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Journal of Parasitology and Vector Biology

Full Length Research Paper

# Identification and prevalence of ixodid tick in bovine at Bedele district, Oromiyia Regional State, Western Ethiopia

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#### Received 14 August, 2015; Accepted 24 August, 2015

A cross sectional study was conducted with the aim of identifying and estimating the prevalence of cattle tick infestation with respect to host related factors in Bedele district, Western Ethiopia. A total of 384 cattle were considered in the study, and both physical examination and microscopical investigation were employed. The study revealed that there was high tick infestation in the study with an overall prevalence of 315 (82%). Four species of ixodidae ticks were identified from the study area. Among the ticks, Amblyomma cohaerens (41.5%) was the most prevalent tick species while Amblyomma variegatum was the least prevalent (6.5%) tick species recorded in the study. All species of ticks had more than one male to female ratio except Rhipicephalus (Boophilus) decoloratus (0.0097:1). There was no statistically significant association between hosts related factors and tick prevalence except for body condition score. Cattle with poor body condition have significantly (p < 0.05) higher tick burden than cattle with the other body condition scores. All tick species were distributed and attached with statistically significant (p < 0.05) variation among different parts of the host body, while all ticks inflict significantly diverse (p < 0.05) types of lesion except A. variegatum. Overall, the present study revealed very high prevalence of tick infestation that could potentially hamper the productivity of cattle in the study area, hence a serious measure should be put in place to control and reduce the adverse effect of tick infestation.

Key words: Bovine, identification, prevalence, ticks.

#### INTRODUCTION

Ticks were considered as parasites of domestic animals as early as 400 B.C. Aristotle in his famous historia animalium, stated that the ticks were disgusting parasites generated from grass. Despite this early realization, little work was done until the latter half of nineteenth century, when a number of parasitologists all over the world started working on taxonomy, prevalence, and bionomics, seasonal and regional occurrence of the ticks (Dobbelarece and Heussler, 1999). Ticks are obligate blood feeding ectoparasites of vertebrates; particularly mammals, birds and reptiles throughout the world (Rajput et al., 2006). They are cosmopolitan in distribution, but occur principally in tropical and subtropical regions with warm and humid climate which are suitable to undergo

\*Corresponding author. E-mail: natenealtamerat@yahoo.com. Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> metamorphosis (Kilpatrick et al., 2007).

There are two well established families of ticks, the ixodidae (hard tick) and the argasidae (soft ticks). Of the ixoidae families, *Dermacntor, Rhipicephalus, Heamaphysalis, Boophlius, Ambylomma, Hyalomma, and Aponomma* genera have a great veterinary importance (Wall and shearer, 2001; Walker et al., 2003). Over 79 different species are found in Eastern Africa but many of these appear to be of little or no economic importance. The highest impact on livestock health is caused by species belonging to only three genera, namely, *Amblyomma, Hyalomma and Rhipicephalus* (Cumming, 1999).

Ethiopia has the largest livestock population in Africa that contributes to 40% of agricultural output/GDP in Ethiopia (CSA, 2013). Even though the livestock sub sector contributes much to the national economy, its development is hampered by different constraints. Ectoparasites are one of the most important constraints that directly or indirectly affect the socio-economic development of poor farmers (Bekele. 2002). Ectoparasites in ruminant causes serious economic loss to small holder farmers, the tanning industry and the country as a whole through mortality of animals, decreased production, down grading and rejection of skin and hide (Tikit and Addis, 2011).

Ticks infestation is severe in different parts of Ethiopia and at a conservative estimate, one million USD is lost annually only through rejection of downgraded hides and skins attributed to tick damage (Gashaw, 2005). Bekele (2002), estimated that an annual loss of USD 5000,000 from hide and skin downgrading from ticks, and approximately 65.5% of major defects of hide in eastern Ethiopia were from ticks. Even though losses due to tick infestation is considerable in Ethiopia, and a number of researchers reported the distribution and abundance of tick species in different parts of the country, there is no work done in estimating the prevalence and distribution of ticks in Bedele district.

Therefore, the current study was conducted with the objectives of estimating the prevalence and identification of ixiodid ticks with respect to host related variables in Bedele district.

#### MATERIALS AND METHODS

#### Study area description

The study was conducted in Bedele district which is located in western Oromia regional state on a distance of 483 km to the west of Addis Ababa. Geographically, Bedele falls between 36° to 28° 80° N latitudes and 36° to 20°79°N longitudes. The total land area covers 1140.57km<sup>2</sup> with the altitude of 1400 to 2010 m above sea level. The annual mean temperature ranges from 12.5 to 27.5°C, and receives the annual rainfall greater than 1400 mm. The farming system of the area is a mixed farming system where 87% of the total population is engaged in agriculture. The livestock population is estimated to be 59,233 cattle, 40,543 sheep, 9,378 goats, 38,386 poultries and 1,878 equines of livestock populations (BWAB, 2006).

#### Study design and study animals

A cross-sectional study design was implemented from November, 2014 to April, 2015 to determine the prevalence of ixodid tick infestation and associated effect in bovines in Bedele district. The study population consists of cattle managed under extensive, semi intensive and intensive management system which constitute exotic, cross and local breeds.

#### Sample size determination and sampling method

The sample size was determined by assuming the expected prevalence of 50% tick infestation. The desired sample for the study was calculated by setting 95% confidence interval at 5% absolute precision (Thrusfield, 2007).Therefore, sample size of 384 cattle were examined in the study. Areas in the district were selected purposively according to accessibility and the cattle within the selected areas were selected and examined randomly from the household.

#### Study methodology

The host related factors like age and body condition were classified into groups for the convenience of the study. The age of the cattle were grouped into young (1 to 2 year), adult (3 to 7 years) and old (> 8 years) according to Gatenby (1991). While body condition score were grouped into poor, medium and good according to Nicholson and Butterworth (1986) after some modification.

#### Tick collection and preservation

The entire body surface of the animals was inspected for the presence or absence of ticks, and half body tick collections on alternative sides were made. Adult ticks were collected from different parts of body regions; dewlap, axillae, udder, groin, shoulders, hump, back, belly, flank, perineum, vulva, anus, under tail, scrotum, teat, prepuce, hind leg and sternum of animals after being restrained using physical handling. Date of collections, address, sites of attachment, associated lesion, breed, age, sex, body condition score and management system of animals were registered.

In addition, lesion inflicted on the animals due to tick infestation was recorded during the study. Ticks were removed from the host skin whilst retaining their good condition for identification using hand manually (Wall and Shearer, 2001). The collected ticks from each body regions were preserved in separate pre-filled universal bottles with 70% ethyl alcohol before transportation to parasitology laboratory for identification.

#### Laboratory examination for tick identification

The collected ticks were identified using stereomicroscope and classified to different genera levels based on size, mouthparts, colour of the body, leg colour, presence and absence of the eye. Furthermore, different morphology tick such as shape of scutum, leg colour, body, coxae one, festoon and ventral plates were considered for species level identification according to Walker et al. (2003).

#### Data analysis

The collected data was entered into Microsoft excel spread sheet and it was transferred to statistical package for the social sciences

Tick species	No. of infested animals	Prevalence (%)	Total no. of collected ticks	Percentage of total ticks
A.cohaerens	159	41.5	1030	51.91
B.decoloratus	124	32.3	620	31.25
R.evertsievertsi	52	13.5	236	11.98
A.variegatum	25	6.5	98	4.9
Total	315	82	1984	100

Table 1. Prevalence of ticks and of percentage tick species from the collected ticks.

Table 2. Identified tick species count and sex ratio.

Tiekenseise		Mala ta Famala Datia		
TICK Species	Male count (no.) Female count (no.)		wale to remale Ratio	
A. cohaerens	634	396	1.6:1	
B. decoloratus	6	614	0.0097:1	
Rh. evertsi evertsi	142	94	1.5:1	
A. variegatum	62	36	1.7:1	
Total	844	1140	-	

Table 3. Number of infested animal and prevalence of tick species with respect to breed and management system of bovine.

	Breed				Management Semi				
Tick species				$x^2(x)$				2()	
	Local	Local Cross Exotic		X (P)	Extensive	Intensive	Intensive	X (P)	
	no. (%)	no. (%)	no. (%)		no. (%)	no. (%)	no. (%)		
A.cohaerens	152 (39.7)	6 (1.6)	1 (0.3)	1.83 (0.4)	154 (40.2)	4 (1.0)	1 (0.3)	2.2 (0.33)	
B.decoloratus	113 (29.4)	7 (1.8)	4 (1)	5.52 (0.06)	113 (30.7)	5 (1.3)	6 (1.6)	11.7 (0.3)	
Rh.evertsi evertsi	51 (13.3)	1 (0.3)	-	2.01 (0.36)	52 (13.5)	-	-	2.61 (0.27)	
A.variegatum	24 (6.2)	1 (0.3)	-	0.41 (0.81)	24 (6.2)	1 (0.3)	-	0.8 (0.67)	
Total	340 (88.6)	15 (4)	5 (1.3)	-	343 (90.6)	10 (2.6)	7 (1.9)	-	

(SPSS) version 20 for analysis. Association between explanatory variables (breed, sex, age, body condition score and management system) and outcome variable (tick infestation) was done using chi-square ( $X^2$ ) test and percent values. In all analysis, all statistics were considered as significant at p < 0.05, while the confidence interval was set at 95% and 5% error probability.

#### RESULTS

The overall prevalence of ticks in the study area was 315 (82%) including single and mixed infestation (Table 1). Four ixodidae tick species were identified from the study area which belong to *Amblyomma* (Figure 1a, b, c and d) and *Rhipicephalus* (Figure 1e, f and g) genera of ticks. *Ambloyomma cohaerens* was the predominant tick species which was collected (1030 in number with 41.5% prevalence), while *Ambloyomma varigatum* was the least prevalent tick (98 in number with 6.5% prevalence)

(Table 1). In the present study, male to female sex ratio for tick species indicated higher number of males than females for all species of tick except *Rhipicephalus* (*Boophilus*) decoloratus which had (0.0097:1) ratio of male to female tick (Table 2). Regarding the host related factors in the study, there was no statistically significant variation (P > 0.05) in prevalence of ticks between the breed, sex, age and management system of the cattle production (Table 3, 4). On the other hand, poorly conditioned animals were significantly (p < 0.05) infested more than the other groups in each species of identified ticks (Table 4).

In this study, tick species attachment site was investigated. *A. cohaerens* mostly tend to attach to genital area (scrotum/prepuce/premium/vulva) (14.9%), *A. variegatum* to groin/hind leg (7.9%), *R. (Boophilus) decoloratus* to dewlap (14.7%) and *Rhipicephalus evertsi evertsi* to anal region (anus/under tail) (14.9%). There



Figure 1. (a) Pictures of identified tick species. *Amblyomma cohaerens* male (dorsal and ventral side), (b) Amblyomma cohaerens female (dorsal and ventral side), (c) *Amblyomma variegatum* male (dorsal and ventral side), (d) *Amblyomma variegatum* female (dorsal and ventral side), (e) *Rhipicephalus evertesi evertesi* male (dorsal and ventral side), (f) *Rhipicephalus evertesi evertesi* female (dorsal and ventral side), (g) *Rhipicephalus (Boophilus) decoloratus* females (dorsal and ventral side).

was statistical significant difference between all tick species and attachment site of ticks to host (p < 0.05) (Table 5). Concerning tick inflicted lesions on the cattle, *A. cohaerens* (35.0%) was the dominant tick species which inflict bite mark followed by *R. evertsi evertsi* (14.1%), while *R. (Boophilus) decoloratus* (6.3%) was the leading tick species in inflicting dermatitis followed by *A. cohaerens* (3.9%). Abscessation, inflamation, skin keratinization and focal hemorrhage were dominantly inflicted by *Amblyomma*. There was statistically significant association (p < 0.05) between the lesion inflicted and tick species infestation except for *A*.

variegatum (p > 0.05) (Table 6).

#### DISCUSSION

In present study, there was high prevalence (82%) of tick infestation. This finding is in agreement with the reports of Alemu et al. (2014) with overall prevalence of 81.5%. However, the prevalence of ticks in the current study is greater than the reports of Gedilu et al. (2014), Tadesse and Sultan (2014) and Abdisa (2012) who reported prevalence of tick infestation with overall prevalence of

Tiels en esias	Sex			Age			BCS			
LICK Species	Female No. (%)	Male No. (%)	Young No. (%)	Adult No. (%)	Old No. (%)	Poor No. (%)	Medium No. (%)	Good No. (%)		
A. cohaerens	91 (23.8)	68 (17.8)	51 (13.3)	82 (21.4)	26 (6.8)	81 (21.1)	63 (16.4)	15 (3.9)		
χ²(p-value)	0.110 (0	0.740)		0.27 (0.893)			7.37 (0.02)			
B. decoloratus	70 (18.2)	54 (14.1)	40 (10.4)	67 (34.5)	17 (4.4)	50 (13)	48 (12.5)	26 (6.8)		
χ <sup>2</sup> (p-value)	0.19 (0.65)		5.52 (0.06)			7.9 (0.02)				
Rh. evertsi evertsi	30 (7.8)	22 (5.7)	14 (3.6)	27 (7.0)	11 (2.9)	27 (7.0)	21 (5.5)	4 (1.0)		
χ <sup>2</sup> (p-value)	0.004 (0.95)		0.004 (0.95) 1.69 (0.42)			3.05 (0.02)				
A. variegatum	12 (3.1)	13 (3.4)	10 (2.6)	13 (3.4)	2 (0.5)	10 (2.6)	13 (3.4)	2 (0.5)		
χ <sup>2</sup> (p-value)	1.11 (0.29)		1.46 (0.48)			4.713 (0.09)				
Total	203 (52.9)	157 (41)	125 (29.9)	189 (66.3)	56 (14.6)	168 (43.7)	145 (37.8)	47 (12.2)		

Table 4. Number of infested animal and prevalence of tick species in relation to sex, age and body condition score of bovine.

Table 5. Number of affected animal and tick prevalence in relation to attachment site on the host.

Tick species	Bite mark no. (%)	Dermatitis no. (%)	Abscessation no. (%)	Inflammation no. (%)	Skin keratinization no. (%)	Focal hemorrhage no. (%)	χ²(p)
A. cohaerens	72 (35.0)	8 (3.9)	11 (5.3)	19 (9.2)	10 (4.9)	37 (18.0)	13.1 (0.022)
B. decoloratus	-	13 (6.3)	-	3 (1.5)	3 (1.5)	-	137.7 (0.00)
R. evertsi evertsi	29 (14.1)	-	4 (1.9)	12 (5.8)	-	2 (1.0)	19.7 (0.00)
A. variegatum	10 (4.9)	-	-	4 (1.9)	2 (1.0)	9 (4.4)	7.3 (0.199)
Total	111 (54.0)	21 (10.2)	15 (7.2)	36 (18.4)	15 (7.4)	48 (23.4)	-

Table 6. Number of affected animals and tick prevalence in relation to lesions inflicted by ticks.

Attachment sites										
Tick species	Face no. (%)	Dewlap no. (%)	Neck/shoulder/ hump no. (%)	Belly/flank/ back no. (%)	Scrotum/perineum/ prepuce/vulva no. (%)	Anus/under tail no. (%)	Axillae/ sternum no. (%)	Groin/hind leg no. (%)	Udder/ teat no. (%)	χ²(p)
Ambloyomm. cohaerens	-	16 (5.1)	1 (0.3)	-	47 (14.9)	17 (5.4)	11 (3.5)	21 (6.7)	46(14.6)	181.1 (0.0)
Rh.(Booph) decoloratus	10 (3.2)	47 (14.9)	28 (8.9)	28 (8.9)	4 (1.3)	3 (1.0)	-	-	3 (1.0)	238.1 (0.0)
Rh. evertsi evertsi	-	-	-	-	5 (1.3)	47 (14.9)	-	-	-	226.3 (0.0)
Ambloyomm. variegatum	-	2 (0.6%)	-	-	7 (2.2)	1 (0.3)	6 (1.9)	1 (0.3)	8 (7.9)	43.5 (0.0)

#### 74, 59.4 and 53.2%, respectively.

In addition, various researcher works has proven to find less prevalence of tick infestation

than the present study including the reports of Addis (2011) and Onu and Shiferaw (2013) who indicated tick prevalence of 25.64 and 14.5%,

respectively. This difference could be due to the difference in the agro climatic condition of the study areas, since tick activity was influenced by rainfall, altitude and atmospheric relative humidity according to Pegram et al. (1981).

*A. cohaerens* was found to be the most abundant tick species with prevalence of 41.5 and 51.86% of total tick collected in the present study (Table 1). Likewise, Belay (2004) had reported high prevalence of *A. cohaerens* (50.5%). On the contrary, Gedilu et al. (2014), Huruma et al. (2015), Alemu et al. (2014) and Abdisa (2012) had reported *A. cohaerens* as the least prevalent tick species with a prevalence of 0.20, 2.4, 5.21 and 7.73% in their respective study. This can be attributed to the great susceptibility of *A. cohaerens* for losses of total body water which ultimately make it to perish rapidly when the humid protection is disrupted according to Gashaw (2005).

On the other hands, *R. (Boophilus) decoloratus* was the second most abundant tick species in the present study with prevalence of 32.3% (Table 1), which is in line with the findings of Alemu et al. (2014), Gedilu et al. (2014) and Bedaso et al. (2014) who reported *Boophilus decoloratus* as the most abundant tick with respective prevalence of 40.86, 47.93 and 26.3%, respectively. This might be due to *B. decoloratus* been abundant in wetter highlands and sub-highlands receiving more than 800 mm rainfall annually according to Pegram et al. (1981). *R. evertsi evertsi* was the third most abundant tick species in the present study area with prevalence of 13.5% (Table 1), which agrees with reports of Alemu et al. (2014) with prevalence of 11.51%.

In another research, *R. evertsi evertsi* was the second most abundant tick species with prevalence of 50.9% according to Abdisa (2012) and it was the most abundant tick species with prevalence of 53.4% according to Huruma et al. (2015) finding. On the other hand, *A. variegatum* was the least abundant tick species in the present study area with prevalence of 6.5% (Table 1), which agrees with reports of Onu and Shiferaw (2013) with prevalence of 4.7% and Abebe et al. (2010) with prevalence of 4.2%. However, the findings of Tadesse and Sultan (2014), Bedaso et al. (2014) and Addis( 2011) were greater than the current finding with prevalence of 32.2, 41 and 45.49%, respectively.

The current study indicates that the numbers of male ticks were higher than the number of females except in *R*. (Boophilus) decolaratus in which the number of females are higher than female ticks (Table 2). This finding was in agreement with the report of Abdisa (2012) and Bedaso et al. (2014) who reported the similar trend. This might be attributed to the fact that male ticks take less food than females but remain longer on the host and can mate with several females. Furthermore, the observed female outnumbering of male ticks in *B. decolaratus* in the current study might be due to the small size of male tick which may not be seen during collection according to Huruma et al. (2015) who reported the same result.

In the current study, animals with poor body condition were highly infested (p < 0.05) than the other body condition groups by each species of the ticks (Table 4).

This finding is in line with the work of Bilkis et al. (2011) and Wolde and Mohamed (2014) who reported cattle with poor body condition were significantly (P < 0.05) infested more than that of cattle with normal body condition. This may be due to the fact that poorly conditioned animals were least resistant to tick infestation and lack enough body potential to build resistance whereas over-conditioned animals showed reasonable combat to the infestation according to Manan et al. (2007). Alternatively, tick infestation might be a cause for poor body condition; hence high prevalence was computed in this group of animals.

Regarding the attachment site of the ticks, there was statistically significant (p < 0.05) difference in attachment site on host in present study (Table 5). The predilection sites found in this study corroborate with those reported by Wolde and Mohamed (2014) at southern part of Ethiopia. Specifically, Kabir et al. (2011) at Bangladesh also reported that hard tick infestation on groin and mammary glands was most prevalent in cattle (48.75%), whereas lowest in face and neck region (30.0%) which is almost in line with present finding. In fact, Stachurski (2000) states that short hypostome ticks like *Ripicephalus* usually prefer upper body parts including nape of neck and margin of anus and under tail while long hypostome ticks like *Ambloyomma* attaches to lower parts of the animal body, which is also the case in the present study.

In the present study, there was statistically significant association (p < 0.05) between the lesion inflicted and specific tick species infestation except for *A. variegatum* (p > 0.05) (Table 6). *R. (Boophilus) decoloratus* (6.3%) was the leading tick species in inflicting dermatitis while *A. cohaerens and A. variegatum* was the most important tick species to inflict the bite mark (wound) and focal hemorrhage in the present study (Table 6). This was probably due to their long mouth part that results in severe bite according to Gebre et al. (2001) and Gashaw (2005).

#### Conclusion

The present study revealed high prevalence of ixodid tick infestation in the study area. These pose huge economical and health constraint to the farmers and the animals in the study area. The direct pathological lesion encountered during the study support the effect of tick infestation on the skin condition of the animals which can be reflected on the tannery industry as a whole. Therefore, systematic intervention and control of tick infestation should be put in place to tackle the diseases.

#### **Conflicts of interest**

Authors have none to declare.

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Full Length Research Paper

# The influence of malaria infection on kidney and liver function in children in Akoko area of Ondo state, Nigeria

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Renal and hepatic dysfunctions are part of the pathological effect of malaria infection common in children and pregnant women. Renal dysfunction is characterized by increase in creatinine, urea, and some of the electrolytes in the serum, while hepatic dysfunction is characterized by increase in liver enzyme activities. Two hundred and seventy children (age ranged 0 to 5 years) were recruited into this study. Malaria positive children were divided into two groups based on the number of parasite per µl. Those that had parasitaemia below 10,000 parasite per µl were considered mild infection, while those that had parasitaemia above 100,000 parasite per µl were considered severe infections. Malaria negative children were used as control. The result showed significant increase (P < 0.05) in serum creatinine and urea level in the malaria positive when compared with the control group. Among malaria positive children, the level of creatinine and urea were higher in the severe group than in the mild group. Sodium, potassium, bicarbonate, and chloride levels were significantly higher (P < 0.05) in the control group than in malaria infected children, with the exception of the potassium which was significantly higher in the severe group than in the control group. Among malaria positive, serum Na<sup>+</sup>, HCO<sub>3</sub> and Cl levels were significantly higher in the mild infection than in the severe infection. Serum protein was also significantly higher (P < 0.05) in the control group than in the infected children. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were significantly higher in the severe group than in the mild and control groups. This study showed that malaria infection has effect on renal and hepatic functions, and the level of dysfunction is determined by the severity of the infection.

Key words: Malaria, liver function, kidney function, serum electrolyte, liver enzymes, children.

#### INTRODUCTION

The prevalence of malaria infection is more pronounced in the tropics, especially in the Sub-Saharan Africa than in other parts of the world (Tauil, 2006). One of the reasons for this, is climatic condition that favours the reproduction pattern of the vector and rapid development of the parasite within the vector and the host (Ross and

\*Corresponding author. E-mail: s\_akanbi@hotmail.com. Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> Smith, 2010). The attitude of the people in the community and lack of basic infrastructural facilities may also be responsible for the rampant prevalence of malaria infections (Modiano et al., 1996). Developed countries have been able to eradicate malaria infection because of the high level of technical knowhow and human resource. The control or eradication of the vectors would go a long way towards the eradication of the malaria infection in the tropics. Despite the concerted efforts of the World Health (WHO), Organization government, and private organizations, the elimination of malaria parasite remains elusive in the tropical region (Akanbi et al., 2014). Because of the development of drug resistant strains of the parasite, the attention on malaria parasite has now been shifted to the prevention of malaria rather than eradication.

Prevalence and severity of malaria infection have been reported to be higher among children and pregnant women than in any other groups (Zaki et al., 2013; WHO, 2010), and it is the main cause of morbidity and mortality in endemic areas (Gomes et al., 2011). Among the malaria parasites that infect man, *Plasmodium falciparum* is the major cause of severe malaria and deaths from malaria cases. Effect of *P. falciparum* varies from asymptomatic to multi-organ manifestation, which could lead to the death of the victim (Zaki et al., 2013). Malaria has been implicated as one of the factors responsible for renal and hepatic dysfunction in malaria endemic area countries (Mishra et al., 2003; Ogbadoyi et al., 2007; Sharma et al., 2004). The malaria parasites usually affect the kidney, liver and brain (Dzeing-Ella et al., 2005).

The level of severity of malaria infection can be determined by both renal and hepatic malfunction. The clinical manifestation of renal involvement is associated with infection by P. falciparum and Plasmodium malariae (Nagvi et al., 2003), and may be responsible for an immune complex mediated glomerular disease leading to nephrotic syndrome. Other implications range from urinary sediment abnormalities, mild proteinuria and electrolyte changes to acute renal failure with metabolic acidosis (Padhi and Mishra, 2012). In addition, renal tubular changes have been reported to be associated to P. falciparum infection more than glomerular changes, and complication may range from minor to acute tubular necrosis and acute renal failure (ARF) accompanied by frequent oliguria and hypercatabolism (Gomes et al., 2010).

ARF can be diagnosed by the presence of oliguria and increased serum creatinine and blood urea nitrogen (Gomes et al., 2010). Malaria has been reported to be one of the factors responsible for acute renal failure among children in malaria endemic areas (Mockenhaupt et al., 2004), and this adverse effect of malaria parasite on the kidney could lead to an increase in blood urea, hyper-nareamia, hyper-kalaemia, low urine specific gravity, metabolic acidosis and low ratio of urinary to

blood urea (Padhi and Mishra, 2012). The sudden increase in the urea level and imbalance in the electrolytes level such as sodium, potassium, bicarbonate, and chloride in malaria infected people could serve as indicators for kidney dysfunction (Uzuegbu, 2011; Ebele et al., 2010; Jasani et al., 2012). Liver dysfunction is a common complication that usually occurs in malaria infection. Some studies have reported a sudden increase in liver enzymes in malaria infected individuals as an indication of liver dysfunction (Jarike et al., 2002; Onvesom and Onvemakonor, 2011). This could be as a result of the invasion of liver cells by the sporozoite during malaria parasite life cycle (Onvesom, 2012).

The changes caused in the hepatic cell by sporozoite can lead to the leakage of parenchymal and membranous enzymes of the liver into the circulatory system, which can be responsible for the increase in liver enzymes (Burtis and Ashwood, 2001). The severity of malaria infection has been linked with the increase in the level of liver enzymes in the body (Onyesom and Onyemakonor, 2011). As a result of high level of complication and death of children due to malaria infection, there is need to evaluate the extent of renal and hepatic dysfunctions in malaria cases so that there will be proper management of malaria infection and its associated complication (Ogbadoyi et al., 2009). The place where this study was conducted is a peri-urban area with high episode of malaria infection, especially among children. There is no information on the pathology of malaria infection in children in this area so far.

Therefore, this study assessed the pathological effect of malaria infection on liver and kidney functions in children in Akoko area of Ondo state, Nigeria.

#### MATERIALS AND METHODS

#### Study centre and subjects studied

The samples for this study were collected at Comprehensive health centre, Akungba-Akoko, Ondo state, Nigeria, and paediatric unit of the specialist hospital, Ikare, Ondo state, Nigeria from May to September 2011 and June to September, 2012, respectively. Two hundred and seventy children within the age range 0 to 5 years who were sick with temperature > 37°C, diarrhoea, headache, vomiting, and some other malaria symptom were recruited for this study. One hundred and ninety were malaria positive, while 80 were malaria negative (control). The children that were malaria positive were divided into two groups based on the number of parasite per µI.

Those that had below 10,000 parasites per  $\mu$ l were considered mild infection, while those that had parasitaemia levels above 100,000 parasites per  $\mu$ l were considered severe infection. The children that were malaria negative after screening for malaria parasite were used as control. The details of the study were explained to the mother or guardian of the children and the verbal consent was sought for and obtained from them. Questionnaires were distributed among the parents who gave their consent in order to get some information about their children. The study was reviewed and approved by Local Institution Review Committee.

#### **Collection of blood**

About 4 ml of blood was collected by venepuncture from each child by a trained technologist under the supervision of the medical doctors using 5 ml needles and syringe. 2 ml of the whole blood was immediately transferred into ethylene diaminetetra acetic acid (EDTA) bottle that was used for the haematological study, while the remaining 2 ml was transferred into plain bottle and was allowed to clot and serum was later separated. The level of electrolytes, total protein, creatinine, urea and liver enzymes were determined from the serum. The parasitaemia was determined using the whole blood.

#### Determination of parasitaemia

Thick blood film was prepared on the slide from the whole blood collected from each child. The slide was stained with Giemsa' stain and left for about 10 min, after which the slide was washed and allowed to dry. The slide was mounted on the light microscope and screened for the presence of malaria parasite. A slide was considered negative when no parasite was found after screening of 200 fields. For those slides that were positive the number of parasites counted per 200 white blood cells was used to calculate parasite density on the basis of 8000 leucocyte per  $\mu$ l of blood.

#### **Kidney function test**

Kidney function test was done by determining the level of creatinine, urea and electrolytes in the serum of all the groups studied.

#### Determination of serum urea

Serum urea was determined by using the method described by Di Giorgio (1974). Urea reacted with diacetylmonoxime to form yellow diazinederivative. The colour change was measured at 520 nm being directly proportional to the concentration of urea in the sample.

#### **Determination of serum creatinine**

Serum creatinine level was measured by the method described by Narayanan and Appleton, (1980). Protein present in 0.5 ml of serum was precipitated with 0.5 ml of sodium tungstate and centrifuged at 3000 rpm for 5 min. The supernatant was separated, and 0.5 ml of alkaline picrate was added to 0.8 ml of supernatant which formed a red complex. The absorbance was read in a spectrophotometer at 520 nm against a reagent blank, after incubation for 15 min at room temperature.

#### Electrolyte assays

The serum electrolytes (Na<sup>+</sup>, K<sup>+</sup>, HCO<sub>3</sub><sup>-</sup> and Cl<sup>-</sup>) levels were determined by standard assay kit procedure from Teco Diagnostics, Anaheim, U.S.A. as described by Cheesbrough (1991).

#### Determination of serum protein ALT, AST and ALP

Serum ALT, AST and ALP activities were used to determine the liver function in the samples using the spectrophotometric

method with standard assay kits obtained from Randox laboratories, UK. ALT, AST and ALP levels were measured by the pyruvate, oxaloacetate and thymolphthalein monophosphate methods, respectively (Christen and Metzier, 1985). Serum protein levels were determined by the Biuret method (Gornall et al., 1949).

#### Statistical analysis

The data were analyzed by the one-way analysis of variance and the means are separated by Duncan's Multiple Range Test with 95% confidence intervals (SPSS 15.0, SPSS Inc., Chicago, IL, USA). The results were expressed as mean  $\pm$  standard deviation (SD).

#### RESULTS

The urea level was significantly lower (P < 0.05) in the control group than in the malaria infected children. The level of creatinine was significantly increased (P < 0.05) in the severe group when compared with the level in the mild and control groups, while it was only marginally higher in the mild group than in the control group (Table 1). Total protein was found to be significantly reduced in the severe group when compared with both mild and control groups, but serum protein level was not significantly higher in the control group than in the mild group (Table 1). The levels of Na<sup>+</sup>, HCO3<sup>+</sup> and Cl<sup>-</sup> were significantly higher (P < 0.05) in the control group than in the mild model.

There was a significant increase (P < 0.05) in the Na<sup>+</sup>, HCO3<sup>+</sup>, and Cl<sup>-</sup> levels in the mild group when compared with the severe group. The potassium was significantly higher (P < 0.05) in the severe groups than in the control and mild group but was only marginally higher in the mild group when compared with the control group (Table 2). Table 3 shows the effect of malaria infection on liver enzymes. The AST and ALT levels were significantly higher (P < 0.05) in the severe group than in the control and mild groups, but they were not significantly higher in the mild group than in the control group. The ALP level was higher in the control group than in the severe and mild groups but it was not significantly higher.

#### DISCUSSION

Malaria is a major parasitic disease that is responsible for a high death rate in children in the world, especially in the tropical region where it is endemic (WHO, 2010). Almost all the complications and death that occur as a result of malaria infection in children are caused by *P. falciparum*. Among complications that are associated with falciparum malaria, renal and hepatic dysfunctions are common in both children and adults living in malaria endemic regions (Ogbadoyi et al., 2009; Uzugbue et al., 2011). These two organs are very important in the body and their impair-

Severity of infection	No.	Urea (mg/dl)	Creatinine (mg/dl)	Protein (g/dl)
Mild	95	18.63±1.3 <sup>a</sup>	0.41±0.1 <sup>a</sup>	27.06±2.5 <sup>a</sup>
Severe	95	24.95±0.3 <sup>b</sup>	$0.47 \pm 0.3^{b}$	16.73±2.5 <sup>b</sup>
Control	80	18.21±2.3 <sup>a</sup>	0.40±0.1 <sup>a</sup>	28.90±1.2 <sup>a</sup>
Total	270	-	-	-

Table 1. Effect of malaria infection on urea, creatinine, and protein level in children.

Values are means  $\pm$  SD. Means followed by similar letters within the same column are not statistically different (p < 0.05 was considered significant). No. stands for number of children enrolled.

Table 2. Effect of malaria infection on the electrolytes level in children.

Severity of infection	Na⁺ (mmol/l)	K⁺ (mmol/l)	HCO <sub>3</sub> <sup>-</sup> (mmol/l)	Cl <sup>-</sup> (mmol/l)
Mild	129.0±1.6 <sup>b</sup>	4.3±0.1 <sup>a</sup>	27.2±0.2 <sup>c</sup>	100.1±3.0 <sup>b</sup>
Severe	123.2±3.4 <sup>c</sup>	4.9±0.2 <sup>b</sup>	26.1±0.3 <sup>b</sup>	91.6±4.3 <sup>°</sup>
Control	133.4±2.3 <sup>a</sup>	4.4±0.1 <sup>a</sup>	29.1±0.5 <sup>a</sup>	110.1±3.1 <sup>ª</sup>

Values are means  $\pm$  SD. Means followed by similar letters within the same column are not statistically different (p < 0.05 was considered significant).

 Table 3. Effect of malaria infection on serum AST, ALP and ALT in children.

Level of infection	No.	AST (U/L)	ALP (U/L)	ALT (U/L)
Mild	95	134.6±3.0 <sup>b</sup>	65.0±9.5 <sup>a</sup>	62.3±3.7 <sup>b</sup>
Severe	95	139.5±3.1 <sup>ª</sup>	67.5±13.1 <sup>ª</sup>	73.9±6.1 <sup>a</sup>
Control	80	130.5±2.1 <sup>b</sup>	69.4±12.5 <sup>a</sup>	60.1±3.5 <sup>b</sup>
Total	270	-	-	-

Values are means  $\pm$  SD. Means followed by similar letters within the same column are not statistically different (p < 0.05 was considered significant). No. stands for number of children enrolled.

rment needs to be detected early and managed appropriately. This study showed a significant increase in the creatinine and urea in children with severe malaria infection when compared with the group that had mild malaria infection and control group. Low levels of creatinine and urea in the group with mild malaria suggests that the severity of malaria infection has great influence on the level of creatinine and urea in the body of infected person. The observed increase in creatinine and urea in the severe group could be a result of sequestration of the parasite into the renal microvasculature bed which may lead to ischemia (Zaki et al., 2013). This indicates that the group with severe malaria may be more likely to have transient renal dysfunction which could be limited for the period of the infection. A study has reported a reduction in the clearance of endogenous creatinine during acute malaria infection returning to normal levels after recovery (Mishra et al., 2007). The serum protein levels were significantly reduced in the severe group when compared with the mild and control groups. A previous study reported a reduction in the serum protein in malaria patients (Akanbi et al., 2014), which was probably a result of intervention of malaria parasite in the synthesis of protein.

Electrolyte imbalances in the body may also serve as an indicator of renal failure and this has been indicated in malaria-infected individuals (Kirk and Horner, 1995). Some of the electrolytes were used as an indicator of renal dysfunction including Na<sup>+</sup>, Cl<sup>-</sup>, K<sup>+</sup> and HCO<sub>3</sub><sup>-</sup> (Uzuegbu, 2011). The levels of electrolyte in this study were reduced in malaria positive children when compared with the control group. This shows that malaria infection could influence the level of body electrolyte in infected individuals. The significant decrease in Na<sup>+</sup>, Cl<sup>-</sup>, and HCO<sub>3</sub><sup>-</sup> levels in the group with severe malaria infection when compared with the mild group in this study shows that the level of kidney dysfunction may be determined by the severity of malaria infection in the infected individuals. The reduction in the level of Na<sup>+</sup> may be due to losses in sweat and urine in the body which may serve to compensate for increased lactase and urea levels commonly found in *P.falciparum* patients (Etim et al., 2011; Mayne, 1994). A significant increase in K<sup>+</sup> level recorded in the severe group in this study could lead to metabolic acidosis (Etim et al., 2011). This result concurs with some previous report of hyponatraemia and hyperkalemia in malaria infected individuals (Etim et al., 2011; Olanivan, 2005). It has been reported that impaired glomerular filtration as a result of malaria infection could be responsible for the reduction in the Na<sup>+</sup> available in the renal tubule for  $K^{+}$  exchange. This may lead to the increase in the serum K<sup>+</sup> level (Wolfswinkel et al., 2010; Etim et al., 2011) as a result of imbalance in hydrogen ions which will lead to the retention of  $K^+$  in the serum.

The invasion and development of the malaria parasite into the liver during the life cycle may be responsible for liver dysfunction by causing organ congestion, cellular inflammation, and sinusoidal blockage (Anyasor and Olorunsogo, 2011). The increase in the serum ALT and AST in the severe and mild group when compared with the control group in this study shows that malaria parasite infection may be responsible for the increase in the liver enzymes, which may lead to liver dysfunction. This agrees with a previous study that showed that P. falciparum infection may be responsible for the increase in the liver enzymes (Onyesom et al., 2011). The increase in serum ALT and AST in malaria positive children could be as a result of leakage of these enzymes from the liver, as a result of damages to liver cell during the liver stage of the life cycle of malaria parasite. The ALT and AST level were higher in the group with severe group as compared with the mild group which is an indication that the level of liver dysfunction may be determined by the level of parasitaemia in the body. This study agrees with the previous study which showed a positive correlation between the level of liver dysfunction and parasitaemia (Onvesom and Onvemakonor, 2011). The ALP level was not significantly higher in the control than in the infected groups. The factors that may be responsible for this contrary result when compared with the previous studies are not known, therefore, there is a need for further study to confirm this.

#### CONCLUSION

This study concluded that malaria infection has an immense impact on the liver and kidney function in children, especially among those who were severely infected.

#### RECOMMENDATION

To avoid the damage done to the liver and kidney of malaria infected children by the malaria parasite, it is therefore recommended that malaria test should be conducted regularly for every child below the age of 5 years, so that appropriate treatment could be applied before the infection becomes severe. Children living in malaria endemic areas should be given intermittent preventive malaria treatment along with other vaccinations as recommended by the WHO. The use of treated mosquito net for the children should be overemphasized to the expectant mothers during antenatal clinic and when the children are brought for routine vaccination.

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#### **Conflicts of interest**

The author has none to declare.

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