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PARASITIC DISEASES TRANSMITTED BY TICKS AND THEIR EFFECT ON SOME HAEMOTOLOGICAL PARAMETERS AMONG SHEEP AND GOAT IN SOKOTO MODERN ABATTOIR

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ABSTRACT

A preliminary study was conducted to determine the Effect of Ticks infestation on some haemotological parameters and parasitic diseases transmitted by ticks among sheep and goat in sokoto modern abattoir. A total of 100 blood samples were collected from sheep (n = 50) and goats (n = 50) from March 2018 to June 2018. Giemsa stained blood smears were prepared and examined under light microscope, to screen for Tick-borne haemoparasite. Packed cell volume (PCV) was determined by microhaematocrit centrifugation technique while haemoglobin (Hb) concentration was determined by Sahli's method. The total white blood cell (WBC) and red blood cell (RBC) counts were estimated with Neubauerhemocytometer while erythrocyte indices were calculated. The results showed 18.0% overall prevalence of tick-borne haemoparasitic diseases in sheep 5(10%) and goats 4 (8.0%). *Anaplasma ovis* and *Babesia ovis* were identified in the study of which *Anaplasma ovis* [4(8.0%)] was prevalent ($p<0.05$) than *Babesia ovis*[1(2.0%)] in sheep, However in goats prevalence of *Anaplasama ovis* as well as of *Babesia ovis* was similar[2(4.0%). The mean values of PCV, Hb and RBC and WBC counts of infected sheep and were lower ($p<0.05$) than the uninfected sheep. Similarly, the mean values of Hb and WBC of were significantly ($p<0.05$) lower in infected goats.

KEYWORDS: Sheep, Goat, Ticks etc.

INTRODUCTION

Ticks are invertebrate animals in the phylum Arthropoda, related to spiders, in the subclass Acari which consists of many orders of mites and one tick order, the Ixodida. All ticks and some mites are parasitic and feed on blood. Some species of mites look like ticks and may be mistaken for larval ticks at infestations, but their feeding mechanisms are distinctive (Sonenshine, 2014).

Ticks are responsible for the variety of losses attached to the host (tick worry) causing injection of neurotoxin produced in the tick's salivary gland; which cause blood loss, general stress, hide damage and irritation, leading to decrease in productivity in terms of milk, meat etc. and weakens the immune function. (Alim *et al.*, 2012; FAO United Nation 2011).

Tick paralysis is the only tick-borne disease that is not caused by an infectious organism. The illness is caused by a neurotoxin produced in the tick's salivary gland. After prolonged attachment, the engorged tick transmits the toxin in its host body. Affected organism can experience severe respiratory distress (similar to anaphylaxis) Gothe (2011).

Although tick paralysis is of concern in domestic animals and livestock, it can also affect human and usually occur in children under the age of 10 years (Schmitt *et al.*, 2013). Tick paralysis occurs when an engorged and gravid (egg-laden) female tick produces neurotoxin in its salivary glands and transmits it to its host during feeding. Experiments have indicated that the greatest amount of toxin is produced between the fifth and seventh day of attachment (often initiating or increasing the severity of symptoms), although the timing may vary depending on the species of tick (Dworkin *et al.*, 2010).

Tick-borne fever (TBF) is now a widespread problem for the sheep industry.(Sumption, 2007) The causative agent has kept taxonomists gainfully employed for decades. It was first tentatively named *Rickettsia phagocytophila*. The protozoa then spent time as *Cytoecetesphagocytophila* and *Ehrlichia phagocytophila*.

It now seems to be most commonly referred to as *Anaplasma phagocytophila* although as a zoonosis the agent is referred to as *Granulocytic ehrlichia*(GE) (Kennedy *et al*, 2007; Sumption, 2007).

Ovine *anaplasmosis* (unlike bovine anaplasmosis) is a mild and often undiagnosed disease of sheep in our regime, the Nigeria, Niger republic, the Mediterranean region, and the Middle East of Africa. Ovine anaplasmosis predominantly affects older sheep in the spring and summer when ticks are active.

Ticks spread *Babesia ovis* and *Babesia motasi* in Nigeria, West Africa and the Middle East (Sumption, 2007). It is an important disease in areas infested with the tick *Rhipicephalus bursa*. Following infection the protozoa directly invades red cells causing intravascular haemolysis. Consequently, affected animals develop anaemia, jaundice and haemoglobinuria. Pyriform parasites are easily demonstrated within red cells of blood smears from clinically affected animals.

The disease is seasonal, based on tick availability and on the breed and immune status of the local sheep (and goat) population. In the acute form of the disease mortality rates can be high and affected animals are febrile (Ponnudurai, 2013).

Ticks are efficient vectors of multiple pathogens due to their potential interactions with several different vertebrate hosts during their life cycle. As a result, they have the opportunity to acquire a large array of different types of organisms that are present in the blood of these hosts. The microbial community in ticks includes viruses, bacteria, protozoa, and fungi, and serves as symbionts, commensals, and pathogens (Pettersson *et al*, 2014). This microbial community can influence the acquisition, transmission, and virulence of human pathogens. Furthermore, as the tick feeds for extended periods, it interacts with its vertebrate host and has the ability to suppress the host's immune system by dampening down the immune response (Pettersson *et al*, 2014).

The prevalence of Tick Born Encephalitis Virus (TBEV) in ticks has mainly been investigated in questing unfed ticks in the field.

The median TBEV prevalence in field collected ticks is about 0.4%, but varies significantly depending on how targeted the collection of ticks is on areas where individuals that have contracted TBE reported to be tick-bitten. In a recent Swedish study, the average prevalence of TBEV in field collected ticks was 0.23%, with a lower prevalence in nymphs (0.1%), than in adults (0.55%), but in a well-known highly endemic Island in the Stockholm archipelago the prevalence was 0.5% in nymps and 4.5% in adults (Pettersson *et al.*, 2014). In 3 German studies, a higher prevalence of TBEV has been observed in ticks detached from humans than in field-collected ticks from the same area (Klaus *et al.*, 2010). Only a small proportion of infected ticks, both from the field and from humans, appear to have a high virus titer. However TBEV can persist at such low prevalence rates in ticks is not completely understood, but mathematical models suggests that aggregation of co feeding ticks on host animals is crucial to maintain virus circulation, and may explain the highly focal and patchy distribution of TBEV (Harrison *et al.*, 2012).

Economically sheep and goat contribute enormously to the protein requirement of most developing local areas (Mandal *et al*, 2007; Muhammad *et al*, 2008). Integration of sheep with crop agriculture usually occurs under sustenance condition on small scale farmers in Sokoto metropolis. They form an integral part of the system, providing milk, meat manure and cash to the farm family during the time of need. Sheep and goat are effectively reared on marginal lands and good users of crop resistive (Fakoya, 2002; Sanni *et al*, 2004). Sokoto located in the Sahel region of Nigeria, is endowed with a formal climate suitable for the proliferation of ticks and tick borne parasite these has been increasing interest among the resident at Sokoto metropolis to keep sheep for economic importance, this has considerably increased the proportion of sheep and goat roaming around the street and house close to them. However, the prevailing socio-economic conditions have made it difficult to many animal owners to urgently provide food, shelter and basic health to the animals.

The animals are therefore left to secure food around the street thereby increasing interaction amongst the susctive and increasing the risk of ectoparasitic infections. The burden of ectoparasite on sheep and goat belong to the resource poor communities and importants communities has been a subject of research interest in many part of the world (Hohn *et al* 1992). Ticks are generally known to be a major transmitter of organism responsible for several diseases of man and other animal.

Since ticks remain the major threat to sheep and goat as a ectoparasite in the prevalent areas and may also be specific and they also transferrable to other animals, including humans, therefore there is a need to identify the various types of ticks that parasitized sheep and goat this research work was focused on diseases transmitted by Ticks among goats and sheep.

MATERIAL AND METHOD

Study Area

Samples for this study were collected from sokoto central abattoir which is located in sokoto north local government area of sokoto state. Sokoto is a city which islocated in the extreme northwest of Nigeria near to the confluence of river sokoto and river rima, sokoto has latitude of $13^0$04'N $5^0$14'E and longitude of 13.067^0N5.233^0E. As 2006 census they have been estimated population of 427,760, sokoto city is the

modern day capital of sokoto state also called seat of caliphate.

Sokoto state is in dry Sahel surrounded by sandy savannah and isolated hills. With an annual average temperature of 28.8^0c (82.9^0f), sokoto is in the whole, a very hot area. However maximum day time temperature are for most of the year generally under 40^0c (104.0^0f) and the dry season make the heat to be variable.

Sample Collection
A total of 100, sheep (n=50) and goat (n=50) blood sample were collected from ticks infested animals, the body of the animals was searched throughout to ensure there is infestations of the ticks before collecting the sample. About 3 ml of blood was collected immediately after slaughter from the severed jugular vein in to vacutainer tube containing 1 ml of ethylene diaminetetra acetic acid (EDTA).

The sex, age, breed and specie of each animal were also identified based on morphometric characteristics and recorded in case book .the samples were transported on ice packs to the Parasitology laboratories, Usmanu Danfodiyo University Sokoto, veterinary teaching hospital for parasitological and haemotological examination.

Identification of Haemoparasites
Preparation of Thin Blood Films
A drop of blood were added at edge of a clean slide, a cover slide was placed at an angle of 30^0-40^0 on the first slide in contact with the drop of the blood until the blood run along the back edge of the second slide, and the slide will be push along steadily and uniformly. A film of blood was left behind and allowed to dry.

3.3.1 Preparation of Thick Blood Films
Two drops of blood was placeed on the center of the clean slide with the aid of the corner of the second slide, and using a circular motion; the drop of the blood will be mixed and spread over and area and was dried in a dust – free environment.

Staining of thin of blood film
The thin blood films prepared were fixed by dipping it in absolute methanol for one minute. Excess methanol was removed by shaking the slides and allow drying. The slides were stained in solution of Giemsa diluted with buffer for about 30 minute. The slides were removed from the stain and washed in distilled water and allowed to dry by placing it in upright position.

Staining of thick blood films
The thick films after drying were washed in buffered water to dehaemoglobinize the erythrocyte for efficient microscopic examination of the parasites. The slides were covered with giemsa's stain for about 30 minutes than, they were removed from the stain and washed briefly in buffered water and finally drained and dried in air by keeping them in an upright position.

Identification of haemoparasites in blood film
The procedure for the identification of parasites in both thin and thick films were the same. To confirm the parasite species or mixed infections after examining the thick film, examination of the thin film was done. Placed was a drop of immersion oil on the feathered edge of the thin film and 10x lens and 100x lens were used for identification. The species of haemoparasites were confirmed to the identification chart for morphological confirmation of each species. (Chaudhary, 2011).

Determination of haemotological paramrters
Determination of packed cell volume (PCV)
The packed cell volume (PCV) was determined by centrifuging heparinized blood in a capillary tube (also known as a micro hematocrit tube) at 10,000 RPM for five minutes and PCV was calculated by measuring the lengths of the layers (Hedin, 2011).

Determination of haemoglobin (Hb)
Procedure: N/10 HCL was placed in diluting tube up to the mark 20 and the blood was taken in the haemoglobin pipette up to 20-cubic-mm-mark and blew into diluting tube. The blood was rinsed thoroughly. After 10 minutes distilled water was added in drops and mixed until it had exactly the same color as the comparison standards. The reading was recorded (Hedin, 2011).

Determination of white blood cells
White Blood Cell Count
The white blood cell count test used to measure the number and of the differential number of white blood cells.

The Whole blood was mixed with a weak acid solution (3% acetic acid) to dilute the blood and hemolyze the red blood cells. Making a 1:20 dilution of blood, 1900 µl of diluting fluid was taken (3% acetic acid solution) into a test tube. 100 µl of well-mixed whole blood was added to the tube. Contents of the tube was mixed for 2-3 minutes. Hemocytometer and cover glass were cleaned with alcohol and dried thoroughly then cover glass was placed on hemocytometer. Hemocytometer was filled approximately 10 µl each side and allowed for 2-3 minutes for WBC to settle prior to count, counting chamber was placed on the microscope stage and focused by using the low power (10X) objective lens.

Starting with the square in the upper left-hand corner, all the the cells in the four corner squares (4W) were counted, in this way the cells that touch the top and left lines were counted, Cells that touch the bottom and right lines were ignored.

Determination of red blood cells
Red Blood Cell count
To facilitate counting, whole blood was diluted with Gower's solution which hemolyze white blood cell and prevent red cell lysis (Muraleedharan, 2008).

Procedure
Blood was diluted in 1:200 dilution with Gower's solution. The counting chamber was cleaned and Filled with 10 µl to each side of the counting chamber with diluted blood. Once the counting chamber was filled, it was left for approximately 3 minutes for the red blood cell to settle prior to counting, then the red blood cell were after placing counting chamber on the microscope stage using 10X objective lens.

RESULTS

Out of 100 blood samples examined for haemo-parasites, 9 were positive which represented the overall prevalence of 9%, out of which 5 samples was positive showing prevalence 10.0% in sheep and 4 samples was positive representing 8.0% in goat. The order of their occurrence was as follows; *Anaplasma ovis* had the highest prevalence of 8.0% followed by *Babesia ovis* with 2.0% in sheep while *Anaplasma ovis* and *Babesia ovis* had the percentage of 2.0% in goat (Table 1).

Age based prevalence showed that the sheep between 3-4 years had the highest prevalence with 17.6% followed by the sheep greater than 4 years with prevalence of 10.0% while the least prevalence was observed in the sheep between 2-3 years with percentage of 7.7%. However sheep between 1-2 years examined in this study were not infected with any tick-borne haemoparasites as presented in (Table 2). The chi square analysis indicates no significant association between the ages of the sheep with respect to infection with haemoparasites.

Occurrence of haemoparasites based on genders of the sheep showed that female sheep had the highest prevalence of 15.4% than the male sheep with 2.1% as shown in (Table 3).

Among the different breed of sheep examined in this study, Balami breed had the highest prevalence of infection with haemoparasites with 13.0%, followed by Yan kasa with prevalence of 11.8% while Uda examined in this study were not infected with any haemoparasites.

Age based prevalence showed that the goat of 4 years and above had the highest prevalence with 23.1% followed by the goat in between 3-4 years with prevalence of 3.8%. However goat between 1-2 years and 2-3 examined in this study were not infected with any tick-borne haemoparasites (Table 5). The chi square analysis indicates no significant association between the ages of the goat with respect to infection with haemoparasites.

Occurrence of haemoparasites does not significantly associate with genders of the goat. Although, female sheep have the highest prevalence of 9.4% then male sheep with 5.6% as shown in (Table 6).

Among the different breed of goat examined in this study, Dwarf had the highest prevalence of infection with haemoparasites with 12.5%, followed by Sahelian with prevalence of 9.1% while Sokoto Maradi red examined in this study had the least prevalence of is haemoparasites. Statistical analysis showed no significant association in breeds of goat examined in this study with respect to infection with haemoparasites (Table 7).

Some hematological parameters of infected and uninfected Sheep and Goats are presented in (Table 8 and Table 9). Mean values of packed cell volume (PCV), haemoglobin (Hb), WBC, and RBC of infected and uninfected Sheep and Goats examined in this study were within normal range. However, there was significant difference ($p>0.05$) in mean packed cell volume, haemoglobin, WBC and RBC of infected and uninfected Sheep and Goats slaughtered at Sokoto central abattoir.

Table 1: Prevalence of tick-borne haemoparasitic infection among sheep and goat.

Animals	Specie of parasites	No positive	Prevalence (%)
Sheep	*Anaplasma spp*	4	8.0
	Babesia spp	1	2.0
Goat	*Anaplasma spp*	2	4.0
	Babesia spp	2	4.0

Table 2: Prevalence of haemoparasitic infection among sheep with respect to their age.

Age	No examined	No infected	Parasite recover	Prevalence (%)	X^2
1-2	10	0	Nill	0	2.06
2-3	13	1	*B. ovis* (1)	7.7	
3-4	17	3	*A. ovis* (3)	17.6	
4 and above	10	1	*A.ovis* (1)	10.0	
Total	50	5		10.0	

$X^2tab= 7.81$, df = 3, $p> 0.05$

Table 3: Prevalence of haemoparasitic infection among sheep based on their gender.

Gender	No examined	No infected	Parasite recover	Prevalence (%)	X^2
Male	37	3	*A. ovis* (4)	8.1	0.51
Female	13	2	*B. ovis* (1)	15.4	
Total	50	5		10.0	

X^2tab= 3.84, df= 1, p> 0.05

Table 4: Prevalence of haemoparasites infection among sheep based on their breed.

Breed	No examined	No infected	Parasites recover	Prevalence (%)	X^2
Yan kasa	17	2	*A. ovis* (2)	11.8	1.26
Balami	23	3	*A. ovis* (2) *B. ovis* (1)	13.0	
Uda	10	0	Nill	0	
Total	50	5		10.0	

X^2tab= 5.99, df= 2, p> 0.05

Table 5: Prevalence of haemoparasites infection among goat based on their age.

Age	No examined	No infected	Parasites recover	Prevalence (%)	X^2
1-2	4	0	Nill	0	5.13
2-3	7	0	Nill	0	
3-4	26	1	*B. ovis* (1)	3.8	
4 >	13	3	*A.ovis*(2) *B.ovis* (1)	23.1	
Total	50	4		8.0	

X^2tab= 7.81, df= 3, p> 0.05

Table 6: Prevalence of haemoparasites of goat based on their gender.

Gender	No examined	No infected	Parasites recover	Prevalence (%)	X^2
Male	18	1	*A. ovis* (1)	5.6	0.21
Female	32	3	*A. ovis* (1) *B. ovis* (2)	9.4	
Total	50	4		8.0	

X^2tab= 3.84, df= 1, p> 0.05

Table 7: Prevalence of haemoparasites of goat according to their breed.

Breed	No examined	No infected	Parasites recover	Prevalence (%)	X^2
S.M Red	31	2	*A. ovis* (2)	6.5	0.31
Sahelian	11	1	*B. ovis* (1)	9.1	
Dwarf	8	1	*B. ovis* (1)	12.5	
Total	50	4		8.0	

X^2tab= 5.99, df= 2, p> 0.05

Table 8: Haemotological parameters of infected and uninfected sheep.

Haemotological parameters	Mean ± S.E		
	Infected	Uninfected	Difference (Control)
PCV	31.0±1.0	42.5±1.8	3.93
Hb	7.5±0.5	11.0±0.7	2.92
RBC	6.5±0.3	12.0±0.35	8.46
WBC	7.5±0.61	9.5±0.35	3.1

Table 9: Haemotological parameters of infected and uninfected goat.

Haemotological parameters	Mean ± S.E		
	Infected	Uninfected	Difference (Control)
PCV	25.5±3.5	35.0±2.0	1.3
Hb	5.5±1.1	10.5±1.5	1.9
RBC	7.0±1.0	14.0±2.0	2.3
WBC	7.5±1.5	12.0±1.0	1.8

DISCUSSION

The results obtained from this study revealed that tick-borne haemoparasites are prevalent with 10.0% in sheep and 8.0% in goat examined at Sokoto modern abattoir.

The study further revealed a numerically higher prevalence in female adult sheep (9.4%) and goats (15.4%) than their male counterparts. The variation in the respective finding of haemoparasitic infections in females against the male sheep and goat could be attributed to the proportion of the female sheep and goat population sampled. It has also been reported that the female ruminants are generally more prone to infection by haemoparasites due to their extended breeding, parturition and milk production as well as the stress of breeding, milking and cyclical hormonal changes associated with gestation, parturition and calving processes. Furthermore, the sheep and goats slaughtered in Sokoto are raised under extensive and semi-intensive management systems in outdoor environments graze alongside with cattle. These increase their exposure to the arthropod vectors. Both *Anaplasama spp* and *Babesia spp* were identified in this study. *Anaplasma spp* was the most prevalent species in both sheep and goats with (10.0%). This finding agrees with previous reports (Okaiyeto *et al.,* 2008; Jatau *et al.,* 2011). The prevalence of these parasites elsewhere in Nigeria was linked with suitable microclimate favoring the propagation of their arthropod vectors (Jatau *et al.,* 2011). Similarly, the prevalence of tick-borne haemoparasites of sheep and goat slaughtered in Sokoto was linked with conditions favoring the bionomics of ticks (Paul *et al.*, 2016). Moreover, *Anaplasma spp* is a ubiquitous organism that has been reported in all the six continents (Rymaszewska *et al.,* 2008) and especially in the tropics and subtropics, due to the abundance of its tick vectors (Jongejan *et al.,* 2004). The low prevalence of *Babesia spp* (8.0%) was recorded in both sheep and goats in this study. This study agreed with previous reports (Bell-Sakyi *et al.,* 2004; Jatau *et al.,* 2011). This finding could be attributed to the enzootic occurrence of *babesiosis* in indigenous animals in Nigeria. Sheep and goats usually develop strong immunity in early life and resist subsequent challenges favorably by preventing establishment of the parasite (Soulsby, 1982).

This finding agrees with those of (Opara *et al.* 2016) who also reported significantly high level of haemoparasites infection rate in adult sheep and goat (100%) compared to the young (0.0%) ones. However, these finding contradicts those of (Opara *et al.*2016) who reported low level of haemoparasites infection rate in lambs (11.5%) compared to the adults (30.4%) sheep and goat. Previous studies have confirmed higher level of haemoparasitaemia in adult sheep reared under nomadism compared to same infection in lambs (Ademola and Onyiche, 2013; Nwoha *et al.,* 2013). This may be due to the fact that the lambs are closely watched and cared for by the Fulani pastoralists during grazing than the adults this may probably reduce infestation rate with arthropod vectors such as ticks and the corresponding disease.

In the present study, screening of breed, prevalence data revealed that cross-bred animals were mostly affected than indigenous animals. Breed susceptibility in this study are in line with observation made in crossbred Sheep of Bangalore North (Ananda *et al.* 2009). Further, it was observed in the present study that the occurrence of these tick-borne haemoparasites diseases were found to be high among the age groups of 3 and above years in both the cross breeds and also in indigenous animals. This study supports the report witnessing animals over 3 years of age are highly affected by tick-borne haemoparasitic diseases in cross-bred animals (Chakraborti, 2002). Most of the cross-bred animals aged around 4 and above years must be in the stage of 3 or 4 lactation as peak milk yielders. The weakening of immunity during high milk yielding stage in addition to genetic makeup and seasonal stress in summer months could be reason for high susceptibility to this tick-borne haemoparasite parasite.

This study revealed that, infection with tick-borne haemoparasites in sheep caused a significant ($p>0.05$) reduction in PCV (3.9), Hb (2.92) and total RBC counts. Furthermore, our results show a significant ($p>0.05$) reduction in the Hb concentration and total WBC count of goats. The anemia observed in this study characterized by a reduction in PCV and Hb concentration of infected sheep and goats, is consistent with previous reports. (Anosa, 1988) reported that, anemia is a predominant feature that often serves as a reliable indicator for severity of haemoparasitic infections. (Rymaszewska *et al.,* 2008) observed that progressive anemia usually develops during *anaplasmosis* and *babesiosis*. Furthermore, Anumol *et al.* (2011), reported that haemoparasites are responsible for most cases of anemia in goats. The pathogenesis of anemia in haemoparasitic infections is multifactorial in nature; emergence of parasites from RBC, mechanical rupture of RBC, spontaneous lysis of RBC due to increased osmotic fragility, direct removal of non-infected erythrocytes by phagocytosis and adsorption of circulating antigen antibody complexes to the surface of RBC, leading to their removal by phagocytosis (Soulsby, 1982). The observed reduction in WBC counts of infected goats in this study has been previously reported. A significant reduction in total WBC counts of dromedary camels infected with *babesiosis* in Saudi Arabia was described by (Swelum *et al.* 2014). This finding could be linked with concurrent infections and stress which may lead to immune suppression. Helminthosis and bacterial infections are usually encountered concurrently with tick-born haemoparasitic infections under field conditions in Nigeria (Okaiyeto *et al.*, 2008; Jatau *et al*., 2011), which may complicate the clinical course of haemoparasitic infections.

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