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VIRAL DETECTION IN TICKS AND THE LEVEL OF TICKS' INFESTATIONS AMONGST SMALL RUMINANTS IN MAIDUGURI, BORNO STATE

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ABSTRACT

Ticks were the first arthropods to be established as vectors of pathogens and are currently recognized, along with mosquitoes, as the main arthropod vectors of disease agents to humans and domestic animals globally due to increased incidences of tick-borne diseases (TBDs) the world over. They are responsible for the transmission of many viral diseases to humans and domestic animals. Therefore, ticks infection and infestation results in great economic losses to resource-poor farming communities, especially in tropical and subtropical regions, including Nigeria, where approximately 80% of the world's cattle population is raised. The current study is aimed to study the distribution, composition and abundance of ticks in Maiduguri, Borno State. Adult ticks were collected from 350 freshly butchered small ruminants (sheep and goats) from three locations within Maiduguri metropolis namely: Kasuwa shanu, Bulumkutu, and Maiduguri Central Abattoir. These were identified using taxonomy identification keys in to three species, *Rhipicephalus, Amblyomma and Hyalomma,* using stereomicroscope for each of the locations. They were crushed using bullet blender after the addition of glass beads and centrifuged. The supernatant from the crushed samples was used for viral detection by inoculating it on to two cell lines, Baby Hamster Kidney (BHK) and African Monkey Kidney (Vero E6), and were observed for cytopathic effects daily for a period of ten (10) days. The results showed a high level of ticks' infestation amongst small ruminants in the study locations. Kasuwan Shanu has the highest distribution of ticks amongst sheep, followed by Maiduguri Central Abattoir while Bulumkutu has the least. More also, Kasuwan Shanu has the highest distribution of ticks amongst goats, followed by Bulumkutu while Maiduguri Central Abbatoir has the least. The three species of ticks investigated were found to comprise of either male or female or both in the different locations, and either of the two sexes is involved in the infestation. The results also showed the presence of virus in some of the tick samples isolated from the small ruminants and inoculated on to the cell lines due to the observed cytopathic effects. It is concluded that there is high level of ticks' infestation among small ruminants in Maiduguri metropolis and a threat of viral disease transmission to humans and other animals.

Keywords: *Ripicephalus spp*, *Amblyomma spp*, *Hyalomma spp*, cytopathic effect, tick-borne diseases, BHK**,** Vero E6.

INTRODUCTION

Ticks are small arachnids of about 3 to 5mm long, belonging to the order parasitiformers (Nicholson *et al.,* 2010). They are obligate-blood feeders requiring an animal host and can reproduce along with mites. They constitute the sub class Acari, and are ectoparasites (external parasites) that thrive by feeding on the blood of mammals, birds, and sometimes reptiles and amphibians (Nicholson *et al.,* 2010).

Ticks are known to transmit many human and animal disease pathogens such as viruses, bacteria, rickettsiae, and protozoa,

which cause significant morbidity, and mortality amongst humans, livestock, companion animals, and/or wildlife (Mansfield *et al*., 2017). This, in turn, may result in major economic lost particularly to the owners of livestock affected by the disease. The relationship between the tick, its host and the pathogens they transmit has been shown to be very complex, and each may benefit or suffer detrimental effects due to the combination of physiological and immune mediated processes each elicits during infestation and infection (Merga and Mohammedkemal, 2022).

Ticks were the first arthropods to be established as vectors of pathogens and are currently recognized along with mosquitoes, as the main arthropod vectors of disease agents to humans and domestic animals globally due to increased incidences of tick-borne diseases (TBDs) the world over (Bukbuk *et al*., 2016; Hassan, 2022).

Many viral diseases such as spotted fever, relapsing fever, Lyme disease, tularaemia, some forms of encephalitis, and Texas cattle fever are transmitted to humans and domestic animals through tick bites during opportunistic feeding or through crushing of ticks by hand and, in some instances, through contact with infected viremic animal or ticks excrement. Therefore, ticks infection and infestation results in great economic losses to resource-poor farming communities, especially in tropical and subtropical regions, including Nigeria, where approximately 80% of the world's cattle population is raised (Gasseer, 2015).

This study seeks to study the distribution, composition and abundance of ticks amongst small ruminants (sheep and goats) in Maiduguri, Borno state, Nigeria to prevent and control tick infestations and tick-borne virus infections being responsible for some of the most serious emerging and re-emerging infectious disease problems facing the world today, which frequently occur in epidemic form.

MATERIALS AND METHODS

Study Area

The study was carried out on Ticks collected exclusively from Sheep and Goats in Kasuwan Shanu, Maiduguri central abattoir, and Bulumkutu area of Maiduguri, Borno State between September and October 2018. Maiduguri is the main capital city of Borno State, located in the Semi-arid zone of Borno State Nigeria with an area of about 69,436km^2 at latitude 10-30^0 N and longitude 12-15^{0}E. It is situated within the savannah vegetation zone with low rainfall. The state comprises of 27 Local Government Areas and shares boundaries with the Republic of Niger to the North, Republic of Chad to the Northeast, Cameroun to the East, Adamawa state to the South and Yobe State to the West. The population of the state is estimated to be 4,151,193 (FGN, 2007).

Inclusion Criteria

1. Only Adult living ticks from Sheep and Goats were sampled for the study.

2. Both soft and hard ticks were included in the study.

Exclusion Criteria

1. Larvae and nymphs of ticks were not included in the study.

2. Ticks from Sheep and Goats outside Maiduguri were not used for this study.

3. Ticks from Cattles and other Animals were not used for this study.

Ticks Identification and Processing

Tick identification

The taxonomy keys as described by Walker (2003) were employed in the identification of the various species of the ticks used for this study. The pooled ticks stored in the freezer at -20^{0}C was removed and separated into their species; *Rhipicephalus, Amblyomma*, and *Hyalomma* using Stereomicroscope. The separated tick samples were pooled together according to

species and areas collected and returned back to the freezer stored at -20^{0}C until required for virus isolation.

Washing of the Ticks

Phosphate Buffer Saline (PBS) plus (+) was dispensed into each tube containing ticks and was allowed to soak for 30 minutes, after which it was soaked in antibiotics for 30 minutes and then rinsed in 3 changes of distilled water.

Crushing of Ticks

The washed ticks were dispensed into a petri dish and cut in to pieces using a sterile blade; these were placed into a bullet blender tube in small numbers, glass beads were added after which the bullet blender machine was allowed to run for 5 minutes to crush the ticks. The crushed ticks were transferred into a 15ml chloroform resistant centrifuge tube to the 3ml mark and 5mls of PBS was added.

Centrifugation/Separation

The tubes were arranged in a cold centrifuge and spun at 3000rpm for 30minutes. The supernatant was separated into 2ml cryo vial and kept at -20^{0}C, until required.

Virus Inoculation/ Virus Isolation

200µl of tick extracts was inoculated onto Baby Hamster kidney (BHK 21) and African Monkey kidney (Vero E6) cell lines. The cells were seeded 48 hours prior to inoculation by adsorption with growth medium (Eagle's MEM supplemented with 10% Fetal Calf Serum). The monolayers were inoculated and observed daily for 10 days under an inverted microscope for cytopathic effects (CPE). The BHK 21 cell line and Vero E6 cell line were observed daily for 10 days. Samples that did not show evidence of CPE after 10 days of inoculation are declared negative whereas cells that showed evidence of CPE (+3 i.e. 75% and +4 i.e. 100% CPE) are declared positive. The positive cells were harvested by freeze thawing and separated into 2ml cryovial, then stored at -20^{0}C Freezer for further investigation.

RESULTS

Distribution of Ticks Amongst Small Ruminants (Sheep and Goats)

The results presented in table 1 below showed the distribution of ticks amongst small ruminants (sheep and goats) in Maiduguri. The results revealed that Kasuwa Shanu has the highest distribution of ticks amongst sheep with (64.1%), followed by (18.5%) in Abattoir, and the lowest distribution in Bulumkutu with (17.3). Amongst goats the highest distribution was recorded in Kasuwa Shanu with (53.5%), followed by Bulumkutu with (33.3%) and Abattoir, had the lowest distribution of the ticks with (13.2%) respectively.

Table 1: The Distribution of Ticks amongst Sheep and Goats

Location	Sheep	Goat	Total	% Sheep distribution	% Goat distribution
Bulumkutu	132	133	265	17.3	33.3
Abattoir	141	53	194	18.5	13.2
Kasuwa Shanu	488	214	702	64.1	53.5
Total	**761**	**400**	**1161**	**100**	**100**

Abundance and Composition of Ticks Amongst Sheep

The results presented in table 2 below showed the abundance and composition of ticks amongst sheep. The results revealed that Sheep in Kasuwa Shanu has the highest abundance of ticks with a total number of 488 ticks, out of which, (22.5%) were males and (10.2%) females for *Amblyomma species*, (12.3%) males and (7.2%) females for *Rhipicephalus species* while, (26.0%) males and (21.7%) females for *Hyalomma species*, followed by Abattoir with total

number of 141 ticks, out of which (28.3%) were females and (0.0%) males for *Amblyomma species*, (36.9%) females and (0.0%) males for *Rhipicephalus species*, then (0.0%) males and (34.8%) females for *Hyalomma species*. The results also showed that Bulumkutu had the lowest abundance and composition of ticks with 132 ticks, out of which, (39.4%) were females and (0.0%) males for *Amblyomma species*, (22.7%) males and (37.9%) females for *Rhipicephalus species*, but no *Hyalomma species* was collected from sheep in Bulumkutu.

Table 2: The Abundance and Composition of Ticks amongst Sheep

S/No.	Location	Animal	Site	No. of ticks collected	Species	Sex	No. of Sex	% abundance
1.	Bulumkutu	Sheep	Ear	132	*Amblyomma spp.*	Male	0	0.0
						Female	52	39.4
					Rhipicephalus spp.	Male	30	22.7
						Female	50	37.9
2.	Abattoir	Sheep	Ear	141	*Amblyomma spp.*	Male	0	0.0
						Female	40	28.3
					Rhipicephalus spp.	Male	0	0.0
						Female	52	36.9
					Hyalomma spp.	Male	0	0.0
						Female	49	34.8
3.	Kasuwa Shanu	Sheep	Ear	488	*Amblyomma spp.*	Male	110	22.5
						Female	50	10.2
					Rhipicephalus spp.	Male	60	12.3
						Female	35	7.2
					Hyalomma spp.	Male	127	26.0
						Female	106	21.7

Abundance and Composition of Ticks Amongst Goats

The results presented in table 3 below showed the abundance of ticks amongst goats. The results revealed that goats in Kasuwa Shanu has the highest abundance with total number of 214 ticks, out of which (32.7%) were males and (19.1%) females for *Amblyomma species*, (24.8%) females and (0.0%) male, for *Rhipicephalus species* and (23.4%) were males and (0.0%) females for *Hyalomma species* followed by Bulumkutu with total number of 133 ticks, of which (70.0%) were males and (30.0%) females for *Hyalomma species* while, *Amblyomma* and *Rhipicephalus species* were not collected from goats. Abattoir had the lowest abundance of ticks with total number of 53 ticks, out of which (52.8%) were male and (0.0%) females for *Amblyomma species*, (47.2%) males and (0.0%) females for *Rhipicephalus species* and (34.8%) were females and (0.0%) male, for *Hyalomma specie.*

Viral Detection in Ticks amongst Sheep

The results presented in Table 4 below showed viral detection in ticks collected amongst sheep. In Bulumkutu, the results showed the presence of cytopathic effects (CPE) on both Vero E6 and BHK 21 cell lines in both *Amblyomma* and *Rhipicephalus species*, while *Hyalomma* were not collected from sheep in the area. In Abattoir, *Amblyomma species* showed the presence of CPE on BHK 21 but no CPE on Vero E6, *Hyalomma species* showed the presence of CPE on both Vero E6 and BHK 21 cell lines, while *Rhipicephalus species* showed no CPE on both Vero E6 and BHK 21 cell lines. In Kasuwa Shanu, *Amblyomma, Rhipicephalus* and *Hyalomma species* showed the presence of CPE on both Vero E6 and BHK 21 cell line.

Table 3: The Abundance and Composition of Ticks amongst Goats

S/No.	Location	Animal	Site	No. of ticks collected	Species	Sex	No. of Sex	% abundance
1.	Bulumkutu	Goat	Ear	133	*Amblyomma spp.*	Male	0	0.0
						Female	0	0.0
					Rhipicephalus spp.	Male	0	0.0
						Female	0	0.0
					Hyalomma spp.	Male	93	70.0
						Female	40	30.0
2.	Abattoir	Goat	Ear	53	*Amblyomma spp.*	Male	28	52.8
						Female	0	0.0
					Rhipicephalus spp.	Male	25	47.2
						Female	0	0.0
					Hyalomma spp.	Male	0	0.0
						Female	0	34.8
3.	Kasuwa Shanu	Goat	Ear	214	*Amblyomma spp.*	Male	70	32.7
						Female	41	19.1
					Rhipicephalus spp.	Male	0	0.0
						Female	53	24.8
					Hyalomma spp.	Male	50	23.4
						Female	0	0.0

Table 4: Viral Detection in Ticks amongst Sheep

Location	Species	Cell Lines	Results
Bulumkutu	*Amblyomma spp.*	Vero E6	Pos. (+4)
		BHK 21	Pos. (+3)
	Rhipicephalus spp.	Vero E6	Pos. (+4)
		BHK 21	Pos. (+4)
	Hyalomma spp.	Vero E6	ND
		BHK 21	ND
Abattoir	*Amblyomma spp.*	Vero E6	Neg.(-)
		BHK 21	Pos. (+)
	Rhipicephalus spp.	Vero E6	Neg.(-)
		BHK 21	Neg.(-)
	Hyalomma spp.	Vero E6	Pos. (+3)
		BHK 21	Pos. (+3)
Kasuwa Shanu	*Amblyomma spp.*	Vero E6	Pos. (+4)
		BHK 21	Pos. (+4)
	Rhipicephalus spp.	Vero E6	Pos. (+4)
		BHK 21	Pos. (+3)
	Hyalomma spp.	Vero E6	Pos. (+3)
		BHK 21	Pos. (+3)

Pos. =Positive (Presence of CPE)

Neg. = Negative (No CPE)

ND = Not detected

Positive [+3 **(75%),** +4 **(100%)**] **=** Cytopathic effects (CPE) i.e. virus

BHK 21= Baby Hamster Kidney Cell line

Vero E6 = African Monkey Kidney cell line

Viral Detection in Ticks amongst Goats

The results presented in Table 5 below showed the results of viral detection in ticks collected amongst goats. In Bulumkutu, the results showed the presence of CPE on both Vero E6 and BHK21 cell lines in both *Rhipicephalus* and *Hyalomma species*, while *Amblyomma species* were not collected from goats in the area. In Abattoir, both *Amblyomma* and *Rhipicephalus species* showed no CPE on both Vero E6 and BHK21 cell lines, while *Hyalomma species* showed the presence of CPE on both Vero E6 and BHK21 cell lines. Results of viral detection for Kasuwa Shanu showed the presence of CPE on both Vero E6 and BHK21 in *Amblyomma, Rhipicephalus* and *Hyalomma species*.

Table 5: Viral Detection in ticks amongst goats

Location	Species	Cell Lines	Results
Bulumkutu	*Amblyomma spp.*	Vero E6	ND
		BHK 21	ND
	Rhipicephalus spp.	Vero E6	Pos. (+4)
		BHK 21	Pos. (+4)
	Hyalomma spp.	Vero E6	Pos. (+4)
		BHK 21	Pos. (+3)
Abattoir	*Amblyomma spp.*	Vero E6	Neg.(-)
		BHK 21	Neg. (-)
	Rhipicephalus spp.	Vero E6	Neg.(-)
		BHK 21	Neg.(-)
	Hyalomma spp.	Vero E6	Pos. (+4)
		BHK 21	Pos. (+3)
Kasuwa Shanu	*Amblyomma spp.*	Vero E6	Pos. (+4)
		BHK 21	Pos. (+4)
	Rhipicephalus spp.	Vero E6	Pos. (+4)
		BHK 21	Pos. (+3)
	Hyalomma spp.	Vero E6	Pos. (+4)
		BHK 21	Pos. (+3)

Pos. =Positive (Presence of CPE)
Neg. = Negative (No CPE)
ND = Not detected
Positive **[**+3 **(75%),** +4 **(100%)] =** Cytopathic effects (CPE) i.e virus
BHK= Baby Hamster Kidney Cell line
Vero E6 = African Monkey Kidney cell line

More also, figure 1, and 3 showed a healthy monolayer Baby Hamster Kidney (BHK) cell line on the date third day after inoculation and figure 2 and 4 indicate the presence of virus in *Rhipicephalus species* and *Amblyomma species* on the third day of inoculation by the visible cytopathic effects observed on these cell lines as shown below.

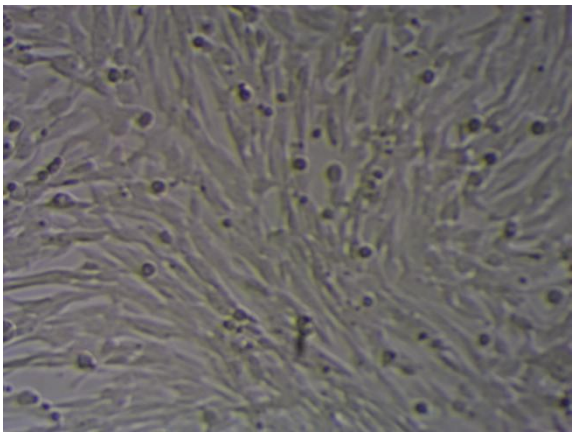

Figure 1: Healthy Monolayer of Baby Hamster Kidney (BHK) Cell line on the 3rd day

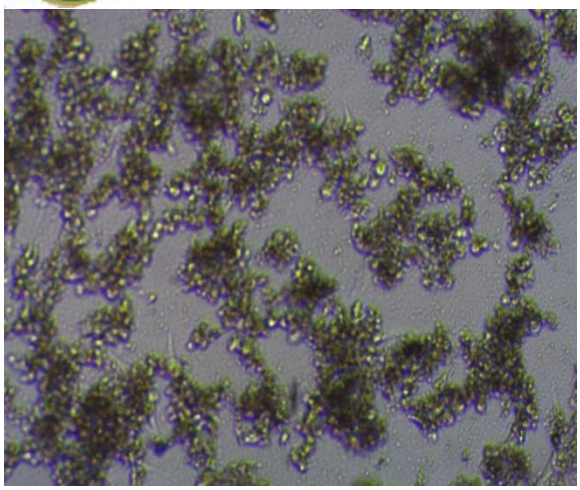

Figure 2: Cytopathic effects from Virus contained in Crushed Inoculated Ticks (*Rhipicephalus species*) on the 3rd day.

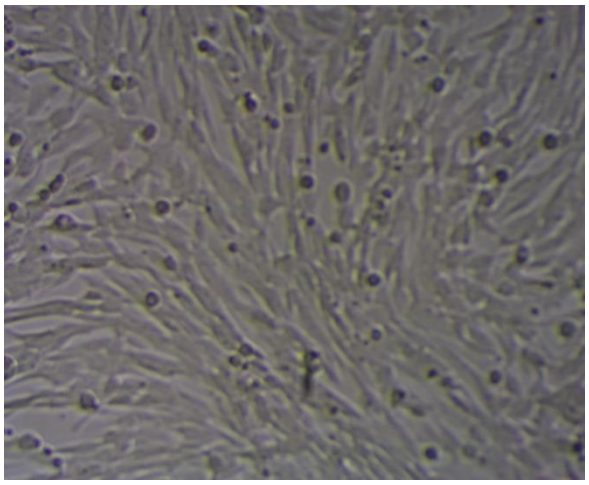

Figure 3: Healthy Monolayer of Baby Hamster Kidney (BHK) Cell line on the 3rd day

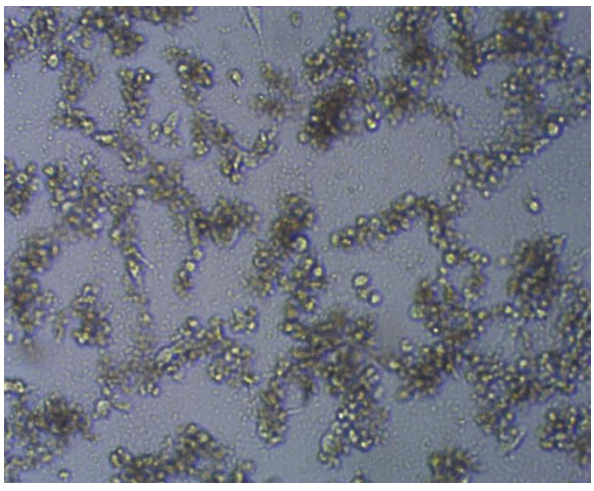

Figure 4: Cytopathic effects from Virus contained in Crushed Inoculated Ticks (*Amblyomma species*) on the 3rd day.

DISCUSSION

Ticks are ectoparasites that thrive by feeding on the blood of mammals, birds and sometimes reptiles and amphibians making them a nuisance as their bites can cause irritation, and in the case of some ticks, paralysis (Nicholson *et al.,* 2010). They are implicated in some of the serious emerging and re-emerging infectious diseases bedeviling the world today, which frequently occur in the form of epidemics. Therefore, Herders, Butchers, and public health professionals (Veterinarians) that come in contact with infectious blood from infected animals are at risk of acquiring these diseases (Bukbuk *et al*., 2016).

The results found in this study indicate that ticks infestation was high amongst small ruminants (sheep and goats) with Kasuwa Shanu having the highest distribution of ticks amongst sheep with (64.1%), followed by (18.5%) in Abattoir, and lowest distribution in Bulumkutu with (17.3%). Whereas, amongst goats the highest distribution was recorded in Kasuwa Shanu with (53.5%), followed by Bulumkutu with (33.3%) and Abattoir had the lowest distribution of the ticks with (13.2%) respectively as depicted in table 1.0. This is in conformity with a report that ticks are widely distributed around the world, especially in warm, humid climates characteristics of the weather of Maiduguri, Borno State (Memishet *et al.,* 2005). This also agreed with findings reported by Musa *et al.* (2014) that there is a high prevalence of tick infestation in Maiduguri. More also, a study carried out in Askira Uba Local Council of Borno State revealed a high level of tick infestation in the area (Musa *et al*., 2014).

This study also showed the abundance and composition of ticks among sheep and goats in Bulumkutu, Abattoir and Kasuwa Shanu area, Maiduguri. Sheep in Kasuwa Shanu have the highest abundance of 488 ticks, out of which, (22.5%) were males and (10.2%) females for *Amblyomma species*, (12.3%)

males, and (7.2%) females for *Rhipicephalus species* while, (26.0%) males and (21.7%) females for *Hyalomma species*, followed by Abattoir with a total number of 141 ticks, having only females but no males in all the three species of ticks studied. Bulumkutu had the lowest abundance and composition of ticks (132), with more female species of *Amblyomma* and *Rhipicephalus* than males but no *Hyalomma species* was collected. The prevalence of various species of ticks in different ecological zones of Nigeria has been reported (Musa *et al.,* 2014). This study is in agreement with previous findings, which showed that *Hyalomma* and *Rhipicephalus* are the much more predominant species of ticks to survive in dry areas and that *Hyalomma dromedarii* appeared to be the most successful desert adapted ixodid tick amongst the *Hyalomma species* (Gabaj *et al*., 1992; Biu and Nwosu, 1998).

Furthermore, Bukbuk *et al*. (2016) who made a survey of hard ticks (Acari: Ixodidae) amongst Sheep in Borno State reported that *Boophilus spp* were the most abundant tick species infesting sheep in the study area and that the predilection site for most of the ticks was the ear, which his is in agreement with this study because virtually all the ticks were collected from the ear.

The preference of a particular site of the animal body by tick species, as is the case of ear in this study, has been suggested by a previous researcher to be as a result of variations in microbial conditions and temperature on the body of the hosts, as well as easy accessibility of blood vessels (Madakan *et al*., 1993).

The results also showed the abundance of ticks among goats, revealed that, goats in Kasuwa Shanu has the highest abundance with total number of 214 ticks, with more males than females *Amblyomma species*, females and no males for *Rhipicephalus species,* and males and with no females for *Hyalomma species,* followed by Bulumkutu with total number of 133 ticks, with more males than females for *Hyalomma species* whereas, *Amblyomma* and *Rhipicephalus species* were not collected from goats in this location. Abattoir had the lowest abundance of ticks with total number of 53 ticks, with males and no females for *Amblyomma and Rhipicephalus species,* and females but no males for *Hyalomma species.* These results is in conformity with that of Aminu (2015) who carried out a survey of Cattle, Sheep and Goat ticks infestation in Katagum Local Government Area of Bauchi State, Nigeria and discovered that goats are mainly infested by four species of ticks, prevalent amongst which is *Rhicephalus sanguinensis* and the less prevalent species was *Amblyomma variegatum*. Another similar findings was that carried out by Ofukwu and Akwuobu (2010) who studied the epidemiology of ectoparasites infestation of sheep and goats in Makurdi, North Central, Nigeria and reported that the number of sheep infested with ticks is higher (40.7%) than in goats (31.5%), a similar trend of which was observed in this study, and that the commonest species of ticks observed were *Rhipicephalus evertsi* and *Amblyomma variegatum.*

The results clearly depict the fact that the composition of ticks in terms of sex and species distribution in the study locations is very irregular, which may suggest their vulnerability to environmental conditions, often requiring very specific conditions of humidity and temperature to thrive as stated by Estrada-Pen˜a *et al.* (2004). More so, several external factors may interact to produce such patterns with a predominant role for climate change due to direct modifications in temperature, rainfall and humidity, and their associated indirect effects on vegetation, host diversity and abundance (Gray *et al.,* 2009). It has been postulated that tick distributions are more limited by climatic conditions than by the presence and abundance of specific host types (Cumming 2002).

The presence of CPE in the ticks extracts inoculated on the cell lines as depicted in tables 4 and 5, and figure 2 and 4, is a clear indication that the virus is circulating among sheep and goats and as such people that domesticate these animals, butchers, and veterinarians are at risk of contracting viral diseases harbored by the ticks that infest these animals.

Recent studies using in vitro models are beginning to identify transcriptional responses in ticks' cell during infection with viruses (Mansfield *et al*., 2017; Maya-Delgado *et al*., 2020).

However, new examples of emerging tick-borne viral diseases that affect man are constantly being reported. In addition, Crimean Congo Haemorrhagic fever virus (CCHFV) is primarily transmitted by the *Hyalomma* species (Mertens *et al*., 2013).

In a similar study conducted by Bukbuk *et al.* (2016) on serological and virological evidence of Crimean-Congo Haemorrhagic Fever Virus Circulation in the Human Population of Borno State, Northeastern Nigeria, reported that there was evidence of the presence of Crimean Congo Haemorrhagic fever virus (CCHVF) antibodies with the positive rates for IgG and IgM of 10.6% and 3.5% respectively among the human population in the study area. From the above findings it could be inferred that CCHVF is endemic in the population of Maiduguri environs, and ticks are the major reservoir of the virus.

Ticks are vectors of a wide-range of human and livestock disease agents. Therefore, any factor that limits their distribution is essential for understanding and predicting disease occurrence and emergence (Thomas *et al.,* 2004; Maya-Delgado *et al*., 2020).

CONCLUSION

This study has shown that there is a high prevalence of ticks infesting sheep and goats in Maiduguri, notably the ticks species found were *Hyalomma, Amblyomma*, and *Rhipicephalus*. *Hyalomma* and *Rhipicephalus* species are the major vector for viruses, and these could be a serious threat to the health status of the populace especially the herders, butchers and other public health professionals like the veterinarians working in the abattoir.

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