iran. Few studies in Ethiopia report dermatophytosis for example 1.89% by Regasa (2003) western Ethiopia (Nekemt) and 0.7% prevalence for Almata Wereda by

Table 3. Prevalence of dermatophytosis with respect to age.

Age	No of sample examined	No of positive samples	Prevalence (%)
Calve	114	71	62.28
Young	124	45	36.29
Adult	118	44	37.29
Old	28	7	25.00
Total	384	167	43.49

χ^2 = 24.7359 P-value = 0.001.

Table 4. Prevalence of dermatophytosis with respect to sex.

Sex	No of Sample Examined	No of Positive Samples	Prevalence (%)
Male	77	36	46.75
Female	307	131	42.67
Total	384	167	43.49

χ^2= 0.4174 P-value =0.51.

Table 5. Prevalence of dermatophytosis based on season.

Season	No of sample examined	No of positive samples	Prevalence (%)
Wet	170	109	64.12
Dry	214	58	27.10
Total	384	167	43.49

χ^2= 52.8165 P-value = 0.001.

Table 6. Prevalence of dermatophytosis based on body condition of the animals.

Body condition	No of sample examined	No of positive	Prevalence (%)
Poor	14	4	28.57
Medium	50	28	56.00
Good	320	135	42.19
Total	384	167	43.49

Aklilu (2008). These differences observed between the present and previous studies, may be due to the difference breed of animal and agro ecological zones.

Statically significant difference was not observed between the disease prevalence and sex of animals because dermatophytosis affects both sexes, even though the proportion of infection was relatively higher in males than in females (Table 4). The highest proportion in males may be due to fact that male animals lack proper caring since the farm purpose is dairy and thus attention is only given to female animals. Differences in prevalence rates of the disease were observed in the different breeds of cattle studied. This is due to disease resistance influenced by diversity and type of genetic resistance (Fries and Ruvinsky, 2006).

In this study there is higher prevalence of dermatophytosis in winter season (Table 5) which conceded with finings of others (Quinn et al., 2002; Songer and Post, 2005), who reported the incidence of dermatophylosis which is higher in winter, possibly because of crowding and increased with carrier animal or contaminated debris in barns. This shows that season is a significant factor affecting the disease prevalence. The main transmission of dermatophytosis is through close contact between an infected animal and a healthy one (Hirsh et al., 2004). High dermatophytosis in winter time due to the short rainy season, favors aggravation of the disease due to the ecology of dermatophytosis which are zoophilic in cold climates, where animals are stabled over long period of time that favor close contact (Radostitis et al., 2007).

The study revealed that the disease was highest in calves and lowest in old age group (Table 3). This may be explained by the fact that old aged animals are highly resistance because they are adapted to the disease. Zoophilc dermatophytosis infection is most often observed in young animals that are kept in proximity to one another. Therefore, calves are more susceptible than adults (Songer and Post, 2005).

CONCLUSION AND RECOMMENDATIONS

The prevalence of dermatophytosis was found higher in the farm. Breed, age and season were found to be at high risk factors, while sex and body condition are not. Based on the results obtained, it is clear that cattle dermatophytosis is a major problem that hampers efficient utilization of production potential of the farm herd. Based on the above conclusion the following recommendations are forwarded:

(1) The farm should seriously implement appropriate control measures like hygienic practice; especially those associated with calve which avoid substitution of one animal place with another animal.
(2) Awareness should be created around the problems especially for personnel working in farm because, the diseases are zoonosis.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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academicJournals

Vol. 9(5), pp. 97-104, May 2017
DOI: 10.5897/JVMAH2017.0567
Article Number: 3DC387E64022
ISSN 2141-2529

http://www.academicjournals.org/JVMAH

Journal of Veterinary Medicine and Animal Health

Full Length Research Paper

Prevalence of bovine brucellosis, tuberculosis and dermatophilosis among cattle from Benin's main dairy basins

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Received 21 February, 2017: Accepted 18 April, 2017

In order to determine the prevalence of bovine brucellosis, tuberculosis and dermatophilosis, a study was carried out in main dairy areas of Benin from April to September 2015. For brucellosis, 780 sera and 78 milk samples were analyzed by indirect enzyme-linked immunosorbent assay (iELISA). For tuberculosis, 780 cattle underwent a comparative intradermal tuberculin test and 78 milk samples were used for Ziehl-Neelsen's staining. About dermatophilosis, 78 samples of scabs were collected for Giemsa's staining. For brucellosis, the overall individual animal seroprevalence was 8.85%. The regions of Borgou with 19.33% and Atlantique with 0% prevalence showed significant differences (p <0.05) with the other regions. For tuberculosis, the overall individual animal prevalence was 2.18%. The regions of Borgou and Alibori, with 0% prevalence each, showed significant differences (p <0.05) with most other regions. Taking into account the individual animal prevalence, Zou (brucellosis 18.33%, tuberculosis 6.67%) and Plateau (brucellosis 10%, tuberculosis 6.67%) were the areas at risk for these two diseases. For dermatophilosis the overall herd prevalence was 23.08%. There was significant difference (p<0.05) between Alibori and Mono but also between Alibori and Zou. It is urgent, therefore, to put in place an adapted control strategy taking into account these geographical realities.

Key words: Brucellosis, tuberculosis, dermatophilosis, prevalence, cattle, Benin

INTRODUCTION

Brucellosis and tuberculosis are considered as the most important and prevalent zoonotic diseases (WHO, 2004). Both diseases are under control in developed countries, but remain prevalent in sub-Saharan Africa, affecting both livestock and human populations (Abbas and Agab, 2002; Schelling et al., 2003; Mostowy et al., 2005; Zinsstag et al., 2007). In addition to being a threat to public health, both diseases can have serious economic implications. Bovine tuberculosis has a negative impact on livestock production in developing countries by reducing production efficiency, seizure of carcasses or organs and restricting international trade. It has implications not only for the economies of livestock communities, but also for human health through the consumption of raw dairy products and / or close contact with infected animals or animal tissues (OIE, 2009). Brucellosis also causes significant reproductive losses in animals (Cutler et al., 2005). Bovine tuberculosis and brucellosis remain a major public and animal health problem in many developing countries, where cattle are a major source of food and income (Omer et al., 2000). Understanding the epidemiology of bovine tuberculosis and brucellosis is therefore essential to develop evidence-based disease control strategies. However, this information is insufficient in Africa's sub-Saharan. Therefore, appropriate preventive measures have not been taken (McDermott and Arimi, 2002). Bovine dermatophilosis is distributed worldwide, but mainly recorded in African countries (Kassaye et al., 2003; Kusina et al., 2004; Hamid and Musa, 2009). The disease leads to great economic losses in African countries due to inferior wool and leather quality, death and culling, decrease meat and milk production (Yeruham et al., 2000). Among the skin diseases, bovine dermatophilosis is one of the common economically important diseases of cattle with high economic significance in decreasing the productivity (Awad et al., 2008). As bovine tuberculosis and brucellosis, it is also a zoonotic disease.

Bovine tuberculosis, brucellosis and dermatophilosis are endemic in Benin. This is from the reports of the Direction of Animal Production (DAP) and authors mentioning cases from slaughter houses for tuberculosis and suspicions of clinical signs for brucellosis and dermatophilosis (Ali-Emmanuel et al., 2002; DAP, 2012, 2013, 2014, 2015, 2016). But there is no control program. However, in order to implement Milk and Meat Support Project (PAFILAV), it was imperative to investigate the current situation of the major pathologies affecting milk and meat production in the Project Intervention Zone (ZIP). The main objective of this project is to improve production systems and competitiveness of milk and meat sectors. Then, bovine brucellosis, bovine tuberculosis and bovine dermatophilosis have been retained to determine their prevalence throughout the national territory. These are diseases for which data on their prevalence in Benin are rare. Indeed, for bovine brucellosis, Akapko et al. (1984) found a seroprevalence of 10.4% in extensive herds. Koutinhouin et al. (2003) studies on herds supervised by Livestock Development Project gave a seroprevalence of 6.20 at 15.21%, while those of Adéhan et al. (2005) gave a seroprevalence of 2.06 to 3.4% on state farms. It should be noted that all these studies have focused on serum analysis only.

Concerning the prevalence of bovine tuberculosis, Farougou et al. (2006) conducted a study at the state farms of Samiondji and Bétécoucocu with single intradermal skin test. Prevalences obtained were 8.25 and 2.64% respectively for Samiondji and Bétécoucou. In addition, Dossou et al. (2016) conducted a study on milk through detection of *Brucella abortus* and *Mycobacterium tuberculosis* in the state farms of Kpinnou, Bétécoucou, Okpara and a private farm in Adjohoun, with no case of infection found. It is clear that all these previous studies, in geographical terms, took far more account state farms. No studies have considered both serum and milk for bovine brucellosis. Similarly, no studies have considered a comparative intradermal skin test and milk for bovine tuberculosis. Concerning bovine dermatophilosis there is no study about it prevalence in Benin. Thus, the aim of our study is to provide information on bovine dermatophilosis herd prevalence and to determine the bovine brucellosis and bovine tuberculosis in the Projet d'Appui aux Filières Lait et Viande (PAFILAV)'s ZIP with the identification of areas at risk through the analysis of serum and milk associated with comparative intradermal skin test.

MATERIALS AND METHODS

Study area

The PAFILAV's ZIP has 27 municipalities out of 77 of the country, and extends throughout the national territory. Benin is part of the intertropical zone. Depending on the latitude in which they occur, rainfall periods combine in different ways to define rainfall regimes. In the south of the 7° 45 'parallel is a bimodal regime with four (4) seasons, two dry and two rainy seasons. North of parallel 8° 30 ', there is a unimodal regime with two seasons, one dry season and one rainy season. Thus the South experiences a climate with four seasons: a great rainy season from April to July; a small dry season from August to September; a small rainy season from October to November and a great dry season from December to March. The North has two seasons: a dry season from November to early May and a rainy season from May to October. The administrative division of Benin comprises 4 hierarchical levels, which are in decreasing order: Regions, municipalities, districts, villages or wards. So we have 12 regions; 77 municipalities; 546 districts and 3557 villages/wards.

The intervention zones are targeted by region and municipality according to the potential in livestock and milk production. The study was conducted in 26 municipalities from eleven of the twelve regions of the country. These selected municipalities are 10 in the northern area and 16 in the southern and central areas of the country (Figure 1). These include:

1. Nikki, Kalalé, Parakou, Bembèrèkè, Gogounou, Tchaourou, Kandi, Banikoara, Bassila and Pehunco in the northern zone;

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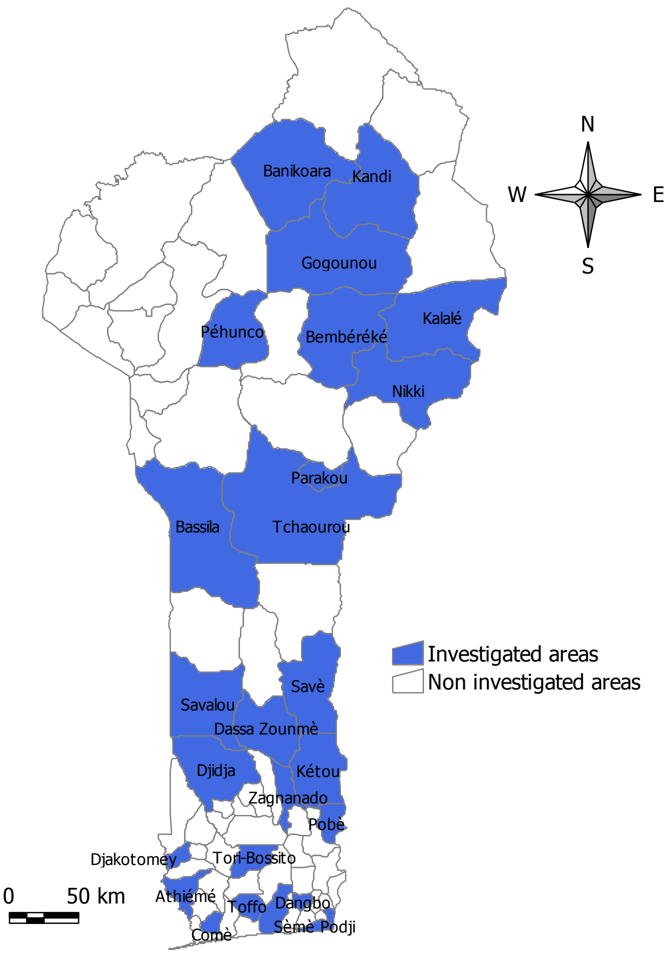

Figure 1. Map of Benin showing locations of the study area.

2. Djidja, Zagnanado,Djakotomey, Comé, Athiémé, Pobè, Kétou, Savalou, Dassa-Zoumè, Savè, Dangbo, Adjarra, Sèmè Podji, Abomey-Calavi, Tori Bossito and Toffo in the southern and central zones.

Sample collection

The sampling was carried out from April to September 2015. Respondents were selected based on their accessibility and availability to cooperate. Recruitment of animals for testing was based entirely on the owner's willingness. From the twenty-six (26) municipalities of the ZIP, three (3) herds were retained in each of them. Ten (10) identified animals were selected in each of those herds. In total, seventy-eight (78) herds and seven hundred and eighty (780) animals were included in the study (Table 1). On each of the 10 animals, blood is taken from the jugular vein and the two tuberculins are injected. In each herd, all animals with exudative dermatitis were examined. From these animals, three samples of scabs were collected in clean, sterile tubes for isolation of *Dermatophilus congolensis*.

For tuberculins' injection, animal is maintained in position of lateral decubitus. Two sites located to the right of the collar (the flat of the neck), at 20 cm intervals, were shaved and the thickness of skin is measured with caliper measurement. A first site is injected with 0.1 ml containing 2500 IU/ml bovine PPD. Similarly, 0.1 ml of avian PPD of 2500 IU/ml was injected into the second site. The injection is done in the dermis using an insulin syringe. After 72 h, the skin thicknesses were measured at injection sites which are:

1. For bovine tuberculin (B): at the limit of the posterior and middle thirds of the neck and approximately equidistant from the upper and lower edges of the latter;
2. For avian tuberculin (A): in front of the preceding one, at the limit of the anterior and middle thirds of the neck, and approximately equidistant from the upper and lower edges of the latter.

In addition, once all herd cows are milked, 50 ml of the milk mixture is collected. During this study, 78 herds including 780 animals (595 females and 185 males) were investigated throughout the national territory (Table 1). For tuberculosis, 780 tuberculinations were performed with 78 samples of milk for Ziehl-Neelsen's staining. For brucellosis, 780 sera and 78 samples of milk were analyzed by indirect ELISA.

Analysis of samples

To analyze serum, milk and scab, the two veterinary laboratories of the country (Laboratory of Parakou and that of Bohicon) were involved.

For bovine tuberculosis

Reading was done 72 h later. We went back into the herd and we measured the skin thickness at the injection sites. The thickness differences between D_0 and D_3 were calculated:

$DB = B_3 - B_0$ for bovine tuberculin
$DA = A_3 - A_0$ for avian tuberculin

The interpretation of the measures is as follows:

If DB - DA is greater than 4 mm: Positive result
If DB - DA is less than 1 mm: Negative result
If DB - DA is between 1 mm and 4 mm inclusive: Doubtful result

The milk samples were subjected to Ziehl-Neelsen's staining.

For bovine brucellosis

Sera and milk were subjected to the indirect Enzyme Linked ImmunoSorbent Assay (iELISA) using *Brucella* smooth lipopolysaccharide (S-LPS) as an antigen. Indirect ELISA results were classified as positive or negative using the manufacturer's recommended values.

For dermatophilosis

Small pieces were taken from the underside of the scab and softened in a few drops of distilled water on a clean microscope slide; a smear was made and stained with Giemsa's staining as described by Scott (1988).

Statistical analysis

Data had been integrated into the Excel spreadsheet and then into the software R version 3.1.2. For brucellosis, individual and herd prevalences were calculated by dividing the number of positive iELISA cases by the number of animals or milk taken.

Table 1. Categories and number of animals sampled for tuberculosis, brucellosis and dermatophilosis according to locations in Benin.

Region	Municipalities	Sampled animal						
		Cow	Bull	Heifer	Bull-calf	Calf	Calve	Total
	Gogounou	18	1	3	1	4	3	30
Alibori	Kandi	18	6	0	0	4	2	30
	Banikoara	18	3	4	4	0	1	30
Atacora	Pèhunco	17	3	3	0	2	5	30
	Abomey-Calavi	16	4	4	3	2	1	30
Atlantique	Tori Bossito	18	2	5	4	0	1	30
	Toffo	18	3	6	3	0	0	30
	Nikki	12	0	5	10	2	1	30
	Kalalé	10	0	9	7	1	3	30
Borgou	Parakou	15	7	2	3	3	0	30
	Bembèrèkè	23	1	0	2	2	2	30
	Tchaourou	27	1	1	0	0	1	30
	Savalou	19	2	2	2	1	4	30
Collines	Dassa Zounmè	22	3	2	3	0	0	30
	Savè	20	4	4	2	0	0	30
Couffo	Djakotomey	16	2	6	3	2	1	30
Donga	Bassila	12	5	6	1	3	3	30
Mono	Comè	12	1	6	5	4	2	30
	Athiémé	10	4	8	4	1	3	30
	Dangbo	13	0	5	4	3	5	30
Ouémé	Adjarra	11	1	4	3	2	9	30
	Sèmè Podji	19	0	5	5	0	1	30
Plateau	Pobè	16	0	3	6	1	4	30
	Kétou	4	1	16	9	0	0	30
Zou	Djidja	15	3	5	1	4	2	30
	Zagnanado	22	0	6	2	0	0	30
Total		421	57	120	87	41	54	780

For dermatophilosis, herd prevalence was calculated by dividing the number of positive cases by the number of scabs taken.

For tuberculosis, differences in skin thickness were calculated. Results from the Ziehl-Neelsen's staining were recorded. Thus individual and herd prevalences were estimated by dividing number of positive cases by the number of tuberculinized animals or the number of milk taken.

In both cases, the individual prevalences obtained by region were compared two by two with Fisher's exact test. For each relative frequency, a 95% confidence interval (CI) was calculated using the formula:

$$CI = 1.96\sqrt{\frac{[P(1-P)]}{N}}$$

Where P is the relative frequency and N is the sample size.

RESULTS

Concerning brucellosis, the overall individual seroprevalence was 8.85%. There was significant difference by sex and also between cow and calve ($p<0.05$). Moreover, according to the regions, and overall, Borgou with 19.33% and Atlantique with 0% (Table 2) showed significant differences ($p<0.05$) with the other regions. Ouémé with 1.11% and Zou with 18.33% also showed some significant differences with the rest of the

Table 2. Prevalence of brucellosis infection among cattle from different regions of Benin.

Region	Individual seroprevalence (%)	Confidence interval	Milk prevalence (%)	Confidence interval
Alibori	10[a]	6.2	66.67[ac]	30.79
Atacora	6.67[a]	8.9	66.67[ac]	53.34
Atlantique	0[b]	0	22.22[cde]	27.16
Borgou	19.33[a]	6.26	66.67[acd]	23.85
Collines	3.33[bd]	3.66	33.33[ac]	30.79
Couffo	0[bd]	0	66.67[ac]	53.34
Donga	13.33[a]	12.16	66.67[ac]	53.34
Mono	6.66[cd]	6.33	16.67[ac]	29.82
Ouémé	1.11[bd]	2.18	11.11[be]	20.53
Plateau	10[a]	7.6	33.33[ac]	37.72
Zou	18.33[ac]	9.76	83.33[ac]	29.82
Total	8.85	2	46.15	11.06

Proportions in the same column followed by different letters differ significantly at 5%.

other regions. Furthermore there was no significant difference (p> 0.05) between the four northern regions (Alibori, Atacora, Borgou and Donga); whereas in the South, with Atlantique, Mono, Ouémé and Plateau, there were significant differences between them. The herd seroprevalence was 37.18% (95% CI 26.45 to 47.91%). The milk prevalence was 46.15% (95% CI 35.1 to 57.2%). It relates only to the cows of the herds investigated. Overall, the region of Ouémé had a significant difference with most other regions. No significant difference was observed between milk prevalence and herd seroprevalence.

Concerning tuberculosis, the overall individual prevalence was 2.18%. There is no significant difference between sex and categories (p> 0.05). But depending on regions, Borgou and Alibori, with 0% each (Table 3), showed significant differences (p <0.05) with most of the other regions. Plateau and Zou, with 6.67% each, also had some significant differences with the rest of the other regions. There was no significant difference (p> 0.05) between the four northern regions (Alibori, Atacora, Borgou and Donga). Moreover in South, with Atlantique, Mono, Ouémé and Plateau, there were no significant differences between them. The herd prevalence was 15.38% (95% CI 7.38 to 23.38%). The milk prevalence was 6.41% (95% CI 0.98 to 11.84%). It is also related to the cows of the herds investigated. There were no significant differences between regions. No significant difference was observed between milk prevalence and herd prevalence.

Concerning dermatophilosis, Table 4 presents the results of herd prevalence by regions. The overall herd prevalence was 23.08% (95% CI 13.73 to 32.43%). There was significant difference (p<0.05) between Alibori and Mono but also between Alibori and Zou. The four northern regions (Alibori, Atacora, Borgou and Donga) had the lowest rates. In addition, taking into account the individual prevalence, Zou (Brucellosis 18.33%, Tuberculosis 6.67%) and Plateau (Brucellosis 10%, Tuberculosis 6.67%) constituted the zones at risk for these two diseases. In the same way, but to a lesser extent, there were also the regions of Mono and Ouémé. Two cows at Pobè and one at Comè were both positive for brucellosis and tuberculosis. Thus mixed prevalence rate was 0.38%. No herds were positive for these three diseases at the same time.

DISCUSSION

For bovine brucellosis, the overall seroprevalence was 8.85%. This result is similar to that obtained by Akakpo et al. (1984). Moreover, it is much lower than that obtained by Koutinhouin et al. (2003) and much higher than that obtained by Adéhan et al. (2005). The Borgou region had the highest rate (19.33%) and there were no significant differences between the four northern regions which showed significant differences with those of the South. It should be noted that the North is characterized by large herds, unlike the South. This would promote close contact between the animals. Furthermore, in this region of North, animals move a lot, especially transhumance in search of water and grazing, whereas in the South the herds are more sedentary. These two situations could favor transmission and maintenance of the disease at this level. This has been noted by some authors (Berhe et al., 2007; Ragassa et al., 2009; Matope et al., 2010; Makita et al., 2011; Megersa et al., 2011). Nevertheless, study of Cadmus et al. (2013) in Nigeria has shown a higher seroprevalence in sedentary herds compared to transhumants. Significant difference was found by sex. This is in agreement with studies of some authors (Traoré et al., 2004; Dinka and Chala, 2009; Adugna et al., 2013). between cow and calf/calve. Indeed, younger animals are more resistant to primary infection and eliminate *Brucella* sp. although sometimes latent infection occurs (Walker, 1999). According to Acha and Szyfres (1989), heifers

Table 3. Prevalence of tuberculosis infection among cattle from different regions of Benin.

Region	CIDT individual prevalence (%)	Confidence interval	Milk prevalence (%)	Confidence interval
Alibori	0[bc]	0	11.11	20.53
Atacora	0[ac]	0	33.33	53.34
Atlantique	3.33[ac]	3.71	0	0
Borgou	0[b]	0	13.33	17.20
Collines	0[bc]	0	0	0
Couffo	0[ac]	0	0	0
Donga	3.33[ac]	6.42	33.33	53.34
Mono	5[ac]	5.51	0	0
Ouémé	2.22[ac]	3.04	0	0
Plateau	6.67[ac]	6.31	0	0
Zou	6.67[ac]	6.31	0	0
Total	2.18	1.02	6.41	5.43

Proportions in the same column followed by different letters differ significantly at 5%.

Table 4. Prevalence of dermatophilosis infection among cattle from different regions of Benin.

Region	Herd prevalence (%)	Confidence interval
Alibori	0[b]	0
Atacora	0[a]	0
Atlantique	33,33[a]	30.79
Borgou	20[a]	20.24
Collines	33,33[a]	30.79
Couffo	0[a]	0
Donga	0[a]	0
Mono	50[a]	40.01
Ouémé	22,22[a]	27.16
Plateau	16,67[a]	29.82
Zou	50[a]	40.01
Total	23,08	9.35

Proportions in the same column followed by different letters differ significantly at 5%

andcows are classified as the most sensitive. In Africa, some authors have recorded rates ranging between 3 and 13% (Traoré et al., 2004; Boussini et al., 2012, Cadmus et al., 2013). These rates are relatively close to ours. In Ethiopia, Tschopp et al. (2013) found 1.7%. But in Zambia, Muma et al. (2013) found 20.7%. It should be noted that in this case, serum samples were taken only from cows. Our herd prevalence was 37.18%. This is close to the 45.9% found in Ethiopia by Asgedom et al. (2016). The prevalence after analysis of the milk was 46.15%. It is above the 16% found in Egypt by Wareth et al. (2014).

For bovine tuberculosis, although comparative intradermal tuberculin test gives more specific results than single intradermal tuberculin test (Monaghan et al., 1994), our study gave an overall prevalence of 2.18%. This rate is similar to that obtained by Farougou et al. (2006) in Bétécoucou farm which was 2.64%, but very far from the rate they obtained in Samiondji's farm which was 8.25%. The highest rates were observed in the regions of the South. It was noted that in this region, there are sedentary herds. Indeed, prolonged contact could favor transmission by aerosols. Factors such as water sharing, grazing, or high promiscuity are potential risk factors for bovine tuberculosis transmission (Thoen and Bloom, 1995). This rate is close to that obtained by Asante-Poku et al. (2014) in Ghana which was 2.48%. However, in Burkina Faso, Traoré et al. (2004) found 27.7%. There is no significant difference about sex. This is in agreement with the study of Traoré et al. (2004) in Burkina Faso. The herd prevalence was 15.38% and the milk prevalence was 6.41%. This difference, although not significant, may be due to the fact that *M. bovis* is rarely isolated from milk, although it is known to be secreted in milk. However, it is not found in milk that has been stored for a few days probably because of competition with lactobacilli (Mariam, 2009). Moreover, the numerous doubtful cases can have several causes. Indeed, considering that the tuberculin test is not a perfect test, some animals would not have been detected, which can lead to an underestimation of the prevalence. In endemic areas, delayed hypersensitivity may not develop for 3 to 6 weeks after infection, and in chronically infected animals with severe disease, tuberculin testing may not respond (OIE, 2010). Thus, it is evident that the initial thickness of the skin fold could confuse the interpretation of reactivity to tuberculin. In Africa, some authors have found relatively low rates ranging from 2 to 6% (Boukary et al., 2011; Boussini et al., 2012; Katale et al., 2013; Muma et al., 2013). In contrast, in Nigeria, Okeke et al. (2014) found 16.17% with PCR on cattle lungs taken from slaughterhouses. In Ethiopia, Tschopp et al. (2013) found 0.3%. The mixed prevalence rate for brucellosis-tuberculosis was 0.38%. It is close to that observed in Burkina Faso by Boussini et al. (2012) which was 0.49%.

For bovine dermatophilosis, about herd prevalence, the four northern regions (Alibori, Atacora, Borgou and Donga) had the lowest rates. This could be in correlation with season. Indeed, it is warmer in the North (only one rainy season) than in the South (two rainy seasons). Dejene et al. (2012) have shown that there was a significant variation between seasons of the year and bovine dermatophilosis which is highly prevalent during the wet season than the dry season. The higher prevalence of the disease during the mentioned season is due to activation of the motile zoospores by rain and increased arthropods population (ticks) so that they may contribute to the occurrence of the disease. Ticks were present in most sampled herds. Furthermore tick *Amblyomma variegatum* had been associated with transmission of the disease (Morrow et al., 1993; Chatikobo et al., 2004) and there was also an association with tick *Boophilus annulatus* (Awad et al., 2008) for which macroclimatic factors play a great role in seasonal dynamics (Singh et al., 2000). In the same way the dry season in the north is usually a period of extensive bush burning. Wilson (1988) observed that the disappearance of vegetation in the dry season had a direct effect on the local abundance of questing adult ticks. He reported that tick abundance was reduced by as much as 88% following removal of vegetation by burning.

Conclusion

Knowledge of diseases is a crucial step in the development of prevention and control measures. This study suggests that the overall prevalence of bovine brucellosis, tuberculosis and dermatophilosis in Benin in general and in the PAFILAV's intervention area in particular is very high and requires urgent intervention. These three diseases are likely to pose a significant risk for the achievement of PAFILAV's objectives. Several recommendations can be made to minimize the risk of spread of these diseases between regions. The first and most important is to disseminate knowledge about brucellosis, tuberculosis and dermatophilosis. Then, educate herders and people involved in the cattle trade on risk factors. Finally train herders on how to deal with any signs of suspicion of disease in their flock. In addition, further studies are needed to determine the actual burden of these zoonoses on public health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to Projet d'Appui à la Filière Lait et Viande (PAFILAV) for financial support.

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