

Evaluation of Enzyme -Linked Immunosorbent Assay (ELISA) and Western Blotting for the immunodiagnosis of hydatid diseases in Sheep and Goats

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Citation

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Abstract

This study was undertaken to investigate antigenic characteristics of hydatid cyst fluid in sheep and goats by Sodium Dodecyl Sulphate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE), to evaluate the sensitivity and specificity of Enzyme Linked Immunosorbent Assay (ELISA) and Western blotting for the diagnosis and determination of seroprevalence of hydatidosis in sheep and goats slaughtered in Kano Abattoir, Northern Nigeria. The SDS-PAGE analysis of sheep hydatid cyst fluids indicated that 8 specific-protein bands were detected at molecular weights of 16, 24, 36, 52, 66, 96, 118 and 150kDa while the goat purified HCF showed 4 specific protein bands of 45, 52, 100 and 118kDa. Western blot analysis showed the dominant components of 12, 45, 50-75 and 100-160kDa in sheep and goats HCF. The most consistent demonstrable protein in *Echinococcus granulosus* occurred as a complex in the 52-62kDa region. Bands of 66,118 and 150kDa persisted among sera of infected animals. The sensitivity and specificity of ELISA were determined as 66% and 86% in sheep and 54% and 73% in goats. Where as corresponding rates for western blotting were determined as 71% and 65% in sheep and 69% and 72% in goats. Out of a total of 268 sera obtained from 130 goats and 136 sheep, 31(23.8%) goats and 50(36.2%) sheep were seropositive, higher prevalences were recorded among older sheep than goats. The study concludes that serology is a useful diagnostic tool for hydatidosis and underscores the need for such standard laboratory facilities in the educational and health sectors for establishing the status of hydatid disease in Nigeria.

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INTRODUCTION

Hydatidosis is a global animal and human health problem of increasing economic and public health importance (Lightowers et al. 2000). It is a helminthic cyclo-zoonotic disease caused by the larval stage (metacestode) of the canid tapeworm *Echinococcus* that require at least one other vertebrate host for completion of its life cycle. Accidental infection of man occurs during the natural transmission of the parasite between the canid definitive hosts and domestic livestock intermediate hosts. The disease is endemic in many parts of the world (Willingham, 2003) it is enzootic in sheep, goats, cattle and camels in Nigeria (Dada & Belino, 1978;1979). In addition large hydatid cysts formed in the liver and lungs of these domestic animals results in

significant economic loss to the meat industry through condemnation of infected organs (Lightowers, 1984).

The geographical distribution of the disease closely parallels the areas of the world where sheep pastoralism is the main occupation. In Africa, the disease is a serious health problem of the nomadic pastoralist tribes of East Africa i.e Kenya, Tanzania, Uganda, Southern Sudan and Somalia (Macpherson et al. 1986) Other endemic areas includes Asia, Europe, Australia and the Mediterranean (Tergut, 2001). To date in spite of the progress achieved in the field of research and control, human and animal cystic echinococcosis remains a considerable problem of public health and livestock economy where pastoralism is common and there is a high dog population (Jenkins et al. 1998; Sotiraki et al. 2003). While the disease persists in many parts of the world and re-emerging in some regions, Jenkins (1998); Eckert et al. (2000). New Zealand has operated a national eradication campaign since 1956 and has successfully eradicated the disease however, live animals imported into the country will

have the potential to re-establish the disease and need to be monitored. The availability of immunological tests with the ability to detect the majority of ruminants infected with *E. granulosus* would be desirable for animal import monitoring and also in countries where control schemes for the disease are operating.

Despite the potential value of serological diagnostic tests for hydatid control programs, Hydatidosis has been studied extensively in humans than animals (Rickard and Lightowlers, 1986). Complement fixation tests, agglutination tests, double gel diffusion immunoelectrophoresis and the enzyme-linked immunosorbent assay (ELISA) have all been used in the serodiagnosis of hydatidosis in sheep and other ruminants (Craig et al. 1981; Dada et al. 1981; Lightowlers et al. 1984; Kittelberger et al. 2002), offering better results with the use of the B/5 rich fraction or partly purified antigen from hydatid fluid (Rogan et al. 1991; Sbihi et al. 1996).

The diagnosis of *E. granulosus* in livestock is most commonly made at slaughter. Abattoirs which are strictly regulated can often provide acceptable prevalence data. However, in many areas where the disease is endemic, home slaughter is practiced and only few abattoirs have adequate veterinary supervision. In addition, post-mortem diagnosis of the disease is of little use in areas of low prevalence. The development of a sensitive, specific and reproducible serological assay for livestock would provide a useful epidemiological tool for the ante mortem study and control of hydatid disease (Lightowlers, 1990).

In the past, diagnosis of the disease in Nigeria has often been based on post mortem findings, Dada & Belino, (1978). Immunoblotting has been reported to yield very sensitive and specific results in diagnosis of hydatidosis (Dioz et al. 2001). The Enzyme linked immunosorbent assay (ELISA) has also been found to be useful for the diagnosis of hydatidosis in man and domestic animals. In view of the fact that the clinical signs of the disease are in apparent, reliable and sensitive serological tests for the diagnosis of the disease in live animals is vital for prompt control and prevention of the disease.

In this context, the aim of this work was to assess the usefulness as well as the influence of the composition of sheep and goat hydatid cyst fluid for the immunodiagnosis of hydatidosis in live animals in Nigeria.

MATERIALS AND METHODS

ANTIGEN PREPARATION

The hydatid cyst fluid (HCF) was obtained from hydatid cysts lodged in the lungs and liver of sheep and goats slaughtered in Kano abattoir, Northern Nigeria. The cyst fluid which is the source of antigen, was aseptically aspirated from the cysts, pooled and centrifuged at 1,500g for 15 minutes at 4 ° C and the supernatant stored in aliquots at 20 ° C until required.

The hydatid cyst fluid antigen (HCF) was processed according to the method of Oriol et al. (1971) in order to obtain a fraction enriched with the subunits of the main antigen (Lightowlers et al. 1989; Leggart et al. 1992; Siracusano et al. 1991).

SERUM SAMPLES

To determine prevalence, sera were obtained from the blood of 138 sheep and 130 goats slaughtered in the Kano (coordinate) abattoir, Northern Nigeria.

To determine the sensitivity, serum samples were obtained from 68 sheep and 60 goats naturally infected with hydatidosis in Kano abattoir. To determine specificity, serum samples were collected from 60 sheep and 52 goats with no hydatid cysts at slaughter. Infection in the animals was determined at the point of blood collection by gross identification of hydatid cysts in the various organs of the animals.

ELECTROPHORESIS AND WESTERN BLOTTING

The hydatid cyst fluid antigen from sheep and goats were subjected to discontinuous electrophoresis in homogenous polyacrylamide gels using 12.5% mini-gels as described previously (Lightowlers et al. 1986; 1989). Proteins were separated according to their molecular weights. The electrophoresed proteins were transferred from unstained gels to 0.22µm nitrocellulose membrane (Biorad Richmond, CA USA) following the standardised procedures of Towbin et al. (1979). Nitrocellulose membranes containing blotted antigens were cut into strips and blocked for 2 hours at room temperature in Tris Buffered Saline (TBS) containing bovine serum albumin (5% w/v BSA). There after, the strips were washed in TBS containing Tween 20 (TTBS-T20) and incubated overnight with the test sera (diluted 1/25). The washed strips were probed with alkaline phosphatase conjugated donkey anti-sheep immunoglobulin G (Sigma) at

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1: 1000 dilution for 1 hour at room temperature, and the strips were developed using nitroblue tetrazolium in aqueous dimethyl formamide (DMF) containing magnesium chloride and 5-bromo-4-chloro-indolyl phosphate in DMF (Sigma) as a substrate. The strips were washed in distilled deionized water.

After separating antigenic proteins by SDS-PAGE, the obtained gel was stained with coomassie blue.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

ELISA procedure was carried out as described by Craig et al. (1986). The *E. granulosus* antigen prepared from sheep and goats were optimally diluted in 0.05M phosphate buffer (pH 7.5) and used to coat (150µl/well) microtitration plates (96 wells, Limbro/Titerlek), incubated at 4 ° C overnight in a moist chamber. Unbound antigens were removed by 5 washes with deionized distilled water and blocked with 0.15M phosphate buffered saline containing 0.05% tween 20 (PBS/T20) at room temperature for 1 hour. 100µl / well of test sera, diluted 1/25 in phosphate buffered saline in tween 20 was incubated for 3 hours at room temperature. After 5 washes, 100µl / well of anti-goat sheep IgG (clone GT-34; Sigma) optimally diluted (10µg/ml) with PBS/T20 were then added and incubated for 1 hour at room temperature. The wells were washed 5 times to remove unbound conjugate, and 100 µl per well of alkaline phosphatase yellow nPP liquid (Sigma) was added, incubated for 30 minutes at room temperature and the reaction was stopped by adding 100µl per well of 3M NaOH. The optimal density (OD) values were read in a microplate reader (Dynatech MR 5000) at 450 nm..

STATISTICAL ANALYSIS

Student t-test was used to determine differences between positive and negative serum. P value < 0.05 was considered to be significant.

RESULTS

The major hydatid fluid proteins revealed were primarily antigen B subunits (8-24kDa) and antigen 5 subunits (36-150kDa) in all the hydatid cyst fluid samples. Goat purified antigen preparation showed five prominent bands of 45 to 52, 66, 100 and 118Kda (Plate 1a) while the purified antigen preparation from sheep contained the 24, 36, 52, 66, 96, 118 and 150 kDa respectively (Plate 1b).

Immunoblotting the hydatid fluid antigen separated by SDS-PAGE on 12.5% gels, showed presence of 16, 24, 36, 52, 66,

96, 118 and 150kDa bands in positive sheep sera, while 32, 58, 62 and 98kDa bands were detected in negative sheep sera. Prominent bands of 45, 52, 100 and 118 kDa were also shown in positive goats sera while 35, 50, and 62kDa bands were detected in negative goat sera. This showed that the 52 and 118kDa bands were specific in sheep tested by western blotting using the antigen prepared from sheep and in goats tested by western blotting using the goat hydatid cyst fluid respectively. The sensitivity and specificity of ELISA were determined as 66% and 86% in sheep and 54% and 73% in goats. Where as corresponding rates for western blotting were determined as 71% and 65% in sheep and 69% and 72% in goats.

Of the 268 sera collected, 50(36.2%) of 138 sheep and 31 (23.8%) of the 130 goats were positive with purified sheep and goats hydatid cyst fluid preparation respectively (Table 1). The overall prevalence of antibody responses in the animals was (81)30.2%. There were statistically significant differences between the prevalence of antibody responses in young and old sheep and goats ($p < 0.05$) and significant association between age and disease prevalence ($p < 0.05$). Antibodies to *Echinococcus granulosus* were encountered in both males and females but there were no associations between the sex of the animals and prevalence of antibodies to *E. granulosus* ($p > 0.05$).

Figure 1

Table 1: Overall Prevalence of antibodies to hydatidosis in sheep and goats slaughtered in Kano abattoir, Kano State using ELISA

Species	Number examined	Number with antibodies	Number without antibodies	Species specific rate (%)
Sheep	138	50	88	36.2
Goat	130	31	99	23.8
Total	268	81	187	30.2

Figure 2

Table 2: Sensitivity of ELISA among sheep and Goats

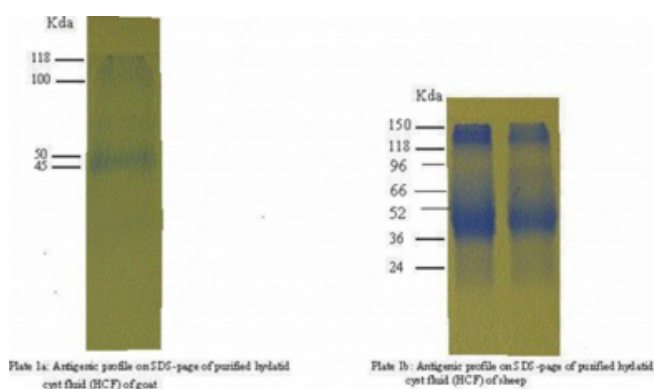
Animal spp.	Number of tested sera	Number positive	Sensitivity(%)
Sheep	68	45	66
Goats	60	37	54
TOTAL	128	82	

Figure 3

Table 3: Sensitivity of Western blotting among sheep and goats

Animal spp.	Number of tested sera	Number positive	Sensitivity(%)
Sheep	68	48	71
Goats	60	41	69
TOTAL	128	82	

Figure 4



DISCUSSION

In this study, hydatid cyst fluid of sheep and goats were used as a source of antigen. Gottstein et al. (1987) tested the cyst fluid of sheep by SDS-PAGE and found seven bands of different molecular weights ranging from 15 to 205kDa. Burgu et al. 2000 detected nine specific protein bands of different molecular weights ranging from 8 to 200kDa. SDS-PAGE analysis of purified hydatid cyst fluids indicated that seven specific bands in sheep hydatid cyst fluids at molecular weights of 24,36,52,66,96,118 and 150kDa and five specific bands were detected in goats HCF at molecular weights of 45,52,66 100 and 118kDa in this study. The 52 and 118kDa were the most evident bands. The 118kDa band was strongly antigenic in the sheep and goat HCF. The antigen was possibly the same as the 116kDa antigen described in sheep, goats, pigs and human cyst fluids Kanwar et al., (1992), and in sheep HCF by Simsek and Koroglu, (2004). The 8kDa band was not detected in this present study. Even studying with the same antigens, it is possible to get different results in SDS-PAGE. These differences might be related to the processing of hydatid cyst fluid before electrophoresis.

The sensitivity of the ELISA in sheep (66%) and in goats (54%) reported here were lower than that found in humans by this assay(100% and 91%) (Kanwar et al. 1992; Poretti

et al. 1999) Specificity of this test in sheep (86%) and goats (73%) in this study was lower than that previously reported in sheep using ELISA test (90%) (Ibrahem et al.1996). The low sensitivity of the ELISA might be related to ages of the sheep and the cyst type.

The sensitivity of Western blotting among sheep(71%) and goats (69%) in this study were found to be lower than the report in sheep (88%) by Simsek and Koroglu, (2004). Many researchers suggested that sensitivity of the assay may depend on the cyst type and location. Hepatic cysts appear to produce a much stronger immunological response than the pulmonary cysts. It is therefore recommended that cyst location and its status be noted and described in detailed in serological studies of hydatidosis. In this study, most of the cysts were collected from the lungs this might be the cause of the low titre values and weak bands obtained in the antigen/sera of some infected animals. Studies have shown that absence or reduced antibody responses could also be associated with the site of hydatid cyst involvement and variation in immunological response in the animal which may or may not lead to production of sustained levels of specific circulating antibody especially among naturally infected animals (Williams et al. 1971; Todorov et al. 1979;; Lightowlers et al. 1989; Leggart et al. 1992). Records have shown that this study is the first to be conducted in this area using the SDS-PAGE and western blotting as diagnostic techniques. The purified hydatid cyst fluid was used as a source of antigen in order to evaluate the specificity and its application in order to analyze echinococcus proteins to know which of the antigens invoke antibody response in the animals and to identify the species specific antigens which might be used for immunodiagnosis of the disease in live animals.

Reports from previous studies conducted on hydatidosis in animals slaughtered in major abattoirs in Nigeria were very few and often based on parasitological or retrospective abattoir records (Dada and Belino, 1978; Anyanwale et al. 1982; Onah et al. 1985; Ajogi et al. 1995; Garba and Maigandi, 1995; Ogunsan, 2000). Studies on the general distribution of the disease in Nigeria is very scarce, (Willingham, 2003; Schantz et al. 1995) making it difficult to compare our findings with existing data in Nigeria.

The results obtained in this present work confirm that the western blotting is suitable for serodiagnosis of sheep and goat hydatidosis. The use of purified antigen yields better result and the site and status of the cyst in the animal is very

useful in serodiagnosis of hydatidosis. More studies on the serodiagnosis of hydatidosis be conducted in live animals in different parts of the country in order to ascertain the actual status of the disease in Nigeria.

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