Enzyme-Linked Immunosorbent Assay (Elisa) In The Serodiagnosis Of Hydatidosis In Camels (Camelus dromedarius) And Cattle In Sokoto, Northern Nigeria

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Abstract

This study was undertaken to evaluate the usefulness of Enzyme Linked-Immunosorbent Assay (ELISA) in determining the current status of hydatidosis in Camels and Cattle slaughtered in the Sokoto metropolitan abattoir, Sokoto State, Nigeria and also to ascertain the sensitivity and specificity of the test. A total of 474 serum samples were examined from 189 camels and 285 cattle. The overall prevalence of Echinococcus granulosus antibody response was 38.6%. The higher prevalence of 59.3% was recorded in camels while cattle had the least (24.3%). The occurrence of antibodies to hydatidosis in both species was significantly different (p<0.05). The sensitivity of ELISA in camels and cattle was 96.4% and 70.5% while the specificities were 80% and 76% respectively. Although a higher prevalence was recorded for older animals than younger ones, there was no statistical significant difference between the age groups and disease prevalence (p>0.05) and no association between age and disease prevalence, however the association between the sex of the animals and disease prevalence was highly significant (p<0.01). The result of this study concludes that hydatidosis is prevalent in camels and cattle in Sokoto and the ELISA is a sensitive and specific assay and could be employed in serodiagnosis of hydatidosis in camels, cattle and other domestic animals particularly for large scale screening purposes.

INTRODUCTION

Hydatidosis is a zoonotic disease caused by larval stages of cestodes belonging to the genus Echinococcus. It is characterised by long term growth of the metacestode (hydatid cysts) in the intermediate host. It is a wide spread infection throughout the world and is found to occur in all domestic livestock including camels and cattle (Ibrahem et al., 2002).

Hydatidosis is a global and human health problem of increasing economic importance (Lightowlers et al., 2000). The public health importance is not only in the areas of endemicity but also in countries or regions without endemicity due to the migration of infected people and livestock exchanges. In particular, the movement of infected livestock increases the potential for transmission and creating new areas of endemicity (Wulamu et al., 2002).

Hydatidosis is highly distributed in underdeveloped countries, especially in rural communities where humans maintain close contact with the dog definitive host and domestic animals that act as intermediate hosts (Mohammad and Nezhat, 2004). Other factors such as agricultural practices, indiscriminate home slaughtering and poor disposal of cysts from infected livestock, lack of adequate control policy, uncontrolled movement and commerce of animals and their products, and the difficulty in early diagnosis have enhanced the distribution of the disease (Dada and Belino, 1979).

In Nigeria, the disease has been recorded in livestock by several workers but its actual status is not known especially in the use of specific and sensitive diagnostic method but rather diagnosis particularly in livestock is commonly made at slaughter and often based on post-mortem recovery of hydatid cysts from infected organs (Dada and Belino, 1978; Ogunsan et al., 2000; Rabi'u and Jegede, 2010).

Serological approaches based on the detection of specific antibodies in infected animal sera by immunoelectrophoresis

(IEP), indirect heamagglutination (IHA), ELISA or immunoblot have been assessed principally in sheep as well as in ovine cases (Craig, 1997; Lightowlers, 1990; Ibrahem et al., 2002). However, serological studies on camel hydatidosis have been limited and application of ELISA or immunoblot for detection of camel antibodies have been limited by lack of commercially available anti-camel immunoglobulin. These reagents have now become available for parasitic infection research study (Azwia, Carter and Woldehhiwet, 1995).

In the present study, ELISA was used to screen serum samples collected from camels and cattle slaughtered in Sokoto abattoir, Sokoto State, Northern Nigeria, for the serological detection of E. granulosus antibody response and to assess its usefulness as an immunodiagnostic tool in this area.

MATERIALS AND METHODS

Serum samples

Blood samples were collected at slaughter and transported to the Public health Laboratory of the Usman Dan Fodio University, Sokoto where they were centrifuged at 2000 rpm for 5 minutes at room temperature to separate serum (Hashemitabar et al. 2006) and stored in 1ml aliquots at -200 C. Each serum sample was numbered and labelled as positive or negative for hydatidosis according to the post-mortem results.

Sera were collected in Sokoto from 84 camels and 5 cattle naturally infected with hydatidosis (based on post-mortem identification) as well as from 105 camel and 280 cattle graded as 'normal' at meat inspection (i.e., without visible hydatid infection).

Enzyme-linked immunosorbent assay (ELISA)

Echinococcus granulosus ELISA (Cat number 8202-3, Diagnostic Automation, USA) was performed in a 96-well micro-titre plate. Anti bovine IgG (Abcam Plc), and Anti camel IgG (Triple J Farms, USA) were respectively used as conjugates during the ELISA. The manufacturer's guide was carefully followed.

The wells had been coated with secreted antigens of E. granulosus with the protein concentrations of 2.5ug/ml phosphate buffer. Sera samples were diluted (1:64) by phosphate-buffered saline (PBS) and 1% bovine serum albumin (BSA) at pH of 7.4. For each micro-titre plate, the first well A1 was left blank and 100ul of the Positive and Negative controls from the manufacturer were loaded into wells A2 and A3 respectively. 100ul of animal sera (camels and cattle) was loaded into the remaining wells (for the two plates for camel and three plates for cattle) and incubated for 10mins at room temperature. The wells were washed 3 times with PBS- Tween-20 (0.05%). After the final washing, the plate was slapped vigorously; with the wells facing downwards on the bench top covered with paper towel. 100ul of HRP-labelled enzyme conjugate (i.e. anti-camel IgG and anti-bovine IgG for camels and cattle respectively) at a 1:1000 dilution in PBS containing 1% BSA was loaded into the wells and incubated for 5mins at room temperature. The plates were washed 3 times as described above to remove the excess conjugate. For colour development, 100ul of chromogen was added to each well as a substrate, and incubated for 5mins at room temperature. The reaction was then terminated after 5mins by adding 100ul of Stop Solution to each well using a multichannel pipette dispenser. The absorbance was read in an ELISA reader (Sigma EIA multi-well reader) at 450 nm. The cut off point was determined according to manufacturer guide which is based on the absorbance values of the positive and negative controls. Samples with absorbance reading less than 0.3 OD units were considered negative while samples with absorbance reading equal to or greater than 0.3 OD units were considered positive.

Statistical analysis

The student t-Test was used to determine significant differences between positive and negative sera. P value < 0.05 was considered to be significant.

The Chi-square and Odds Ratio (O.R) were used to determine association between age, sex and occurrence of antibodies to hydatid disease in the animals.

RESULTS

The sensitivity and specificity of ELISA were determined as 96.4% and 70.5% in camels and 80% and 76% in cattle. The comparison of ELISA test results with actual hydatidosis status determined at post-mortem in Camels and cattle is shown in tables 1 and 2. Of the 474 sera collected, 112 representing 59.3% of camels and 71representing 24.3% of the cattle were positive (Table 3). The overall prevalence of antibody response in the animals was 183(38.6%). The occurrence of antibodies to hydatidosis in both species was significantly different (p<0.05). Higher prevalence was

recorded in older animals than younger ones, but there was no statistical significant difference between the age groups and disease prevalence ($p>0.05$) (Table 4). . However, in camels, seroprevalence increased with age ($r = 0.9711$). Antibodies to E. granulosus were encountered in both males and females with highly significant association ($p<0.01$) between the prevalence of antibodies produced against E. granulosus and sex of the animals (Table 5).

Table 1

Comparison of ELISA results with actual hydatidosis status at post-mortem in Camels

ELISA Test	Hydatidosis	
	Positive (n=84)	Negative (n=105)
Positive (n=112)	81	31
Negative (n=77)	3	74

Table 2

Comparison of ELISA results with actual hydatidosis status at post-mortem in Cattle

ELISA Test	Hydatidosis	
	Positive (n=5)	Negative (n=280)
Positive (n=71)	4	67
Negative (n=214)	1	213

Table 3

Seroprevalence of Cystic hydatidosis in Camels and Cattle slaughtered in Sokoto abattoir

Animal Species	No. Examined	Positive Cases	Negative Cases	Prevalence	O.R	C.I	P (95%)
Camel	189	112	77	59.3			
Cattle	285	71	214	24.3	4.39	1.2-5.30	0.02*
Total	474	183	291	38.6			

*= Significant

Table 4

Seroprevalence of hydatidosis in relations to Age of Camels and Cattle slaughtered in Sokoto abattoir

Animal		Age (years)	Number Examined (n)	Number With Antibodies	Number Without Antibodies	r	P (95%)
Cattle		< 4	87	12(13.7%)	75		
		>4	198	59(29.7%)	139	1	0.682^{NS}
Camel		< 2	22	6(27.3%)	16		
		2-8	48	27(56.3%)	21	0.971	0.130^{NS}
		>8	119	79(66.3%)	40		

F = 3.069 (Camel), r = Correlation coefficient, NS = No Significant difference

Table 5

Seroprevalence of hydatidosis in relations to Sex of Camels and Cattle slaughtered in Sokoto abattoir

Animal	Sex	No.w. A	No wt.A	Total Exam	O. R	C. I	X^2	P(95%)
Camel	Male	21(30.40%)	48	69				
	Female	91(75.8%)	29	120	7.14	3.52-14.73	35.54**	0.001
Cattle	Male	47(20.3%)	184	213				
	Female	24(44.4%)	30	54	3.13	1.60-6.13	12.33**	0.004

X^2 = Chi square; O.R = Odds ratio; C.I = Confidence Interval, ** = Highly Significant association, No.w.A= Number with antibodies, No.wt.A= Number without antibodies, Total Exam= Total number Examined.

DISCUSSION

The prevalence rate of 59.3% recorded in camels and 24.9% in cattle in this study was high compared with those of previous studies and this could be due to an increasing rate of infection of camels and cattle slaughtered in the Sokoto abattoir and this calls for concern. These results although higher, compared favourably with the 55.5% and 26.2% obtained by Dada and Belino (1978) and Ogunsan et al. (2000) in camels slaughtered in Kano and Sokoto abattoirs Northern Nigeria respectively and more recently 38.9% prevalence recorded by Luka et al. (2010) in Kano abattoir. The same trend applies for cattle where lower prevalence rates have been recorded. Dada and Belino (1978) reported a prevalence of 14.7% in Kano abattoir while Rabi'u and Jegede (2010) reported a prevalence of 0.66% in the same abattoir. The result from this study however corroborates with that of Luka et al. (2010) who reported a seroprevalence of 31.2% in cattle slaughtered in Kano abattoir, Nigeria.

Another reason for the high prevalence recorded in this

study could be as a result of the serologic technique used which has proven to be more sensitive. Previous studies in Nigeria except that of Luka et al. (2010) have been based mostly on the morphological detection of infection during post-mortem meat inspection by palpation of organs for the presence of cysts. This method has been shown to have limitations since small cysts and to some extent large cysts are missed during palpation. When this happens, such animals are recorded as negative (Macpherson and Miller, 2003). However, serologic techniques such as ELISA are able to detect antibody responses in these infected animals even when cysts are small and as such will give a more dependable result on the status of the disease as shown in this study. The difference in prevalence in the two species (i.e. camels and cattle) could be attributed to age factors as the camels slaughtered were much older than cattle. Gusbi et al. (1990) reported that the higher prevalence recorded in camels in most studies as compared to other domestic animals might be due to the fact that camels often grow to maturity before they are being slaughtered; this enables the hydatid cyst to be fully developed and fertile. Furthermore, records have shown that camels are unlikely to be slaughtered before 8 or 10 years old and therefore the risk of acquiring infection is relatively greater (Ibrahem and Craig, 1998; Kebede et al., 2009). This result further indicates the suitability of the dromedary camel as susceptible intermediate hosts of E. granulosus (Lightowlers, 1990; Ibrahem and Craig, (1998).

The seroprevalence of infection was found to correlate with age, ie the older animals showed a higher rate of infection than the younger animals. In camels particularly, the result indicate increase as age advances. This could be attributed to the fact that older animals were exposed to the disease (parasitic ova) over a long period of time with an increasing possibility of acquiring infection. Furthermore, this age related difference in prevalence is associated with older animals having a greater chance of ingesting larger numbers of E. granulosus eggs and the cyst being likely to increase in size and become matured in this long lived host (Ahmed, 1991; Ibrahem and Craig, 1998; Larrieu et al., 2001; Luka et al., 2010).

Both males and females of the two species elicited antibodies to hydatidosis but prevalence was significantly associated with the females in both animals. This is a new observation and may be due to the fewer number of males slaughtered as compared to female camels and the fewer number of females slaughtered as compared to males cattle. Again this significant association of hydatidosis to the female sex may be due to immunological factors. May be the males are more resistant to the infection in terms of their defensive mechanism or other immunological factors inherent in the females could be responsible for this. There is a need for further studies in this direction.

The sensitivity and specificity of the ELISA in serodiagnosis of hydatidosis in camels and cattle in Sokoto to the best of our knowledge is being recorded for the first time in this study in Nigeria and can be used as a reference point in further studies. The sensitivity and specificity obtained in this study although lower, compares favourably with that of Mahmoud et al. (2008) who recorded a sensitivity of 100% in camels and donkey and a specificity of 97.6% and 95.9% respectively in Egypt. The difference in sensitivity and specificity may be associated with the purity of the antigens. Notwithstanding, this result thus, may indicate the usefulness of ELISA in serodiagnosis of hydatidosis particularly for screening purposes in live animals as most of the positive and negative cases in this study were detected accurately.

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