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Echinococcus granulosus Antibodies in Dogs and Breeder practices promoting spread of infection in Plateau State, Nigeria

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ABSTRACT

Background: Echinococcosis caused by *Echinococcus granulosus* is a zoonotic disease of public health significance, but there have been few studies of the infection in dogs in Nigeria. This study aimed to establish the seroprevalence of *E. granulosus* in dogs in four Local Government Area of Plateau State, Nigeria.

Methods: A total of 179 dog sera were examined for the presence of *E. granulosus* antibody using an ELISA kit between May to October 2017.

Results; Eleven of the sera (6.1%) were seropositive with a prevalence of 7.4% and 2.3% for dogs less than 2 years and those above 2 years of age respectively. More male dogs (7.5%) than females (4.1%) were seropositive. Seroprevalence was associated with <2years than > 2 years dogs (OR=3.33, p=0.46) and male than female dogs (OR=1.90, p=0.53) but association were not significant. Dogs from Bassa had the highest seroprevalence (15.2%), followed by Mangu (5.9%), Kanke (4.8%) and Jos South (2.0%). Odds ratio showed an association which was not significant between Bassa (OR=3.3), Kanke (OR=2.50).

Conclusion: This study shows that *E. granulosus* infection is present in dogs in some parts of Plateau State. More studies should be done to ascertain echinococcus infection in dogs in Nigeria to aid the formulation of control programmes to forestall its public health impacts.

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Keywords: *Echinococcus granulosus*, dogs, Antibody, seroprevalence zoonotic, Plateau

INTRODUCTION

Cystic echinococcosis (CE) is a world-wide important emerging parasitic, neglected and zoonotic disease caused by *Echinococcus granulosus* (Deplazes *et al.*, 2017; Cerda *et al.*, 2018; Jara *et al.*, 2019) and affects humans and animals (Alvarez Rojas *et al.*, 2014) (leading to serious public health and socioeconomic problems in endemic countries (McManus *et al.*, 2012; Grosso *et al.*, 2012). The disease is considered the second most significant helminthic disease in the world (Sangaran & John, 2009). The disease is reported as endemic in many countries including Sub Saharan Africa especially in sheep and cattle grazing rural areas where humans, dogs and livestock co-exist, promoting the perpetuation of the disease as dogs are fed raw infected livestock offal (Romig *et al.*, 2011; Otero-Abad and Torgerson 2013; Jackson *et al.*, 2017).

The infection cycle of *E. granulosus* require definitive hosts (dogs and other canids) which harbour the adult worm and the intermediate hosts such as deer, sheep, goats, cattle and camels (Carmena & Cardona, 2013). Humans are accidental or aberrant host as they do not play any role in the transmission of the disease except in rare cases. Suitable intermediate hosts become infected while grazing on vegetation contaminated with the eggs of *E. granulosus* while humans become infected if they accidentally ingest infective eggs from contaminated soil, water, vegetation diet and hair of infected definitive host (Craig *et al.*, 2017).

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In the intermediate host, ingested eggs develop and migrate via the blood stream to different organs where they eventually develop into hydatid cysts. The cycle is continued when the definitive host ingests organs of an infected intermediate host. Infection with *E. granulosus* may be asymptomatic or may be life threatening in humans depending on the location, size of the cyst as well as complications related to cyst rupture and spread of parasite material (Brunetti *et al.*, 2011). Although CE is a significant and widespread zoonotic disease in developed and developing countries, the epidemiology of CE is poorly understood in Sub-Saharan Africa (Romig *et al.*, 2017) to date, there are only very few studies using antibody ELISA to determine the presence of *E. granulosus* infection in dogs and humans in Nigeria (Adediran *et al.*, 2014; Bitrus *et al.*, 2020). Thus there is paucity of information on *E.granulosus* infection in animals in West Africa (Mauti *et al.*, 2016) including Nigeria This study aims to determine the seroprevalence of *E. granulosus* using antibody ELISA of dogs in Jos and environs, Plateau State.

MATERIALS AND METHODS

Study area and sample collection

The study was carried out from May to October 2017 in four local government areas (Bassa, Jos south, Mangu and Kanke) located in two senatorial districts of Plateau state, Nigeria. The choice of the designated study areas was based on accessibility. The study population included domestic (security, companion/farm and breeding) dogs that are more than 3 months old. Based on the reported prevalence of 12.4% of *E. granulosus* in dogs in South West Nigeria (Adediran *et al.*, 2014) a minimum of 167 sample is required for this study (Thrusfield 2005). Therefore, 179 dogs were sampled for the study. Prior to dog sampling, advocacy and sensitization visits were made to community heads, youth and religious leaders to create awareness and educate them on the basic facts on *E. granulosus* infection and the zoonotic importance of the disease.

Ethical clearance

Ethical clearance for this study was obtained from the Animal Use and Care Committee (AUCC), National Veterinary Research Institute (NVRI), Vom, Plateau state, Nigeria.

Questionnaire interview

Dog owners who assented and signed the consent forms were verbally interviewed using a questionnaire to obtain the animal rearing and management practices as well as respondent's knowledge of *E. granulosus* infection. Equally, information on age, sex, breed and the use of the dogs was obtained. Each dog was identified by house number along with a sequential numbering depending on the number of dogs per household. The services of dog catchers were employed to ensure adequate restraint of dogs before sample collection.

Blood collection

About 5mls of blood was collected through the cephalic vein of each dog into a labelled plain capped glass tubes without anticoagulant and stacked in a slant position in a cold box and transferred to the Parasitology Laboratory, NVRI, Vom. Samples were allowed to clot in slant position on laboratory bench undisturbed before they were centrifuged at 1500 revolution per minute for 10 minutes. The supernatant was carefully separated and transferred into a clean appropriately labelled micro centrifuge tubes and stored at -20^{o}C until required.

Faecal sample collection

Faecal sample was collected through the rectum with gloved hands. Approximately 5gms of fresh stool was scooped into a plastic container sealed and appropriately labelled. In situations where the dog(s) had already defecated, freshly voided faeces was collected from sites of defecation if identified for the individual dog, otherwise pre-labelled specimen bottles were distributed to dog owners who assisted in the collection of faecal samples. The samples were transported to the laboratory within 4-12 hours and processed immediately or kept at + 4^{o}C. Dewormers meant for dogs and humans were given to respondents as an incentive for their co-operation to participate in the study.

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Serological analysis

The dog serum samples were analysed for *Echinococcus granulosus* antibodies using Enzyme Linked Immunosorbent Assay (ELISA) technique. A commercial kit, *Echinococcus* Antibody (IgG) ELISA Kit (Canine) (OKCA00258) from AVIVA SYSTEMS BIOLOGY CORPORATION, San Diego USA was used according to manufacturer's instructions.

Microscopic examination of faeces

Faecal samples were also processed using the standard flotation and sedimentation techniques using saturated salt (NaCl, specific gravity 1.2) solution (WHO 2003). Each sample was examined microscopically at 10× and 40× magnification under a Zeiss Axiolab light microscope. The identification of parasite eggs/cysts/ova was done based on morphology (Soulsby, 1982).

Statistical analysis

Epi Info calculator was used to determine the odds ratio, confidence Interval, chi square and P-Value. The Chi-square test was used to compare the difference in frequencies of Echinococcus infection between locations, age and sex with a level of significance set at p value < 0.05.

RESULTS

Seroprevalence of *Echinococcus* Antibody (IgG) in dogs in some localities in Plateau State

Eleven out of 179 (6.1%) dog sera examined were positive for *Echinococcus* antibody (IgG). Dogs below two years of age accounted for (7.4%) of positive samples while those above two years old accounted for 2.3%. Odds ratio showed association between *Echinococcus granulosus* infection in dogs and age (OR:3.33, p; 0.464),however association is not statistically significant. More male (7.5%) than female (4.1) dogs were positive for *E. granulossus* antibodies and Odds Ratio showed association between gender and *Echinococcus granulosus* infection in dogs, however association is not statistically significant (OR:1.90, 0.53) as indicated in Table 1. In respect of location, dog sera from Bassa had the highest seroprevalence (15.2%) followed by Mangu (5.9%) and Kanke (4.8%), while Jos South had the least prevalence of 2.0%. Odds Ratio showed association between *Echinococcus granulossus i*nfection and dogs from Bassa (OR: 3.30, P: 0.26) and Kanke (OR: 2.50, P: 0.76) however association was not statistically significant. Contra wise, there was no statistically significant association between infection and dogs from Mangu (OR: 0.40, P: 0.45) and Jos South (OR: 0.33, P: 0.73) as indicated on Table 1.

Table 1. Seroprevalence of *Echinococcus* Antibody (IgG) in dogs in some localities in Plateau state Nigeria

Variable	Number sampled	Number Positive	Positive Percentage	OR	95% CI	X^2	P-value
Age							
Young	136	10	7.4	3.33	0.41, 26.81	0.7	0.464
Adult	43	1	2.3				
Gender							
Male	106	8	7.5	1.9	0.48, 7.43	0.4	0.53
Female	73	3	4.1				
Location							
Mangu	34	2	5.9	0.4	0.07, 2.10	0.6	0.45
Bassa	33	5	15.2	3.3	0.73, 14.70	1.54	0.26
Kanke	62	3	4.8	2.5	0.25, 24.71	0.09	0.76
Jos south	50	1	2	0.33	0.02, 3.80	0.12	0.73

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Table 2 Dog keeping practices in some localities among dog owners in Plateau State

Variables	**Frequency (Percent)**
Why do you keep dogs?	
Companion	51 (31.9)
Guard/security	71 (44.4)
Breeding and guard dogs	17 (10.6)
Consumption	9(5.6)
Breeding, guard and consumption	12 (7.5)
Dog's feeds	
kitchen left over	63 (30.3)
kitchen left over with grain and bone	76 (47.5)
Commercial feed	21 (13.1)
Slaughter slabs in the locality?	
Yes	70 (33.7)
No	138 (66.3)
Does your dog(s) scavenge?	
Yes	124 (77.5)
No	36 (22.5)
Does your dog(s) accompany livestock for grazing?	
Yes	71 (44.4)
No	89 (55.6)
Do you deworm your dog(s)?	
Yes	61 (38.1)
No	99 (61.9)
Seen clear fluid on organs of slaughtered livestock	
Yes	66 (31.7)
No	142 (68.3)

Data presented as frequency (percent)

Dog keeping practices among dog owners in some localities in Plateau State, Nigeria.

The result respondents who keep dogs are as follows: about 44.4% (71/160) of them keep dogs for security, 31.9% (51/160) keep them for companionship, 5.6% (9/160) keep them for consumption, 10.6% (17/160) keep them for breeding and guard dog while 7.5% (12/160) keep them for breeding, guard and consumption. In respect of feeding, 47.5% (76/160) of the respondents fed them with kitchen waste, bone and grain while 30.3% (63/160) fed them with only kitchen waste, however only 13.1% (21/160) fed them with commercial feed as indicated in Table 2. Furthermore, most of the dog owners 77.5% (124/160 of the dog owners allow their dogs to scavenge while only 22.5% (36/160) do not allow them to scavenge. About 44.4% (71/160) of the respondents allow their dogs to accompany their animals for grazing while 55.6% (89/160) of them do not. Only few 38.1% (61/160) of the dog owners deworm their dogs while majority of 61.9% (99/160) as indicated in Table 2.

Prevalence and type of Gastrointestinal Parasites in Dog in Plateau State, Nigeria.

An overall prevalence of 24.6% (44/179) of GI parasites was recorded for dogs examined in this study. The highest prevalence was obtained for

Table 3: Prevalence and type of gastrointestinal parasites (GI) parasites detected by microscopy in dog faeces in Plateau State

		Parasite type, No. positive (%)				
Variables	**No. sampled**	***Eimeria spp***	***Toxocara canis***	***Taenia sp.***	***Ancylostoma caninum***	**Total**
Location						
Mangu	34	0(0.0)	12(35.3)	2(5.9)	12(35.3)	26(76.5)
Bassa	33	4(12.1)	5(15.2)	0(0.0)	5(15.2)	14(42.4)
Kanke	62	0(0.0)	0(0.0)	2(3.2)	0(0.0)	2(3.2)
Jos south	50	0(0.0)	0(0.0)	0(0.0)	2(4.0)	2(4)
Age						
Adult	41	1(2.4)	2(4.9)	1(2.4)	3(7.3)	7 (17.1)
Young	138	3(2.2)	15(10.9)	3(2.2)	16(11.6)	37 (26.8)
Gender						
Male	126	2(1.6)	11(8.7)	3(2.4)	13(10.3)	29(23)
Female	53	2(3.8)	6(11.3)	1(1.4)	6(11.3)	15(28.3)
Total	179	4(2.2)	17(9.5)	4(2.2)	19(10.6)	44(24.6)

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Ancylostoma caninum (10.6%) followed by *Toxocara canis* (9.5%) and 2.2% each for *Eimeria* spp., and *Taenia* spp. A higher prevalence of gastrointestinal parasites was obtained in young than older dogs (26.8% vs 17.1%), and a higher prevalence was observed for female than male dogs (28.3% vs 23%) as indicated in Table 3.

DISCUSSION

The seroprevalence of 6.1% *Echinococcus* antibody in dog sera screened in this study is lower than the 12.5% reported in a similar study conducted in South West state of Nigeria (Adediran *et al.*, 2014). Studies from other countries showed a similar prevalence of 6.57% by PCR compared to 2.77% by conventional floatation methods in stray and domesticated dogs in Akola district of Maharashtra state in India (Ingole *et al.*, 2018). Higher prevalence of 16.5% was obtained in domestic dogs in China by copro-multiplex PCR assay (Liu *et al.*, 2018). However, lower prevalence of 2.6% was obtained in Southern China by ELISA (Liao *et al.*, 2016).

Generally, there appears to be scant information on echinococcosis/hydatid disease in both definitive and intermediate host in Nigeria compared to developed countries where series of studies have been carried out using modern biological and molecular techniques in all aspects commensurately to the public health significance of the disease. There are reports of higher prevalence of *E. granulossus* in several parts of the world based on species specific PCR assays which underscores the advantage of using modern over classical diagnostic methods. Sadly, most of the studies reported in Nigeria were based on retrospective and prospective studies of hydatid disease of food animals especially at abattoirs/slaughter slabs (Magaji *et al.*, 2011). These diagnostic techniques have relatively low sensitivity and specificity, hence, data obtained may underestimate the true prevalence of the disease in Nigeria. This calls for more comprehensive studies using modern methods to elucidate the epidemiology, socio-economic and public health impact of the disease in Nigeria.

The association between seroprevalence of echinococcus infection and location in the present study was not statistically significant, contrary to the findings of other workers (Oba *et al.*, 2016; Wang *et al.*, 2016), which showed a significant difference in respect to the prevalence of echinococcus infection in dogs and location. The non-significant variations in the seroprevalence of *E. granulosus* in the various study locations recorded in the present study could be attributed to the level of enlightenment of dog owners and availability of veterinary services. The seroprevalence of *E,granulosus* infection in the present study is higher in males **(** 7.5%) compared to females (4.1%).

Furthermore, the association of seroprevalence with male dogs reported in this study is in tandem with other studies (Weng *et al.*, 2020) which is contrary to the findings of a study (Harriott *et al.*, 2019) which found higher prevalence in female dogs. The association of higher prevalence with male dogs was thought to be due to the tendency of male dogs to roam freely especially during mating season. Similarly, male dogs are more frequently used as guard dogs during extensive grazing, exposing them to scavenging and predations which predisposes them to infection with *E. granulosus* cysts. The current study showed that young dogs had a higher seroprevalence of 7.4 % while adult had a seroprevalence of 2.3%.

Age related seroprevalence has been reported by several authors. A study (Acosta-Jamett *et al.*, 2014) documented that older dogs have a lower probability of positivity with *E. granulosus* infection as a result of some degree of acquired immunity or age-related variation in dog's behaviour or management. Contrary to our findings, some studies reported higher prevalence in adult dogs than younger dogs, which was attributed to continued exposure to *E. granulossus* in the environment by adults or immune suppression due to concurrent infections (Craig *et al.*, 2017; Liu *et al.*, 2018). An important finding in this study which agrees with other studies outside Nigeria, was the feeding of dogs by their owners. It was observed that most dog owners could not provide good quality feed to their dogs due to poverty therefore, allowing the dogs to scavenge, thereby exposing them to *E. granulossus* infection (Conceição *et al.*, 2017; Thevenet *et al.*, 2020). The current study showed that 39.3 %(63) of the dog owners fed their dogs with kitchen left over This was similar to studies of (Weng *et al.*, 2020) as they showed that most dog owners provide dogs with human food leftovers while others fed them livestock viscera when available.

The current study showed that only 13.1% fed their dogs with commercial feed which agrees with the findings of das Neves *et al.*, (2017) who reported that feeding commercial feed to dogs are not normally practiced in tropical forest communities, which is most likely due to poverty/poor socio-economic status or cultural behaviour. A study (Liu *et al.*, 2018)) similarly reported that majority of rural families are less likely to provide nutrient-rich food to dogs. The scavenging (77.5%) behaviour of most of the dogs screened in this study agrees with the findings of some authors that free-roaming dogs were risk factors of echinococcus infection (Thevenet *et al.*, 2020; Van Kesteren *et al.*, 2013) as they were responsible for the maintenance of a permanent parasitic infection pressure of zoonotic importance (Conceição *et al.*, 2017)). It was observed that dogs around the slaughterhouses and rural areas were more prevalent compared to domesticated dogs residing in towns (Ingole *et al.*, 2018).

The practice of home slaughter of other livestock species by dog owners without proper inspection and disposal of infected offal predisposes dogs to parasitic infections as earlier reported in some studies (Oba *et al.*, 2016; Singh *et al.*, 2014). Deworming of dogs seems to be a difficulty to dog owners as the current study showed that 38.1% (61) of the respondents deworm their dogs while 61.9% (99) do not deworm their dogs which was also similar to reports of (das Neves *et al.*, 2017). It was established that the frequency of deworming was irregular which is tantamount to not clearing off the parasitic burden from the dogs. Studies of (Wang *et al.*, 2016) also showed that deworming frequency was a significant factor responsible for Echinococcus infection as respondents in their study had never given their domestic dogs anthelmintic. Contra wise a study in Xiji County of Ningxia Hui in China showed that 87.8% families carried out dog de-worming (Liu *et al.*, 2018). The situation is further complicated by the high level (68.3%) of ignorance about *E. granulosus* infection among dog owners in this study. Knowledge gaps in the epidemiology of disease has been identified as a risk factor for disease perpetuation in a community (Adediran *et al.*, 2014; Oba *et al.*, 2016).

Traditionally, *E. granulosus* infection in dogs has been determined by identifying the egg or worms in faecal samples (Ingole *et al.,* 2016). However, this method is not very sensitive. Hence, the present study did not find egg or worms of *E. granulosus* in the faecal samples examined similar to the study of (das Neves *et al.*, 2017). Although *E. granulosus* egg was not observed microscopically, in the present study, four important intestinal parasite genera were identified. Importantly, *Ancylostoma caninum* and *Toxocara canis* detected from dogs in this study have zoonotic potentials. Several other studies have reported high prevalence of GI parasites in dogs in Sudan, Mali and Turkey similar to this study (Mauti et al., 2016; Omer et al., 2018) . Such findings could be attributed to the preponderance of risk factors for GI parasite transmission in the communities and the lack of veterinary care.

CONCLUSION

Considering the public health significance and socio-economic impact of *E. granulossus* infection, proactive steps need to be taken to curtail the spread of this disease and safeguard the health of the public. Furthermore, there is need for enlightenment campaign to promote personal hygiene, responsible dog ownership and sanitation geared towards the control of GI parasitism.

COMPETING INTEREST

Authors declare that they have no competing interests.

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