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REGULAR ARTICLE

Prevalence and risk factors of *Echinococcus granulosus* infection in dogs in Moroto and Bukedea districts in Uganda

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Abstract A cross sectional study was conducted in Moroto and Bukedea districts of Uganda from May to September 2013 to determine the prevalence and risk factors of *Echinococcus granulosus* infection in dogs. Fresh dog faecal samples were collected, preserved in 70 % ethanol, and later screened for presence of taeniid eggs using zinc chloride floatation method. Positive samples were confirmed by a copro-PCR (polymerase chain reaction) for *E. granulosus* using NADH dehydrogenase sub-unit 1 gene (NADH1) as a target molecular marker. Structured questionnaires and focus group discussions were used to collect quantitative and qualitative data for risk factor identification. Study sub-counties were selected by simple random sampling. Overall apparent prevalence of taeniid infection in dogs of 14.9 % (39/261, confidence interval 10.6–19.2) in both districts was recorded using the faecal floatation test. The sensitivity of the faecal floatation test was found to be 78 % (25/32), while the specificity was 93 % (215/229). Copro-PCR results revealed a true prevalence of 14.4 % (9.91–19.0, 95 % CI) in dogs in Moroto district and 7.4 % (2.14–12.60, 95 % CI) in Bukedea district. The overall true prevalence of cystic echinococcosis (CE) was 12.2 % (8.70–15.76, 95 % CI) in both districts. The major risk factors identified using logistic regression were uncontrolled access of dogs to animal slaughter facilities, higher cattle herd sizes and lack of knowledge about the disease. It was recommended that restricting dog access to infected tissues and public health education about epidemiology of CE should be done.

Keywords *Echinococcosis granulosus* · Prevalence · Risk factors · Dogs

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Introduction

Cystic echinococcosis (CE) is a zoonotic parasitic disease affecting both domestic and wild animals caused by the metacestodes of the dog tape worm *Echinococcus granulosus.* Studies show that CE is becoming an increasing public health and socioeconomic concern in many countries. Human CE caused by *E. granulosus* and alveolar echinococcosis caused by *Echinococcus multilocularis* are important public health threats in many parts of the world (FAO 2011; WHO/OIE 2001). In 2005, the World Health Organization (WHO) included echinococcosis and cysticercosis as part of a neglected zoonosis sub-group for its 2008–2015 strategic plans for the control of neglected tropical diseases (NTDs). Both CE and cysticercosis are also to be included in a review of the Global Burden of Disease Study (Gemmell et al. 2001; Torgerson et al. 1995).

CE is highly endemic in sub-Saharan Africa (Berhe 2009; Romig et al. 2011). In Uganda, limited information exists on the magnitude of echinococcosis infection in dogs. As a result, appropriate interventions are difficult to formulate and implement. Echinococcosis surveillance in dogs, livestock and humans provides information for establishment of pre-intervention baseline and assesses efficacy of control programmes. A study was therefore conducted to establish the prevalence of *E. granulosus* infection and to identify

major risk factors for its persistence in dogs, which could guide the formulation of appropriate control measures.

Materials and methods

Study areas

A survey was conducted in Moroto district, located in Karamoja region and Bukedea district in Teso sub-region. Moroto district was chosen for the study on account of its close proximity to Turkana region of North-western Kenya where a high prevalence of CE has been reported (Macpherson et al. 1983). Bukedea district was chosen to represent a mixed crop-livestock agro-pastoral area for comparison. The dog population in Bukedea district was estimated to be 1320 dogs (Bukedea District Veterinary Report, 2013) and 2250 in Moroto district (Moroto District Veterinary Report, 2012).

Study design and sample size determination

In each of the study districts, sub-counties were randomly selected for the study. In Moroto district, three sub-counties were randomly selected and these were as follows: Moroto municipality, Rupa and Nadunget. In Bukedea district, the four sub-counties randomly selected were as follows: Kachumbala, Kolir, Bukedea Town Council and Malera. Based on previous surveys by Inangolet et al. (2010) and Ernest et al. (2009), a formula by Thrusfield (2005) was used to determine the required sample sizes of 196 dogs in Moroto and 72 dogs in Bukedea district, giving a total sample size of 268 dogs.

Sampling design and sampling of study dogs

A list of households that kept dogs was obtained from the area veterinarian. Assuming that each household kept at least one dog, the households were then selected by systematic random sampling, with every third household being considered. The selected households were then visited for collection of faecal samples from dogs.

Collection of faecal samples and administration of questionnaires

In households that kept 1 to 2 dogs, both dogs were sampled. On occasions where more than 3 dogs were kept, proportionate sampling of 30 % of dogs was done. Dogs were restrained with a mouth gag, and faecal samples were taken directly from the rectum with gloved hands. The faecal samples (≈5 g) were kept in 10-ml plastic containers containing 70 % ethanol and transported to a laboratory in the Department of Veterinary Preventive Medicine and Public Health at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Kampala, Uganda, for laboratory analysis. A structured questionnaire was then administered, and focus group discussions held with key informants about the knowledge gaps, practices and risk factors for persistence of echinococcosis in dogs in their area.

Laboratory analysis of faecal samples by floatation and Copro DNA PCR

The taeniid eggs were recovered using the floatation technique described by Mathis et al. (1996) and Huttner et al. (2009). A positive sample was considered if it had a typical taeniid egg that was ovoid, brown striated appearance with characteristic hooklets (WHO/OIE 2001). Identification and confirmation of *E. granulosus* eggs was done according to the polymerase chain reaction (PCR) protocol described by Huttner et al. (2008) and (2009).

Data analysis

Data was entered into Excel 16.0 and analysed using R statistical software version 3.1.2 (The R Core Team 2014). The relationship between the exploratory and response variables was examined at 95 % confidence interval. Univariate and multivariate logistic regression analysis was done to identify key risk factors. Values of $p<0.05$ were considered statistically significant.

Results

The overall prevalence of taeniid infection using floatation of 14.9 % (39/261, CI 10.6–19.2) in both districts was determined. Details were as shown in Table 1. Of the studied sub-counties, the highest prevalence of 21.4 % (95 % CI 13.8–29.0) was recorded in Moroto Municipality in Moroto district, with the lowest being Kachumbala sub-county in Bukedea district.

PCR results

On PCR analysis of the samples assumed positive by floatation test, 81.25 % for Moroto district ($n=26$) and 85.7 % for Bukedea district ($n=6$) were confirmed to be *E. granulosus* infection. This gave a sensitivity of 78 % (25/32) and a specificity of 93 % (215/229) of the faecal floatation test when compared to Copro-PCR test (a gold standard). In Moroto district, the true prevalence of CE infection in dogs was found to be 14.4 % (26/180; 9.91–19.0 %, 95 % CI) while it was 7.4 % (6/81; 2.14–12.6 %, 95 % CI) in Bukedea district. Of the 39 samples that were identified as positive by the faecal floatation method in both districts, 82 % ($n=32$) samples were

Table 1 Prevalence of taeniid infection in dogs by faecal floatation method

District	Sub-county	No. faecal samples collected	Total positive	Percent prevalence	95 % conf. interval
Moroto	Municipality	112	24	21.4	13.8–29.0
	Rupa	48	6	12.5	3.1–21.8
	Nadunget	20	2	10	3.1–23.1
Bukedea	Kachumbala	20	1	5	4.5–14.5
	Kolir	21	2	9.5	3.4–22.4
	Bukedea T/C	20	2	10	3.1–23.1
	Malera	20	2	10	3.1–23.1
Total		261	39	14.9	10.6–19.2

confirmed to be *E. granulosus*. Thus, the overall true prevalence of *E. granulosus* in dogs in both Moroto and Bukedea districts was found to be 12.2 % (n=32; 8.6–16.7 95 % CI).

Risk factors for *Echinococcus granulosus* infection in dogs

In Bukedea district, 74.3 % (n=55) while 65 % (n=42) of the respondents Moroto district had never heard of CE infection in dogs. As for the reasons given for keeping dogs, security was a major reason 82.4 % (n=61) in Bukedea and 52.3 % (n=34) in Moroto district followed by hunting which is 14.8 % (n=11) in Bukedea and 44.6 % (n=29) in Moroto district.

The proportion of dogs used for hunting in Moroto was significantly higher ($p<0.05$) than that in Bukedea district. Results showed that 85 % (n=53) and 96.9 % (n=70) of domesticated dogs were free to roam in Moroto and Bukedea districts, respectively. Bukedea district had a significantly higher proportion of dogs that were free to roam ($p<0.05$) compared to Moroto district. In Moroto district, nearly all (96.9 %, n=63) of the respondents never routinely removed dog faeces from their compounds as compared to only 21.3 % (n=16) of the respondents in Bukedea district (χ^2=19.9, $p<0.001$). In Bukedea district, 77 % (n=50) of the respondents claimed dog droppings were taken to the pit latrine or placed in a dugout pit on a nearby ground and covered with soil.

Practices of communities towards tissue cysts

In Moroto district, only 40 % (n=26), while 24 % (n=18) of respondents in Bukedea district, had prior information about cysts. However, a majority were unaware of the potential dangers of presence of cysts in animal tissues. In Moroto, 92.3 % (n=60) of the respondents and 83.8 % (n=62) in Bukedea district could either throw cysts away or feed them directly to the dogs.

Results of univariate regression analysis of risk factors for CE infection

Table 2 shows the results from univariate analysis. The fogs from Moroto counties were more likely to be infected with *E. granulosus* than those in Bukedea county ($p<0.05$). This was reflected by the higher prevalence of the infection in

Table 2 Summary of univariate regression analysis of potential risk factors for infection of dogs with Echin*ococcus granulosus* in Moroto and Bukedea districts

Potential risk factor	Variable category	Factor present	Factor absent	χ^2 statistic	Fishers exact test OR (95 % CI)	p value
Origin	Moroto Bukedea	14/65 5/74	51/65 69/74	6.407	3.75 (1.181–14.19)	0.01137*
Observations of cysts in animal tissues (yes)	Moroto Bukedea	41/65 24/74	24/65 50/74	–	3.525 (1.671–7.62)	0.00030**
Access to slaughter facilities	Yes No	15/57 4/82	42/57 78/82	–	6.865 (2.018–30.25)	0.00067**
Herd size (higher)		10/112	18/27	15.76	–	0.00127*
County of origin		5/75	42/52	7.5806	–	0.0226*
Meat inspection (no)		9/19	63/120	–	0.9948 (0.3316–2.94)	1.000
Type of feed given to dogs		18/120	110/128	05187	–	0.9148

*$p<0.05$ statistically significant; **Statistically significant at $p<0.01$

Table 3 Difference in prevalence of *Echinococcus granulosus* in dogs according to different variables (district, access to slaughter facility and cattle herd size)

Variable		Infection status		χ^2 stat	*df*; *p* value
		Infected	Not infected		
District	Moroto	32	148	6.407	*df*=1; 0.01137*
	Bukedea	7	74		
Access to slaughter facility	Yes	15	42	13.09	*df*=1; 0.0007**
	No	4	78		
Cattle herd size	0–16	10	102	15.76	*df*=3; 0.00127**
	17–32	5	9		
	33–49	4	0		
	Beyond 50	0	4		

*Statistically significant at $p<0.05$; **Statistically significant at $p<0.01$

Moroto than in Bukedea district. The dogs kept in sub-counties located in the urban settings (19.6 %, $n=26$) were significantly ($\chi^2=4.7499$, $p=0.029$) more infected than those in the rural areas (10 %, $n=13$). It was further shown that observations of cysts in animal tissues and access of dogs to livestock slaughter facilities were potential risk factors of infection ($p<0.05$), as was the county of origin of the dog.

Table 3 shows differences in prevalence of *E. granulosus* according to different variables (district, access to animal slaughter facilities and herd size).

The results of the multivariate analysis were as shown in Table 4. In this model, herd size (OR=1.06) and access of dogs to livestock slaughter facilities (OR=11) were significantly ($p<0.001$) associated with infection of dogs and were therefore identified as key risk factors for infection of dogs with *E. granulosus.*

Discussion

The dogs in Moroto district had a higher prevalence of *E. granulosus* infection than those in Bukedea district. These findings were lower than those reported by Inangolet et al. (2010), who found a prevalence of 24.6 % (20.1–29.5 95 % CI) in semi-domesticated and 32.4 % (27.5–37.7, 95 % CI) in domesticated dogs in Moroto district on postmortem examination. The prevalence of CE in dogs found in this study of 12.2 % could be lower than the actual situation, given the lower sensitivity of the faecal floatation method which was used as an initial positive taeniid egg screening test. The prevalence reported in this study was comparable to that reported by Ernest et al. (2004) and Magambo et al. (2006) in dogs (10 %) in Ngorongoro district, Tanzania. However, in eastern Ethiopia, Mersie (1993) found a prevalence of 22 % in dogs when examined by postmortem. In another study by postmortem examination of adult stray dogs, a prevalence of 16.7 % in Tigray region, Northern Ethiopia, was recorded (Kebede 2009). Adediran et al. (2014) found a prevalence of 12.45 % in Southwest Nigeria by direct ELISA. In contrast, Macpherson et al. (1985) found that more than 50 % of dogs were positive for *E. granulosus* when investigated by postmortem. While Gathura and Kamiya (1990) reported a prevalence of 13–70 %. These findings significantly differed from ours probably because of the application of gold standard methods (postmortem). Taken together, majority of the prevalence findings ranged between 10–22 % in concordance with our findings, a fact attributable to low sensitivity of the faecal flotation method.

Our finding revealed that access of dogs to livestock slaughter facilities significantly increased the risk of infection (OR=11; $p<0.001$). The dogs which had access to livestock slaughter facilities were more likely to be infected with *E. granulosus* than those which did not ($p<0.05$). In both districts, the higher proportion of roaming dogs (about 90 %) that had access to slaughter facilities could explain this finding. This agreed with previous findings by Otero-Abad and Torgerson (2013) who reported higher *E. granulosus* coproantigen positives among dogs fed on condemned viscera or raw offal. Similarly, Buishi et al. (2006) revealed that dogs that had access to condemned offal were 12 times more likely

Table 4 Multivariate regression analysis for risk factors of *Echinococcus granulosus* infection

Coefficients	Log of odds ratio	Std. error	*Z* value	Pr(>|z|)
Intercept	2.2165	0.5191	4.27	2e−05***
Herd size	0.0598	0.0182	−3.28	0.00102**
Access to slaughter facilities	2.4018	0.6940	3.46	0.00054***
Meat inspection (yes)	−1.0193	0.6008	−1.70	0.08975

Statistically significant at $p<0.01$;*statistically significant at $p<0.001$

to be infected with *E. granulosus*. This showed that lack of control of dogs to access slaughter facilities was the major infection factor of dogs with CE.

Higher cattle herd sizes were associated with increased likelihood of echinococcal infection. We attribute this to increased cattle slaughter rates, which increased chances of feeding on infected tissues. This agrees with a previous study, which found that frequency of home slaughters was a risk factor for dog infection (Wahlers et al. 2012). Knowledge gaps existed, as shown by 92.3 % of respondents in Moroto and 83.8 % of respondents in Bukedea, who could either throw cysts away or feed them directly to the dogs. This also agreed with a study by Adediran et al. (2014), which found a high prevalence of echinococcosis in hunting dogs, since they were more likely to be fed raw viscera due to ignorance of the owners. It was concluded that the key risk factors of *E. granulosus* infection in dogs were free access of dogs to livestock slaughter facilities, higher cattle herd sizes and limited knowledge about the parasite.

Livestock slaughter facilities should be strictly controlled to limit access by dogs. Meat inspection regulations should be enforced to limit access of dogs to condemned animal tissues, which may perpetuate the parasite. Public health education is crucial to improve understanding of the parasite's transmission dynamics. We recommend further studies in the region to guide the formulation of appropriate control measures.

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Compliance with ethical standards

Statement of animal rights All applicable international, national and institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no competing interests.

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