Serodiagnosis of hydatidosis in sheep slaughtered at Sokoto abattoir, Sokoto state, Nigeria

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Abstract
Serological screening for hydatidosis was carried out on sheep slaughtered at the Sokoto abattoir, Northwestern Nigeria. A total of 186 serum samples obtained from randomly selected animals were analysed for antigen-antibody responses using Echinococcus granulosus IgG Enzyme Linked Immunosorbent Assay (ELISA) kit (RIDASCREEN®). Postmortem inspection for the presence of cyst was also carried out on the selected animals. The study did not observe any antigen-antibody reaction in any of the samples screened and no cyst was found at post-mortem inspection of the selected sheep. The zero prevalence recorded suggests the need to employ some other more sensitive diagnostic techniques to ascertain the result obtained in this study.

Keywords: abattoir, hydatidosis, serodiagnosis, sheep, sokoto

Introduction
Parasitic diseases, including hydatidosis are limiting factors in food animal production and hamper the realization of meat supply to meet the ever increasing demand for animal protein by human population (Srivastava et al., 1983). Hydatidosis is a parasitic zoonotic disease caused by the metacestode of the tapeworm Echinococcus spp (Soulsby, 1982). The larval stage has a wide range of domestic and wild mammals (NAHIS, 2004). In the intermediate hosts, the larval stage develop to a large fluid filled cysts referred to as hydatid cyst (Soulsby, 1982). The lungs and liver are the most favoured predilection sites for the developing cyst (CAB International, 1989; Schantz, 1990; Biu & Abagwe, 2001). Other organs often affected are the brain and bone (Shantz, 1990). Three forms of hydatidosis are known to occur, these include Cystic, Alveolar and Polycystic hydatidosis with Echinococcus granulosus, Echinococcus multilocularis, Echinococcus vogeli as the respective etiologic agents. E. oligarthrus is also reported to cause Polycystic hydatidosis (NAHIS, 2004). Dogs become infected by eating infected carcass of the affected intermediate host. Hydatidosis has a worldwide distribution. Agricultural practices, indiscriminate home slaughtering and poor disposal of cysts from livestock, lack of adequate control policy, uncontrolled movement, trading of animals and their products and difficulty in early diagnosis enhance the distribution of the disease (Dada and Belino, 1979). Infection is often associated with economic losses due to livestock mortality, morbidity, and organ and meat condemnation at meat inspection. It also poses a serious threat to public health where close association exists between dogs, man, and food animals (Blaha, 1989). Several studies have shown that these diseases are significant concern to public health and are regarded as the current emerging or re-emerging diseases (Dada et al., 1979).

In Nigeria, there is paucity of information on hydatidosis in domestic livestock and most of the documented reports are based on postmortem findings from abattoir. The prevalence of this infection in domestic livestock varies from one
location to another and among different breeds of livestock (Dada et al., 1979; Dada and Belino, 1979; Ogunsan et al., 2000).

Abattoir records are important source of information during hydatid studies. This is particularly important because ante-mortem inspection of animals for hydatid cyst is unreliable, because most infections are asymptomatic especially at early phase. Postmortem inspection is valuable when cyst has develop to a size that can be visualized or palpated.

Serological tests for diagnosis of hydatidosis are useful because of ease of performance and sensitivity. The ELISA is considered an effective method overall to evaluate the immune status of animal and human. This diagnostic approach is more reliable and gives more correct disease prevalence when employed as a diagnostic tool.

In this study, the Enzyme Linked Immunosorbent Assay (ELISA) technique using a commercially prepared E. granulosus IgG ELISA kit (RIDASCREEN Enzyme Linked Immunosorbent Assay (ELISA) of r-biopharm, Germany) was used for sero-epidemiological study of ovine hydatidosis along side with a postmortem examination for cystic lesion on randomly selected sheep slaughtered at the Sokoto abattoir.

Materials and methods
Study Area
The study was conducted in Sokoto state, Nigeria. The State shares common borders with Niger Republic to the North, Kebbi state to the South, and Zamfara state to the East. The Sokoto abattoir is a slaughterhouse that serves Sokoto town and neighbouring villages.

Sample collection and Postmortem Examination
The animals used for the study were randomly selected at slaughter. The sex, age and breed of each slaughtered animal were recorded. Following slaughter each animal undergoa thorough postmortem examination for the cystic lesion. Blood samples were collected from each selected sheep into labeled sample bottles accordingly. The blood samples were then transported to Veterinary Public Health laboratory of Usmanu Danfodiyo University, Sokoto, Nigeria. The sera were harvested for the qualitative determination of IgG antibodies against E. granulosus.

Enzyme Linked Immunosorbent Assay (ELISA)
RIDASCREEN® E. granulosus IgG micro well ELISA test is an immunoassay for the qualitative determination of IgG antibodies against E. granulosus in serum. The micro well plates are coated with purified antigens from E. granulosus cysts commercially obtained. Fifty (50) ml of the washing buffer (phosphate-buffered NaCl solution) was dissolved in 950mls of distilled water and stored at room temperature. Ten (10) µl of the serum samples were diluted in a 490µl of the sample buffer solution (phosphate-buffered NaCl solution), one hundred (100) µl of each of the diluted samples in buffer solution was placed on micro titre well plate coated with specific E. granulosus antigens. One hundred (100) µl of each of the positive controls (IgG positive control, human serum) and negative controls (IgG negative control, human serum) were pipetted into each of the corresponding wells and incubated at a temperature of 25°C for 15 minutes. The plate was washed 5 times after incubation using washing buffer. One hundred (100) µl of the conjugate (anti-human IgG conjugate) was pipetted to each of the wells and incubated at 25°C for 15 minutes and the plates were washed 5 times. Fifty (50) µl of each of the substrate (urea peroxidase) and chromogen (tetramethylbenzidine, TMB) were pipetted into the wells and incubated at 25°C for 15 min and 50µl of the stop reagent (0.5M sulphuric acid) was added to each of the wells. There was a colour change from blue to yellow in each of the control wells only. The optical density (OD) values were read in a microplate reader (Dynatech MR 5000) at 450nm, ELISA cut off values of all serum samples were set at the mean OD values. OD reading greater than 0.9 were considered to be positive, those less than 0.3 were considered negative and those in between were interpreted as equivocal.

Results
A total of 186 sheep were sampled and screened during the study. This comprised of 123 (66.13%) males and 63 (33.87%) females. No antigen-antibody reaction was observed in all of the samples. The breed of sheep involved in the studies were Ouda, Balami, Yankasa and Sudanese cross and the age variation range from less than a year to above five years (Table 1). No hydatid cyst lesion was found during postmortem examination of the corresponding carcasses. Therefore, the overall prevalence of E. granulosus IgG antibodies in sheep slaughtered at Sokoto abattoir during this study was found to be zero.
**Table 1: Serodiagnosis of Echinococcus granulosus in Sheep slaughtered at Sokoto abattoir**

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. Sampled</th>
<th>No. Positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ouda</td>
<td>174</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Balami</td>
<td>7</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Yankasa</td>
<td>3</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Sudanese cross</td>
<td>2</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. Sampled</th>
<th>No. Positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>123</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>Female</td>
<td>63</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. Sampled</th>
<th>No. Positive</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1 yr</td>
<td>81</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;1 &lt;2 yrs</td>
<td>59</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;2 &lt;3 yrs</td>
<td>10</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;3 &lt;4 yrs</td>
<td>12</td>
<td>0</td>
<td>0.00</td>
</tr>
<tr>
<td>&gt;4 ≥5 yrs</td>
<td>24</td>
<td>0</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Discussion

Hydatidosis is an important zoonotic disease and is currently tagged a disease of unrecognized increasing importance (Jenkins, 1998). The application of serodiagnostic techniques for epidemiological studies of hydatidosis provides more accurate information on the prevalence of the disease because the technique could detect asymptomatic cyst carriers. This study found a zero prevalence of E. granulosus IgG antibodies in sheep slaughtered at Sokoto abattoir. The result contradicts similar studies on sheep conducted in Yobe state, north eastern Nigeria, where Tijjani et al. (2010) reported a prevalence of 0.01%. It also contradicts the results of Luka et al. (2009) who reported a prevalence of 36.2% in Kano, Nigeria. Ouda and Balami were the most predominant breed of sheep found to be infected in this reported studies (Tijjani et al., 2010). The result also disagree with earlier studies conducted in this area where Dada and Belino, (1979) observed a prevalence of 18.9%.

The zero prevalence recorded in this study may be attributed to several factors. This may be due to the fact that natural intermediate host animals produce very poor antibody responses to infection due to antibodies of the parasite produced at early stage compared with the relatively high levels of specific antibodies seen in human infection (Lightowlers & Gottstein, 1995).

The zero prevalence in this study may also be as a result of periodic treatment of small ruminants with antihelminthes by the pastoralists who now patronize veterinary services (Tijjani et al., 2010) due to increase awareness on the benefits of deworming their animals, sheep brought in for slaughter are usually in good body condition, since they are slaughtered in order to fetch more money for subsistence. Dogs serve as the definitive host of the parasite and a prevalence of 26.69% of the parasite was recorded in dogs at Sokoto (Magaji, 2011), they get infected when they feed on infected offals from the intermediate hosts thrown away from abattoir or slaughter slabs or during festivities.

In conclusion, the zero prevalence obtained suggests low prevalence of the disease in the study area, however, an alternative and more sensitive diagnostic technique should be employed to ascertain the status of the disease in other food animals and humans in the study area.

References


linked immunosorbent assays (ELISAs) for the detection of serum antibodies in sheep infected with *Echinococcus granulosus*. *Veterinary Parasitology*, **110**:57-76.


