Journal of Parasitology and Vector Biology

Volume 9 Number 8, August 2017
ISSN 2141-2510

Academic Journals

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Journal of Parasitology and Vector Biology

Table of Content: Volume 9 Number 8 August 2017

ARTICLES

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Vol. 9(8), pp. 106-110, August 2017
DOI: 10.5897/JPVB2017.0290
Article Number: E9580BD65397
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

Seroepidemiology of *Toxoplasma gondii* infection among slaughtered pigs, cattle and goats for human consumption in Bobo-Dioulasso, Burkina Faso

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Received 23 April 2017; Accepted 19 June, 2017

Toxoplasmosis is a major foodborne infectious disease with substantial adverse impact on population health and economy. Human infection is usually secondary to the consumption of contaminated raw or undercooked meat. Recent studies have reported a high prevalence of the infection in slaughterhouse animals in sub-Saharan Africa but few data exist for Burkina Faso. The aim of this study was to assess the prevalence of *Toxoplasma gondii* infection in animal from Bobo-Dioulasso, Burkina Faso. A total of 962 animal (including 423 pigs, 197 cattle and 342 goats) blood samples were collected in slaughterhouses in Bobo-Dioulasso between August 2013 and May 2014. Serum samples were tested for *T. gondii* antibody detection using the modified agglutination test (MAT). The overall seroprevalence of *T. gondii* was 28.8% in pigs, 13.2% in cattle and 34.8% in goats. Females animals were more infected than males. More than 60% of older animals (higher than 5 year-old animals) were infected. The study reported a high seroprevalence of *T. gondii* infection in pigs, cattle, and goats in Bobo-Dioulasso and is, therefore, of public health concern. The consumption of raw or undercooked meat should be regarded as an important risk factor for *T. gondii* infection in the study area. However further studies are needed to design appropriate control measures.

Key words: Pigs, cattle, goats, seroprevalence, *Toxoplasma gondii*, Bobo-Dioulasso, Burkina Faso.

INTRODUCTION

Toxoplasmosis is an important food-borne parasitic disease caused by the protozoan parasite *Toxoplasma gondii* that causes severe illness in immunocompromised individuals. The infection is found globally, affecting

almost one-third of the human population (Dubey and Jones, 2008). In human, the mode of transmission varies depending on culture behaviors, environmental factors, eating habits and/or religious practices (Dubey and Jones, 2008). The consumption of raw or undercooked meat containing viable cysts represents a major route of human contamination (Dubey, 2010, 2013; Pereira et al., 2010). *T. gondii* infection has a significant impact on animal production particularly in goats and pigs (Dubey, 2009, 2013). In fact, most infections among these animals are asymptomatic, but however can seriously affect their health. The clinical signs vary considerably and include abortions, stillbirths and fetal abnormalities (Kim et al., 2009; Dubey, 2009, 2010).

Consequently; animal reproductive performance is affected leading to heavy economic losses for livestock producers (Mahboub et al., 2013). In addition, livestock products represent an important source of nutrition around the world (Dubey, 1986, 2009; Chikweto et al., 2011).

Studies have shown that pigs, cattle and goats are at higher risk of *T. gondii* infection and the reported prevalence in slaughtered animals is higher in pigs, goats and sheep, than in cattle, and varies worldwide (Dubey, 1986; Gharekhani, 2013; Hosein et al., 2016). However, only very few studies have been conducted in Burkina Faso on the question and very little information is known about the prevalence of *T. gondii* infection in livestock in Bobo-Dioulasso (Bamba et al., 2012, 2013, 2016).

Traditional procedures of meat quality control rely on clinical examination mainly visual inspection, which leads to lot of misdiagnoses. Serological testing is considered to be an appropriate method for the diagnosis in slaughtered animals. The aim of this study was to investigate the seroprevalence of *T. gondii* infection in slaughtered pigs, cattle, and goats in Bobo-Dioulasso, Burkina Faso, using MAT.

MATERIALS AND METHODS

Study area

This study was carried out in Bobo-Dioulasso, the second largest city located in the southwest part of Burkina Faso, a sahelian landlocked country of 274,000 km^2 in West Africa. The country is strongly dependent on the vagaries of the climate and its economy is based on agriculture and livestock. The region of Bobo-Dioulasso has an average annual rainfall of 1000 to 1300 mm, with a temperature ranging from 16 to 45°C. These conditions are necessary for sporulation and survival of *T. gondii* oocysts when excreted with cat feces in the external environment (Dubey, 1990; Yilmaz, 1972).

Study designs and sample size

A cross-sectional study was carried out from August 2013 to May 2014 in commercial slaughterhouses across the city of Bobo-Dioulasso. Pigs, cattle, and goats of both sex and different age groups were included in the study. Blood samples were collected during animal bleeding and serum samples stored at -20°C till analysis in November, 2015.In the absence of data on *T. gondii* infection in pigs in Burkina Faso the required sample size was calculated by assuming 50% infection prevalence in pigs and goats and 14% in cattle (Bamba et al., 2013). With an expected prevalence (P), a 95% confidence interval (Z score of 1.96), and a precision (d) of 5%, the sample size was calculated as $N = (Z)\ 2P\ (1\text{-}P)/d^2$. The total sample size (N) was 423 pigs, 197 cattle and 341 goats.

Sample collection

Blood samples were aseptically collected from ear vein (pigs), coccygeal (cattle) and jugular (goats) venipuncture using sterile vacuum tubes without anticoagulant, the blood samples were labeled and immediately stored in ice boxes with ice packs and transferred to the laboratory of Institute for Research in Health Sciences in Bobo-Dioulasso. Samples were centrifuged at 3200 RPM for 10 min and the separated sera were transferred to Eppendorf tubes and kept at -20°C until analysis.

Serological test

The modified agglutination test (MAT) was used to detect *T. gondii* antibodies in animal serum samples. *T. gondii* tachyzoites were fixed using formalin for the quantitative assessment of IgG. The mercaptoethanol was used to inhibit the IgM-like antibodies that interfere with the assay specificity. All sera were serially diluted to 1:25, 1:50, 1:100, 1:1600 and 1: 3200 before testing. Positive sample antibodies titers threshold was set at 25 (Dubey and Desmonts, 1987).

Data analysis

The EpiData software version 3.1 and the Epi Info software version 6.04 were used for data entry and analysis respectively. The Chi^2 test was used for statistical comparison and a $p\ value < 0.05$ was considered statistically significant. The seroprevalence of *T. gondii* infection was assessed regarding the animal age and gender. The national ethics committee of health research has approved the study (protocol 07/2013) before the implementation of the study related activities.

RESULTS AND DISCUSSION

Overall seroprevalence

A total of 962 animals including 423 pigs, 197 cattle and 342, goats were assessed and serum samples collected

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Table 1. Seroprevalence of *Toxoplasma gondii* infection in pigs, cattle and goats from Bobo-Dioulasso as determined by the MAT.

Animal	No. tested	No. positive	Seroprevalence (%)	*CI 95%*
Pigs	423	122	28.8	24.6 - 33.3
Cattle	197	26	13.9	8.9 - 18.4
Goat	342	119	34.8	29.8 -39.9

for IgG anti-*T. gondii* analysis. Two hundred and sixty seven animals composed of 122 pigs, 26 cattles and 119 goats respectively were positive.

The overall seroprevalence of anti-*Toxoplasma gondii* IgG antibodies in animals studied was 27.7% (95%CI: 29.4 to 30.6%) using MAT. Regarding the technical approach, MAT was chosen for the serological assay because of its good sensitivity and specificity for the diagnosis of latent infections compared to other serological tests (Dubey et al., 1995). Previous studies conducted in slaughtered animals in Bobo-Dioulasso have reported a seroprevalence of *T. gondii* antibodies of 58% in sheep (Bamba et al., 2012), and 14.3% in cattle (Bamba et al., 2013) using high direct sensitivity agglutination assay (HDSA) analogous to the modified agglutination test (MAT). Moreover, previous findings have shown that the MAT is a specific technique for searching *T. gondii* antibodies in pigs and no cross-reaction with other parasites was reported when using this test (Dubey, 1997).

The seroprevalence of 28.8% (95%CI: 24.6 - 33.3%) in pigs, 13. 2% (95%CI: 8.9 - 18.4%) in cattle and 34.8% (95%CI: 29.8 - 39.9%) in goats were reported. Comparing the seroprevalence rate in each group, it appears to be lower in cattles than pigs and in goats (Table 1). This might be due to the difference in feeding habits of these animals. In fact, goats are more likely to be infected from the pasture as they graze close to the ground than cattle which prefer browsing. In other hand, pigs could be contaminated by *T. gondii* oocysts eliminated with the cat faeces in the environment (soil, grass, and water).These finding show that *T. gondii* is present among these animals slaughtered in Bobo-Dioulasso depicting the widespread environmental contamination by *T. gondii* oocysts in this country.

The current seroprevalence (28.8%) indicate the major role of pigs as intermediate hosts of *T. gondii*. The high seroprevalence of *T. gondii* in the present study might be associated with farm management systems and access to free roaming cats on pig farms. Furthermore, the prevalence was 13.2% (Table 1) in cattle group using MAT. According to previous studies, *T gondii* has been rarely isolated from naturally infected cattle (Dubey et al., 1986; Dubey, 2010). Indeed, calves and cows infected with high doses of oocysts by oral inoculation indicate that *T. gondii* is eliminated or greatly reduced in cattle tissues, some animals being negative during infection (Dubey and Thulliez, 1993). Therefore, cattle are considered a poor intermediate host of *T. gondii.*

Otherwise, our findings noted that over 30% of goats from Bobo- Dioulasso were seropositive for *T. gondii* antibodies (Table 1). Toxoplasmosis is a significant cause of reproductive failure in small ruminants such as goats. However, there is no available data on *T. gondii*-associated abortions in Burkina Faso. The high prevalence of *T. gondii* antibodies among goats in our study (34.8%) shows high levels of exposure to the parasite. In addition, it is known that tissue cysts persist throughout the host life (Dubey 1998). Therefore, this could be a potential risk to public health. However, in Burkina Faso, meat is consumed cooked or overcooked usually a common practice which minimizes the risk of *T. gondii* transmission.

Characteristics of animals

According to the sex, a predominance of female animals in each animal group was noted in this study (Table 2). The male to female sex ratio was 0.9 (197/226), 0.7 (86/111) and 0.6 (128/214) respectively for the pigs, cattle and goats (Table 2). This would be linked to the religious practices in Burkina Faso that make the majority of the male animals kept in the farms, sold, slaughtered or sacrificed outside the slaughterhouse during religious or traditional ceremonies. This justifies the high proportion of the female animals on slaughter chains in our study.

Table 3 shows age group repartition of animals with a predominance of older animal (5 years old) in all groups. The average age was 2.6 years (±1.3) for pigs, 2.1 years (± 1.1) for cattle and 1.7 years (± 0.12) years for goats. The animals more than 5 years old were the most represented (> 60%) in each group (Table 3). This observation could be justified by the fact that the consumption of piglet, calf, and little goat is not part of culinary habits in Burkina Faso.

Serological data according to characteristics of animals

According to animal sex, females were more infected

Table 2. Seroprevalence of *T. gondii* infection regarding age and MAT titer.

Animal	Age (Year)	No. tested	No. positive (%)	No. positive with MAT titers of							
				25	50	100	200	400	800	1600	3.200
Pigs	< 2	184	33 (17.9)	17	4	5	5	1	0	1	0
	3-5	218	76 (34.8)	11	26	7	12	6	11	2	1
	> 5	21	13 (61.9)	2	1	2	1	1	4	2	0
Cattle	< 2	31	7 (22.6)	5	2	0	0	0	0	0	0
	3-5	154	11(7.1)	8	3	0	0	0	0	0	0
	> 5	12	8 (66.7)	6	2	0	0	0	0	0	0
Goats	< 2	257	83 (32.3)	17	13	16	15	13	7	2	0
	3-5	58	17 (29.1)	2	5	3	4	1	1	1	0
	> 5	27	19 (70.4)	4	2	3	1	6	1	2	0

Male vs female Pigs: Fisher's exact test: The two-tailed P value equals P = < 0.0001 was considered to be statistically significant; Male vs female Cattle: Fisher's exact test: The two-tailed P value equals P = 0.065 was considered not statistically significant. Male vs Female cattle: Male vs Female goats: Fisher's exact test: The two-tailed P value equals P = < 0.0001 was considered to be statistically significant.

Table 3. Seroprevalence of *T. gondii* infection in pigs, cattle and goats from Bobo-Dioulasso (Burkina Faso) according to sex.

Pigs					Cattle				Goats			
Sex	No. tested (%)	No. positive (N=122)	Sero-prevalence	*P value*	No. tested (%)	No. positive (N=26)	Sero-prevalence	*P value*	No. tested (%)	No. positive (N=119)	Sero-prevalence	*P value*
Male	197 (46.6%)	27	13.7% CI 95% (9.4-19.1%)	*P = < 0.0001*	86 (43.7%)	7	8.1% CI 95% (3.6-15.4%)	*P = 0.065*	128 (37.4)	22	17.2% CI 95% (11.3-24.4%	*P = < 0.0001*
Female	226 (53.4%)	95	42.1% CI 95% (35.7-48.5%)		111 (56.3%)	19	17.11% CI 95% 10.9%-24.9%		214 (62.6%)	97	45.3% CI 95% 37.8%-51.1%	

than male. About 42.1% (CI 95%: 35.7-48.5%) of female pigs, and 45.3% (CI 95%: 37.8-51.1%) female goats were infected with p value *P = < 0.0001* in each group (Table 2). The high susceptibility of female animals reported in the current study corroborates with previous study reports and is related to a higher female animal's susceptibility to protozoan infections (Alexander and Stinson, 1988).

In addition, the seroprevalence of *T. gondii* increased according to the age with more than 60% of older animals infected in each group (Table 3). These findings suggest postnatal acquisition of *T. gondii.*

Regarding the level of dilution, positive MAT tests at the titers of 1:200 or higher were reported in 21.3% of pig and 45.4% of goats. None of the cattle serum samples was positive at the 1:200 dilutions (Table 3). Our results noted that none of the cattle was positive in 1:200 serum dilutions using MAT assay. The low MAT titers among

cattle indicate not the persistent infection but an exposure of *T. gondii*.

Furthermore, authors suggest that a titer of 1:25 in the MAT is considered specific for the detection of *T. gondii* in pigs, goats, sheep, and other livestock (Dubey, 2010), unlike cattle.

Conclusion

An epidemiological study was carried out to evaluate the seroprevalence of *T. gondii* in pigs, cattle, and goats slaughtered for human consumption in Bobo - Dioulasso. In Burkina Faso, livestock represents the second most important source of revenue in the country's primary sector.

Breeding in the study area is generally characterized by a traditional and extensive management system. Majority of slaughtered animals are destined for local human consumption. This study reported a high seroprevalence of *T. gondii* in pigs, cattle, and goats in Bobo-Dioulasso, and is, therefore, of public health concern. The consumption of raw or undercooked meat should be regarded as an important source of infection to people in the study area. However further studies are needed to design appropriate control strategies in Burkina Faso. Moreover, consumer knowledge should be strengthened in order to reduce the impact of the disease.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Vol. 9(8), pp. 111-115, August 2017
DOI: 10.5897/JPVB2017.0287
Article Number: F349BA365399
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

The Circadian and seasonal biting patterns of *Anopheles gambiae* sl in Bayelsa State, Nigeria

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Received 9 April, 2017; Accepted 30 June, 2017

Accurate knowledge on the biting pattern of *Anopheles gambiae* sl is a prerequisites for mounting long lasting control intervention in malaria endemic areas. A descriptive study was undertaken to determine the hourly biting cycle of *A. gambiae* in some randomly selected communities in Bayelsa State, Nigeria during January, 2014 and December, 2015. The two methods used for mosquito collection were human baits (indoor and outdoor). Hourly mosquito collections were undertaken twice quarterly from 1900 to 0400 h. Mosquitoes collected were identified morphologically following a standard key. The hourly and seasonal biting rates of *A. gambiae* were calculated. Two thousand and seven female *A. gambiae* were identified in 16 man-night. The total bites were 62.72 bites/person/night. The mosquito biting rates were higher outdoor than indoor collections. Similar results were recorded for seasonal and ecovegetation collections. Wet seasons had higher mosquito biting rates (71.58 bites/person/night) than that of the dry seasons collection (36.358 bites/person/night). The biting rates of *A. gambiae* sl were 2-fold higher in fresh water than in brackish water swamp forest and mangrove coastal water forest. The hourly biting rates of *A. gambiae* peaked at 2300 h, while the seasonal biting rates peaked at 2300 h and 4 am. This result has demonstrated the inefficiency of the indoor residual spray (IRS) and long lasting insecticidal nets (LLNs) as the only malaria control measure in this area. Outdoor protective control measure is recommended alongside indoor IRS and LLNs.

Key words: Circadian, Seasons, biting patterns, *Anopheles gambiae*, Bayelsa State.

INTRODUCTION

Anopheles gambiae is the principal malaria vector confined exclusively in sub-Saharan Africa (Gillet, 1972; Coetzee, 2004). Their vectorial competence is controlled by several factors, such as ability to locate their host and initiate infective blood meal bite (Mboera et al., 1997), availability of breeding sites (Okiwelu and Noutcha, 2012), poor sanitation habit through urban expansion (Nwoke and Eboh, 1991) and climatic conditions that enhances the parasite development (Theresa et al., 2006).

Malaria accounts for over 1.09 million deaths mostly in children under the age of 5 years around the globes (WHO, 2006) and 90% of total deaths in Africa (NIH, 2001). In Nigeria alone, malaria account for over 60%

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of the total outpatient visits to health facilities, 30%childhood death and 11% of maternal death (NDHS, 2011). Every year, the nation loses over N132 billion for cost of treatment and absenteeism from work, schools and farms (FMH, 2005). In Bayelsa State, malaria infection account for 102 deaths out of 210 recorded from 35 different diseases in the year 2011 (Ambah, 2012).

The menace of mosquito borne diseases has been controlled with different strategies around the globe (WHO, 1995; Najera, 2000). A strategy targeted at reducing the mosquito-man-contact has proven to be most promising (Lwetoijera et al., 2013; Trig and Wemndorfe, 1995). Insecticide treated bed nets and long lasting insecticidal nets are the most widely used intervention in Nigeria to break vector human contact and reduce incidences of malaria (WHO, 2005). Despite the enormous effort to control mosquito bites in Nigeria, the incidences of malaria still remain unchanged.

Insecticide treated bed nets, long lasting insecticidal nets and residual spray have demonstrated a positive potent in reducing man mosquito contact in many malaria endemic areas (WHO, 2007). However, these interventions have also changed the behavior of *Anopheles* mosquitoes from biting indoor to biting outdoor (Moiroux et al., 2012). In Bayelsa State, a personal interaction with most people showed that the use of the non-treated bed net are more predominant than the treated net especially among the rural dwellers. This may have accounted for the high incidence rates of malaria recorded in previous studies in the same area (Ebenezer et al., 2014). Good knowledge on the biting pattern of *A. gambiae* is a pre-requisite for monitoring control measures of malaria infection in endemic areas (Kabbale et al., 2013). Studies have demonstrated the biting behavior of *A. gambiae* in some parts of Nigeria (Awolola et al., 2002; Awolola et al., 2003; Oyewole et al., 2007; Okwa et al., 2009). This information is scarce in Bayelsa State. This pioneering study shall therefore provide base line information on the hourly biting cycle of *Anopheles gambiae* in Bayelsa State.

MATERIALS AND METHODS

Study area

Bayelsa State (5°22′- 6°45′E and 4° 10′- 5° 23′N) copies the lower deltaic plan of Nigeria (Alagoa, 1999). The study was conducted in two randomly selected communities across the 3 eco-vegetation. Details about the study locations have been extensively described (Ebenezer et al., 2012).

Mosquito collections

Two collection methods were adopted: indoor and outdoor human catch. A modified bed net trap in Kabbale et al. (2013) was employed. The bed net trap was constructed to the shape of a pyramid with a diameter of 6 × 4 × 5 ft. The bed net trap was constructed with non-treated nets. Each side of the pyramid was made up of 3 layers of the non-treated net. The second layer was perforated into 8-10 inches holes. The third layer was permanently fixed while the first layer was designed in the form of flip over on the second layer. The first layer serves as door, which are closed against the second layer after an hourly interval. This is in a bid to reduce mosquito escape at the time of collection. The third layer that is permanently fixed gives protection to the human bait. The whole pyramid was set up over a wooden bed with human sleeping on it as bait.

Two sets of human bait volunteers were involved; one serving as indoor catchers and one serves as outdoor catchers. A total of 16 volunteers were involved in the study; two collectors per night per station. They were made to sleep on the bed net trap at some distance apart. Mosquito collection was made from 1900-0400 h for two consecutive nights at each quarter during January, 2014 and December, 2015. In each night, the catchers were made to sleep on the constructed net, leaving the first layer of the trap totally flipped open. Mosquitoes attracted to the human bait were trapped in the second layer. At each hour, the first layer of the net construction was flipped down and the mosquitoes trapped at the second layer were collected using mouth aspirator. The hourly collection represents the mosquito that actively sought for blood meal bite from the host in that particular hour.

Mosquitoes collected were placed in different Petri dishes duly labeled against the hour of collection. Morphological identification of the mosquitoes was undertaken at the Entomology Laboratory of the Department of Animal and Environmental Biology, University of Port-Harcourt. The identification followed standard key in Gillies and Coetzee (1987). *Anopheles gambiae* were preserved dried in silica gel in a labeled 1.5 ml Eppendorf tube for further studies.

Ethical consideration

Verbal consents were obtained from the community heads and house hold heads. Consents were also obtained from human bait volunteers after properly informing them on the purpose of the study. Each volunteer was treated with anti-malaria drugs on and after each collection night.

Data analysis

Data were analyzed in SPSS version 20.3 software. Chi-square and ANOVA were the statistical tools used.

RESULTS

A total of two thousand and seven female *Anopheles gambiae* were identified in 16 man – night of an indoor and outdoor collections. Details are shown in Table 1. The biting rate of *A. gambiae* was 62.72 bites/person/night. The outdoor mosquito abundance (70.5%) was higher than that of the indoor mosquito abundance (29.5%) with statistically significant difference ($F=50.241$ $df= 1$; $p<0.05$). The biting rates of *A. gambiae* of outdoor collection (88.44 bites/person/night) was higher than that of the biting rates of indoor collections (37.00 bites/person/night) with significant difference ($t=12.083$ $df=1$ $p<0.05$).

A. gambiae biting rates vary across seasons. In wet seasons, *A. gambiae* biting rates was 71.58 bites/person/night. During dry seasons, the biting rate was 36.13

Table 1. Biting rates of *A. gambiae* in Bayelsa State, January, 2014 and December, 2015.

Variables	No. of catchers	No. of night	Man- night	No(%) of Anopheles	Biting rates
Collection methods					
Indoor	8	8	16	592 (29.98)	37.00
Outdoor	8	8	16	1415(70.50)	88.44
Total	16	16	32	2007	62.72
Seasons					
Wet					
Indoor	6	6	12	543 (31.60)	45.25
Outdoor	6	6	12	1175(68.40)	97.93
Total	12	12	24	1718(85.60)	71.58
Dry					
indoor	2	2	4	75 (26.00)	18.75
outdoor	2	2	4	214 (74.40)	53.50
Total	4	4	8	289 (14.40)	36.13
Ecovegetation					
FWSF					
Indoor	8	8	16	290(28.88)	18.13
Outdoor	8	8	16	714(71.12)	44.63
Total	16	16	32	1004(50.02)	62.75
BWSF					
Indoor	8	8	16	181(33.39)	11.31
Outdoor	8	8	16	361(66.61)	22.26
TOTAL	16	16	32	542(27.01)	16.94
MCWF					
Indoor	8	8	16	144(31.24)	9.00
Outdoor	8	8	16	317(68.72)	19.81
Total	16	16	32	461(22.97)	14.41

FWSF = Fresh water swamp forest; BWSF = brackish water swamp forest; MCWF = mangrove coastal water forest.

bites/person/night. The differences were significant ($F=9.685$ df=1 $p<0.05$). The biting rates of *A. gambiae* in fresh water swamp forest were 2-fold higher than those in brackish water swamp forest and mangrove coastal water forest, respectively. The differences were significant ($X^2=9.488$ df=4 $p<0.05$).

The hourly biting patterns of *A. gambiae* vary across seasons (Figure 1). The biting pattern was said to be biphasic peaking at 2300hrs and 4am.

DISCUSSION

The biting rates of *A. gambiae* in this present studies was 62.72 bites/person/night. This value was 17-fold higher than the 3.51 reported by Bradley et al. (2015) in Bioko, Equatorial Guinea. The significantly higher outdoor biting rates than indoor biting rates correspond with the report of Kenea et al. (2016). The observed higher outdoor biting rate is an indication that the indoor control measure could be rendered ineffective (Bradley et al., 2015). Although, in this study, speciation of the *A. gambiae* were not undertaken, the higher outdoor catches may be unrelated to the already existing plasticity of most vectors to host preferences (Noutcha and Anumudu, 2009; Sane et al., 2016). It is possible that most of the *A. gambiae* caught were rather host seeking than resting. *A. gambiae* showed higher propensity to biting outdoor.

Studies have demonstrated that the biting behavior of A. *gambiae* was altered when IRS or LLNs were used as indoor control interventions (Moiroux et al., 2012). Surprisingly, such intervention is not feasibly monitored in these study locations. A personal interaction with most rural dwellers showed that people relied mostly on the use of the untreated bed net than the treated net for mosquito control. Both outdoor and indoor mosquito density reported in this study highlighted the adaptive indoor and outdoor biting behavior of *A. gambiae* (Taye et al., 2016).

The hourly seasonal biting rates of *A. gambiae* in this

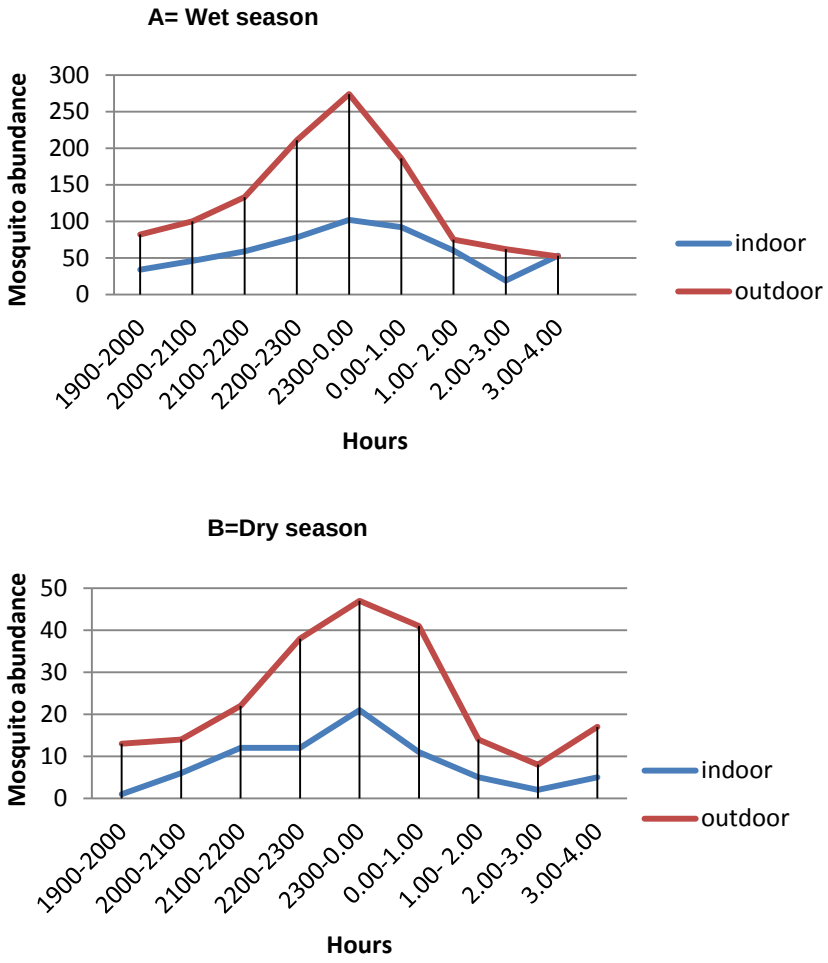

Figure 1. Seasonal circadian *A. gambiae* biting rates.

present study highlighted the importance of suitable timing for mosquito control intervention (Kabbale et al., 2013). The high biting rates during wet seasons is an indication that availability of mosquito breeding sites can increase the propagation of larva population that transits to adulthood. High humidity during the raining seasons is responsible for the high density of adults (Sande et al., 2016).

Mosquitoes in the study locations bite all-night. The biting pattern was said to be biphasic peaking at 23:00 h and 3am. This biting pattern has been reported elsewhere (Amerasinghe and Indrajith, 1995; Sande et al., 2016; Kenea et al., 2016). However the report contrasted the findings of Taye et al. (2016) who reported a biphasic cycle that peaked at 8 pm and 3 am. The peak biting period of *A. gambiae* in this present study corresponds with the exposure time of the inhabitants to fishing activities. The double peak period correspond exactly with late fish trapping period and the early fish harvesting period at the study locations. This timing has been reported by Cooke et al. (2016).

Hourly biting rates was higher outdoor. This highlights the possibilities of acquiring vector-borne infections even before and after bed time; Fishing and trading activities are the two major occupations in the area (Alagoa, 1999). These activities exposes people to outdoor biting of *A. gambiae,* which could also be one of the determinant of higher incidence rates of malaria recorded in previous studies (Ebenezer et al., 2014*).*

CONCLUSION

A. gambiae in this study have shown the tendency of biting all night. This is a concern for public health intervention. However, outdoor biting rates were higher than those of the indoor biting rates. This behavioral plasticity may undermine the conventional indoor control intervention with IRS and LLNS. More so, since the peak biting rates of the mosquitoes synchronize with fishing and trading activities of inhabitants in the study locations, it is recommended however that an outdoor protective

coat impregnated with pyrethroid could be the best control measure for outdoor activities when the IRS and LLNs are used indoor.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are sincerely grateful to community heads and house-hold heads for permitting this study in their locality. They also appreciate all the volunteers that assisted in the mosquito collections.

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academicJournals

Vol. 9(8), pp. 116-121, August 2017
DOI: 10.5897/JPVB2015.0215
Article Number: D88406A65485
ISSN 2141-2510

http://www.academicjournals.org/JPVB

Journal of Parasitology and Vector Biology

Full Length Research Paper

A study on prevalence of canine babesiosis in and around Jimma Town, Western Ethiopia

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Received 15 July, 2015; Accepted 5 October, 2015

Cross sectional study was carried out from November, 2014 to March, 2015 to investigate the prevalence of canine babesiosis, associated risk factors and the species of *Babesia* affecting dogs in eight peasant associations found in and around Jimma town Western Ethiopia. Blood examination conducted on 384 randomly selected dogs showed an overall prevalence of 15.9% (61/384). The prevalence of canine babesiosis in and around Jimma town was 10.8 and 20.6%, respectively. The species of *Babesia* encountered in the current study were *Babesia canis and Babesia gibsoni which* accounted for 9.9% and 6% of the overall infection, respectively. The prevalence of canine babesia infection did not show any significant difference between dogs of different ages, origin and sex groups (*P* > 0.05 in each case). There was a significant (*P*< 0.05) association between canine *Babesia* infection to body condition score (BCS). The significant impacts of *Babesia* infection on dogs should not be neglected. Therefore, a large survey and other highly sensitive and specific molecular diagnostic tools are recommended in Ethiopia to investigate the prevalence of *Babesia* infection, and its associated risk factors in different areas of the country.

Key words: Babesiosis, canine, Ethiopia, Jimma, prevalence.

INTRODUCTION

Babesiosis is a tick borne blood protozoan disease of domestic and wild animals, which occurs in the southern USA, central and South America, Africa, Asia and Europe. *Babesia* species are tick-transmitted apicomplexan parasites that infect a wide range of vertebrate hosts. The identification of individual species has traditionally been based on the host specificity and on the morphology of the intra-erythrocytic forms (piroplasms) (Taboda, 1998; Uilenberg, 2006).

Members of the genus *Babesia* readily parasitize the red blood cells of dogs. Canine *Babesia* are morphologically classified into large (3.0 to 5.0 µm) and small (1.5 to 2.5 µm) forms, both exhibiting a worldwide distribution. *Babesia canis* , *Babesia vogeli* and *Babesia rossi* are large *Babesia* spp. detected in the USA while *Babesia gibsoni* and *Babesia annae* are small *Babesia* spp. that have been documented to infect dogs (Birkenheuer et al., 2004**).** The three main species of large *Babesia* are antigenically distinct, transmitted by different vectors and differ widely in pathogenicity and geographic distribution (Uilenberg et al., 1989).

B. vogeli is the least pathogenic. It occurs in France,

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Australia, Japan, Brazil, South Africa and the USA, and usually causes mild disease in adult dogs, but severe disease in some puppies (Matjila et al., 2004). *B. rossi* occurs predominantly in southern Africa, and is ostensibly the most virulent of the subspecies. Improved polymerase chain reaction (PCR) techniques have lately allowed for better definition of these parasites (Matjila et al., 2008). The smaller parasite, *B. gibsoni* occurs principally in the Middle East, southern Asia, Japan, Africa, and South America and is an emerging infectious disease in the USA, as well as having been detected lately in Italy, Hungary and Australia (Muhlnickel et al., 2002). A more virulent subspecies of *B. gibsoni* has recently been identified in California. A *Babesia microti* like piroplasma, *B. annae* (also known as *Theileria annae*) has been found to be endemic in dogs in northwest Spain (Camacho et al., 2003).

Various species of ticks such as *Rhipicephalus sanguineus*, Dermacentor *spps*, *Haemaphysalis ellipticum* can transmit the large *Babesia* of dogs, whereas *B. gibsoni* is transmitted by *Haemaphysalis bispinosa* and *Haemaphysalis longicornis*. *Babesia annae* is thought to be transmitted by ixodes hexagonus (Lobetti, 2006). Both transtadial and transovarial transmission can occur, and ticks are believed to remain infective for several generations. Babesia spp. can also be transmitted by blood transfusion. Strong circumstantial evidence exist that *B. gibsoni* is transmitted by dog bites (Birkenheuer et al., 2005), whilst transplacental transmission from dam to offspring has recently been proven as an additional mode of transmission (Fukumoto et al., 2005).

A small subset of dogs presents with high haematocrits (relative haemo concentration), despite vigorous haemolysis, due to presumed shifting of fluid from the intravascular to the extra vascular component. These dogs are at increased risk of developing ARF or cerebral complications, as well as other organ failures (Welzl et al., 2001).

Large surveys on canine babesiosis are scarce, but numbers of reports suggest that the parasite infects dogs worldwide. In India, a variable prevalence of canine babesiosis has been reported viz. 0.66 to 8.9% in referral clinics canines in Uttar Pradesh (Chaudhuri, 2006); 21.7% in Assam (Chandhuri and Varshney, 2007), 5.4% in Hissar, Haryana (Bansal et al., 1985), and 3.17% of *B. gibsoni* and 1.26% *B. canis* in Punjab (Eljadar, 2010). However, the prevalence of canine babesiosis in the study area is not yet known. Therefore, this study is crucial to know the status of the disease and its associated risk factor, and even tremendously very important to take measures to control the disease in the study area. The objectives of the study were:

1. To investigate the prevalence of canine babesiosis in and around Jimma town.

2. To assess major associated risk factors of canine babesiosis.

MATERIALS AND METHODS

Study area

The study was conducted in Jimma zone, southwestern part of Ethiopia at Jimma town. Jimma town is the capital city of Jimma zone located in Oromia region. It is located 352 km south west of Addis Ababa at latitude of about 7°13' to 8°56'N, and longitude of about 35°52' to 37°37'E and at elevation ranging from 880 to 3360 m above the sea level. The study area receives a mean annual rain fall of about 1530 mm, which comes from the long and short rainy seasons. The annual mean minimum and maximum temperature during the study period were 12.7 and 26.7°C, respectively. The study was conducted in eight kebeles/Peasant associations such as Furstale, Bosa kitto, Sato samora, Ifa bula, Dedo dima, Bebella kera, Gudeta bula and Doyo bikila.

Study animals

The study was conducted on dogs randomly selected from four kebeles in Jimma town: Furstale, Bosa kitto, Sato samora and Ifa bula and other four PAs around Jimma town such as: Dedo dima, Bebella kera, Gudeta bula and Doyo bikila from November, 2014 to March, 2015.

Study design

A cross-sectional (observational) study was conducted from November, 2014 to March, 2015 to determine the prevalence of babesiosis in the study animals. The animals were selected randomly and restrained by owners for sampling. Blood sample was collected from cephalic vein, collected into heparinized tubes and examined under with light microscope.

Sampling method

Sample size determination

384 samples were collected from the study area by using simple random sampling methods and 95% confidence interval with required 5% precision, the sample size was determined by the formula of Thrusfield (1995).

$$n = \frac{1.96^2 P_{exp}(1-P_{exp})}{d^2}$$

Where; n = required sample size, Pexp = expected prevalence, d = required precision

The expected prevalence of canine babesiosis was 50% to get the maximum number because there was no previous work of canine babesiosis in the study area. The precision was decided to 5 (0.05) to 95% confidence level. By substituting the value in the above formula, the study got the sample size:

$$n = \frac{1.96^2 \times 0.5(1-0.5)}{(0.05)^2}$$

= 384 dogs

Sampling strategy

A total of 384 samples were collected during the study period from animals in and around Jimma town by using simple random sampling. The species of animals sampled were only dogs of any age. Blood samples were collected randomly from dogs, thin blood smear was made on clean slide and PCV determination was performed. The collected blood samples were put in iceboxes box and were transported to Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) veterinary parasitology for immediate laboratory examinations.

Study methodology

Blood examination

Blood samples were collected aseptically from cephalic vein in vials containing anticoagulant (EDTA and study design above). A thin blood smear was prepared for each sample, a drop of blood was placed on a clean glass slide, air dried, fixed in methanol, stained with Giemsa (Coles, 1986) and examined under light microscope by using the oil immersion objective to identify and to examine the morphology. *Babesia* species were identified by morphological characteristics using thin blood smear.

Statistical analysis

The data were collected from the study area, result obtained from blood examination was recorded in the format developed for this purpose and later on entered into Microsoft Excel 2007. Dogs were grouped based on age, sex, origin and body condition to determine whether these factors were associated with the prevalence of canine babesiosis. Statistical evaluations were carried out using statistical package for the social sciences (SPSS) 20.0 and the mean PCV of infected and non-infected dog was compared using independent T-test at 95% confidence level ($p<0.05$). Differences were considered significant when $p < 0.05$.

RESULT

Blood examination conducted on 384 randomly selected dogs showed prevalence of babesiosis of 15.9% (61/384).The prevalence of canine babesiosis was not statistically significant association with sex and age ($p>0.05$) but a statistically significant association was seen ($p<0.05$) with body condition score (BCS) of dogs (Table 1). The distribution of *B. canis* and *B. gibsoni* were identified in each kebeles (Table 2). The prevalence of canine babesiosis in eight PAs is shown in Table 3. This difference in prevalence was statically not significant ($p>0.05$) (Table 3). To assess the relationship between *babesia* infection and packed cell volume (PVC) determination using hematocrit and also the mean PCV of infected and non-infected dogs were calculated. The association of infection with anemia was found to be statistically significantly associated ($p<0.05$) (Table 4).

DISCUSSION

This is the first report in dog in and around Jimma town, in which infection rate of canine babesiosis was evaluated and confirmed. From a total of 384 blood samples collected from dogs, 61 samples were positive for canine babesiosis with light microscopy. The data collected show that the overall prevalence of canine babesiosis based on blood smear evaluation was 15.9 % in and around Jimma town. The result of this study is different from findings by Oduye and Dipeolu (1976) in Nigeria, who found a very high prevalence of Canine babesiosis (47%).

In this study the prevalence of babesia in female dogs (18.12%) in and around Jimma town was not significantly higher than in male dogs (14.47%) (Table 1). This means both male and female are equally susceptible and are equally exposed to the disease. This finding differs from finding by others (Bashir et al., 2009), who found that male dogs have significantly higher prevalence than female dogs.

Babesia can infect dogs of all ages thus in the present study, it was found that young dogs ($\leq$ 1 year) have prevalence rate of 12.37%, while the infection rate of adult (>1 and $\leq$3 years) and old (>3 years) dogs were 19.04 and 20.93% respectively (Table 1). In this study dogs that were $\leq$ 1 year were affected by *Babesia canis* (11.22 %) while *Babesia gibsoni* affects adult (6.8 %) and old dogs (11.63 %). The age distribution of dogs that were positive for babesia in this study was different from the finding of Oduye and Dipeolu (1976) in Nigeria and Bashir et al. (2009) in Pakistan (which the prevalence was 3.59, 4.46 and 3.85% in young, adult and old dogs, respectively). This might be due to the habit formed by the young animals of playing on the grasses around where they pick up a waiting tick that was ready to attach itself to scavenging host as observed during the study.

There was a significant difference in prevalence rate among poor and good body condition that is the result observed revealed the marked effect of babesiosis on body condition of dogs (Table 1). The prevalence of canine babesiosis in Dedo dima, Bebella kera, Gudeta bula, Doyo bikila, Furstale, Sato samora, Bosa kitto and Ifa bula were 20.45, 23.64, 20.83, 17.31, 14.04, 2.94, 2.56 and 18.18%, respectively (Table 3). Based upon this, the prevalence of canine babesiosis was higher in Bebella kera and lower in Bosa kitto kebele. Peaks in the proportion of babesia positive dogs were observed in around Jimma town (20.6 %) compared with in Jimma town (10.81 %). This can be due to different reasons. In Jimma town most of the dogs rather than stray dogs were kept in door, while the dogs found around Jimma town are more likely to roam in search of mates and this can increase their likelihood of contact with tick vectors as well as being involved in dog fights and contracting the infections (Bashir et al., 2009).

During the study period, the mean PCV of infected and non-infected dog was compared using student t-test at 95% confidence level ($p<0.05$). Dogs with PCV value <37 % were considered as anemic and those with PCV value

Table 1. Prevalence of canine babesiosis with sex, age and body condition score.

Risk factor		No. examined	No. of positive	Prevalence (%)	X^2	P-value
Sex	Male	235	34	14.47	0.911	0.340
	Female	149	27	18.12	-	-
	Total	384	61	15.9	-	-
Age	Young	194	24	12.37	-	-
	Adult	174	28	19.04	3.712	0.156
	Old	43	9	20.93	-	-
	Total	384	61	15.9	-	-
BCS	Poor	180	53	29.44	46.619	0.000
	Good	204	8	3.92	-	-
	Total	384	61	15.9	-	-

Table 2. Prevalence of *B. canis* and *B. gibsoni* in eight peasant associations.

Origin/Kebele	Species of *Babesia* (n= 61)		Total
	B. canis	*B. gibsoni*	
Dedo dima	3	6	9
Furstale	4	4	8
Sato samora	1	0	1
Bosa kitto	1	0	1
Ifa bula	7	3	10
Bebella kera	9	4	13
Gudeta bula	6	4	10
Doyo bikila	7	2	9
Total	38 (62.3%)	23 (37.7 %)	61 (100%)

Table 3. Prevalence of canine babesiosis in different origins.

Origin/Pas	No. examined	No. positive	Prevalence (%)	X^2	P-value
Dedo dima	44	9	20.45	-	-
Furstale	57	8	14.04	-	-
Sato samora	34	1	2.94	-	-
Bosa kitto	39	1	2.56	13.925	0.053
Ifa bula	55	10	18.18	-	-
Bebella kera	55	13	23.64	-	-
Gudeta bula	48	10	20.83	-	-
Doyo bikila	53	9	17.31	-	-
Total	384	61	15.9	-	-

≥37% were taken as normal (Kamani et al., 2011). The result of the present study showed that a mean PCV of 30.56 and 42.45% for infected and noninfected dogs respectively. It was generally accepted that PCV value is affected by many factors other than babesiosis. However, these factors likely affect both infected and non-infected dogs. The PCV values for positive and negative samples were compared and the result was found statistically strongly associated. The mean PCV value of studied animals was statistically significantly different ($p<0.05$)

Table 4. Association of the mean PCV of dogs with Babesia infection.

Condition	PCV range (%)	Mean PCV (%)	SD	CI	T-test	P-value
Infected	<37	30.56	4.786	10.55-13.23	17.18	0.000
Non-infected	37-55	42.45	4.988	10.53-13.25	-	-

between positive and negative dogs (Table 4). The prevalence of infected dog with PCV value <37% is 15.9%, while the prevalence of non-infected dog with PCV value <37% is 2.08%. The mean PCV value of the infected dogs was significantly lower than those of non-infected dogs. This finding agrees with the works of Kamani et al. (2008) and Shitta (2009) who observed a lower mean PCV value in infected dogs than non-infected dogs. The appearance of babesia negative dogs with PCV value <37% may due to inadequate detection method, other hemoparasite disease, helminthes parasite and delayed recovery of anemia after treatment.

There has been very limited research and no publications on babesiosis from Ethiopia. Finding of annual average babesia prevalence of 15.9% by microscopic examination in laboratory blood smear in this study is lower than findings by other researchers in Australia, who found babesia seroprevalence of 35.7% (Trapp et al., 2006). This can attributes to the fact that serology is more sensitive method of detection of previously, current and sub clinical infection than microscopic examination.

CONCLUSION AND RECOMMENDATIONS

The present study revealed that canine babesiosis is a major problem in the study area, and the result confirms the disease was more common around Jimma town rather than in Jimma town. The prevalence of Canine babesiosis was associated with several risk factors. Canine babesia infection was more likely associated with body condition of dogs. Those dogs affected by babesia parasite become poor in body condition, and also they become prone to anemia. The study also concludes that *B. canis* and *B. gibsoni* were the two species of babesia identified in the study area, strategic tick control should be practiced at the study area. Also the following recommendations are forwarded:

1. Since there was no publication on canine babesiosis in Ethiopia, a large survey is necessary in Ethiopia to investigate the rate of *babesia* infection and its associated risk factors in different areas of the country.
2. Detection of *babesia* parasite by serology and molecular examination should be more sensitive to find the parasite in blood.
3. Strategic tick control should be done to prevent canine babesiosis.
4. The stray dogs should be controlled so as to know the sample frame of the study animals in the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interest.

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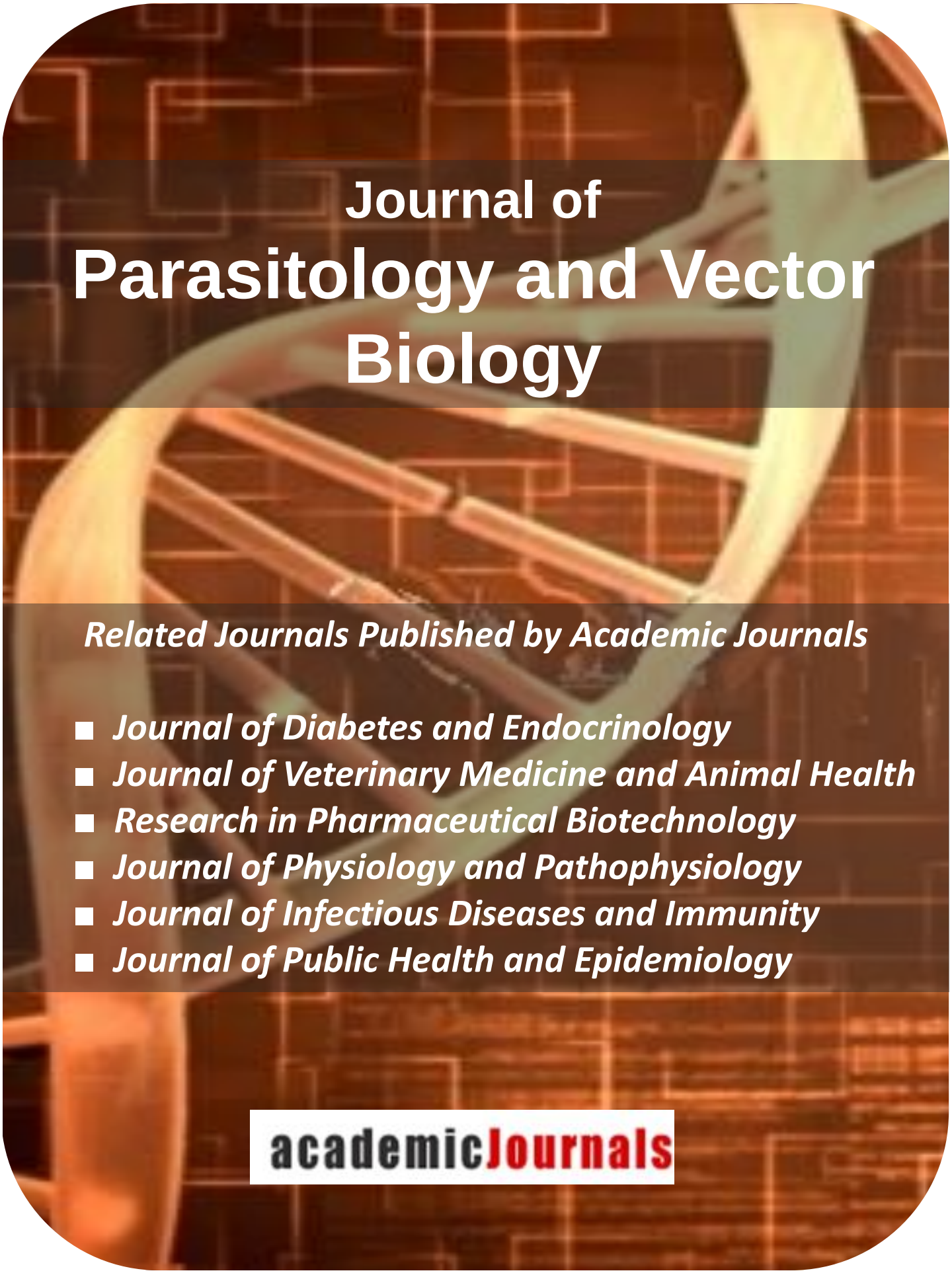

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