ASSOCIATION OF TRYPANOSOME INFECTION WITH CIRCULATING ZONA PELLUCIDA (ZP) ANTIBODIES IN WEST AFRICAN DWARF (WAD) GOATS

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Sera from 967 adult female West African Dwarf (WAD) goats previously screened for Trypanosome infection by some diagnostic laboratories around Ibadan metropolis, in Southwestern part of Nigeria, were assayed for zona pellucida (ZP) antibodies by the enzyme-linked immunoassay (ELISA) technique. Of the 967 female goats, 534 (55.22%) were positive and 433 (44.78%) negative for Trypanosome infection. Out of those that were positive for Trypanosome infection, 346 (64.79%) and 188 (35.21%) were positive and negative for ZP antibodies respectively. These represented 35.78% and 19.44% of the total number of animals screened respectively. The group that was negative for Trypanosome infections had 149 (34.41%) and 284 (65.59%) positive and negative for ZP antibodies, representing 15.41% and 29.37% of the total number of animals screened, respectively. Seropositivity for ZP antibodies was positively correlated with Trypanosome infection (P<0.001). There is the possibility of damage to the blood-ovary barrier as a result of trypanosome infection thereby exposing the zona pellucida to the immune system and being recognized as foreign. The possibility of zona pellucida antibodies as a cause of infertility in domestic animals should always be considered in infertility investigations.

Key words: Trypanosomes, zona pellucida antibodies, and infertility, Goats.

INTRODUCTION

Goats are raised extensively and semi-intensively in Nigeria as a source of animal protein for human nutrition. The West African Dwarf (WAD) breed is noted for its ability to survive the harsh environmental conditions of heat stress and humidity (Devendra and Mcleroy, 1982, Osuagwu and Akpokodje, 1981) and it is the predominant breed in the humid Southwestern part of the country. The WAD goats are kept in small flocks, typically numbering 2 — 6 animals, per household as an adjunct to the main business of cropping, thereby contributing significantly to the economy of third world countries (Upton, 1985; Ademosun, 1985). Despite the importance of this species of animal (goat); it has received very little attention (Okere et al., 1982) and there is low productivity, which has been attributed to factors including nutrition, infectious and non-infectious agents (Laing et al., 1988, Cullen, 1990). Low productivity can be caused by infertility and immunological reactions had been associated with infertility especially antibodies produced against the zona pellucida in humans and animals (Sacco, 1979, Wood et. al, 1981 Sacco, et al, 1981, Haseguwa, 1995).

Trypanosome infections have been reported to cause infertility due perhaps to ovarian pathology in goats (Ikede and Akpavie, 1981). The ovary produces follicles which contain ova surrounded by zonae pellucidae. The zona pellucida is an acellular glycoprotein which is antigenic (Gupta et.al., 1997, Brown et al., 1997) but ordinarily will not stimulate antibody production because it is protected by the blood-ovary barrier which when disrupted will lead to antibody production. The ZP antibody production has been associated with infertility. It has not been established if damage to the ovary can lead to damage to the blood-ovary barrier thereby stimulating ZP antibody production. The ZP antibody has not been correlated with Trypanosome infection.

The objective of this study was therefore to check whether there is any correlation between trypanosome infection and ZP antibody production

MATERIALS AND METHODS

Serum samples previously collected and screened for Trypanosome infections by the
private veterinary clinics, Government and University Laboratories in the Southwestern part of Nigeria were used for this investigation. The samples were randomly selected from the pool without previous reference to the trypanosome screening results until the results of the ZP antibody assay were ready. The samples were taken in ice-packs and air-freighted to the laboratories in the University of Minnesota, St. Paul, Minnesota, U.S.A for ZP antibodies assay.

**Preparation of Antigen**

Caprine ovaries were collected from slaughterhouses at goat farms around St. Paul, Minnesota, U.S.A. The follicles were punctured and 1 ml. tuberculin syringes with 26 gauge needles were used to aspirate the follicular fluid into Petri dishes. The ova were collected in 0.5mls 0.1M phosphate buffered saline (PBS) using a stereomicroscope. The ova were transferred into a test tube containing 0.01% w/v sodium citrate in PBS and shaken for 60 seconds to remove the cumulus cells. The zona pellucidae were then separated from the eggs using glass pipettes with bores a little narrower than the diameter of the egg.

The zonae were washed three times in PBS, resuspended at a concentration of 200 zonae/ml and then sonicated 20 strikes with a sonicator model W380 (HeatSystems Inc.). The sonicates were then centrifuged twice using PBS at 1200g for 20 minutes at 4°C. The optical density OD of the second supernatant was estimated and adjusted to 0.2. This was equivalent to 0.15mg/ml protein and was used as the zona pellucida antigen. The antigen was divided into aliquots and stored in the cold room at —196°C until used for the assay of antibodies.

**Assay of Zona Pellucida Antibodies**

A total of 967 serum samples were assayed for antizona pellucida antibodies using the methods of Henderson et al. (1987) with slight modifications. The zonae antigen was dispensed at 50µl well into 96 — well polyvinyl microplates (Falcon 3912 Micro Test III, Becton Dickinson) and left overnight at 4°C. On the second day, the fluid was decanted and the antigen fixed using 50µl 0.1% gluteraldehyde in PBS-Tween 20 (Sigma) for 5 minutes. The plates were then washed three times in PBS before incubating overnight with 100 1% Bovine Serum Albumin (BSA) at 4°C. After this the plates were washed three times again before dispensing 50µl/well of various dilutions of the test and standard negative samples and incubating for 1 hour at 37°C. The standard negative samples being sera taken from 2-week old kids. The plates were then washed in PBS-Tween 20 and incubated for 30 minutes with addition of 50µl/well Biotin-labeled rabbit-anti-goat IgG (KPL, 1:4800) at 37°C. This was followed by washing and incubation for 30 minutes with 50µ streptavidin peroxidase (KPL, 1:9600) at 37°C. The plates were then washed three times before adding 50µ/well substrate for 15 minutes in the dark dancer. The substrate consisted of equal volumes of 2,2, azino-di (3-ethylbenzthiazoline sulfonate) (ABTS) and hydrogen peroxide (H The optical density was read at 405nm with a micro ELISA reader model MR380 (Dynatech).

The mean OD of the standard negative sera was calculated and used as the benchmark. Any sample with twice the value of the calculated mean was taken as positive.

**Correlation with Trypanosome Infection**

The results of the selected samples in term of trypanosome infection status were collected from the laboratories of origin and compared with the results of the ZP antibodies assay.

**Statistical Analysis of Results**

The antibody assay results were correlated with the trypanosome infection status of the samples using the Panacea statistical package, University of Minnesota

**RESULTS**

Table 1 shows the absolute numbers and proportions of animals that were positive and negative for Trypanosome infection and ZP antibodies. Out of 967 animals tested 534 (55.22%) were positive compared to 433 (44.78%) that were negative for Trypanosome infection.

Those positive for both ZP antibody and Trypanosome infection were 346 (35.78% of Total) while those positive for ZP antibodies but negative for trypanosome infection 149 (15.41% of Total). The animals that were negative for ZP antibodies but positive for trypanosome infection were 188 (19.44% of Total) while those that were negative for ZP antibodies and negative for trypanosome infection numbered 284 (29.37% of Total). Of the total number of 967, 495 (51.19% of Total) were positive for ZP antibodies compared to 472 (48.8 1% of Total) that were negative for the antibodies.
Table 1:  
Proportions of animals positive and negative for Trypanosome infection and zona pellucida antibodies.  
<table>
<thead>
<tr>
<th></th>
<th>Positive for Trypanosome</th>
<th>Negative for Trypanosome</th>
<th>Total</th>
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<tbody>
<tr>
<td>No. Positive for ZP</td>
<td>346 (35.78%)</td>
<td>149 (15.41%)</td>
<td>495 (51.19%)</td>
</tr>
<tr>
<td>No. Negative for ZP</td>
<td>188 (19.44%)</td>
<td>284 (29.37%)</td>
<td>472 (48.81%)</td>
</tr>
<tr>
<td>Total No Tested</td>
<td>534 (55.22%)</td>
<td>433 (44.78%)</td>
<td>967 (100%)</td>
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</table>

Table 2:  
The proportions of Animals positive and negative for zona pellucida antibodies in each of the groups that tested positive and negative for Trypanosome infection.  
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<td>No. Negative for ZP</td>
<td>188 (35.21%)</td>
<td>284 (65.59%)</td>
</tr>
<tr>
<td>Total</td>
<td>534 (100%)</td>
<td>433 (100%)</td>
</tr>
</tbody>
</table>

Table 3:  
The proportions of Animals positive and negative for Trypanosome infection in each of the groups that tested positive and negative for zona pellucida (ZP) antibodies.  
<table>
<thead>
<tr>
<th></th>
<th>Positive for ZP Antibodies</th>
<th>Negative for ZP Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. Positive for Trypanosome</td>
<td>346 (69.90%)</td>
<td>188 (39.83%)</td>
</tr>
<tr>
<td>No. Negative for Trypanosome</td>
<td>149 (30.10%)</td>
<td>284 (60.17%)</td>
</tr>
<tr>
<td>Total</td>
<td>495 (100%)</td>
<td>472 (100%)</td>
</tr>
</tbody>
</table>

Table 2 shows the proportions of animals that were positive and negative for zona pellucida antibodies in each of the groups positive and negative for Trypanosomosis infection. Of the 534 that were positive for trypanosomosis infection, 346 (64.79%) and 188 (35.21%) were positive and negative for ZP antibodies respectively. In the group that were negative for trypanosomosis infection totaling 433, 149 (34.41%) and 284 (65.59%) were positive and negative for ZP antibodies respectively.  

Table 3 shows that out of the 495 animals that were positive for ZP antibodies, 346 (69.90%) and 149 (30.10%) were positive and negative for trypanosomosis infection respectively. Of the 472 that were negative for ZP antibodies, 188 (39.83%) were positive compared to 284 (60.17%) that were negative for trypanosomosis infection. The proportion of animals positive for trypanosomosis infection was significantly higher than those negative for the infection (P<0.01). The proportion of animals that were positive for ZP antibodies was significantly higher than those that were negative for the ZP antibodies (P<0.01). Seropositivity to ZP antibodies was positively correlated with trypanosomosis infection (P<0.001).  

DISCUSSION  
The results show that in the group positive for trypanosomosis infection, a significantly higher proportion was seropositive for ZP antibodies (P<0.001). Also in the group that was negative for trypanosomosis infection the proportion that was seropositive for ZP antibodies was significantly lower than those that were seronegative to the antibody (P<0.001). Seropositivity was positively correlated with trypanosomosis infection.  

The zona pellucida has been shown to be antigenic (Subramanian et al, 1981; Dunbar, et al. 1989, Skinner, et al, 1999) but does not ordinarily stimulate production of antibodies because of the blood-ovary-barrier. Reproductive disorders have been associated with trypanosomiasis infections in man and animals (Apted, 1970). Ruminants infected with Trypanosoma brucei, T. congolense or T. vivax showed that the infection can lead to irregular oestrous, infertility and intrauterine infection with abortion in females (Ike and Akpavie, 1982). The pathogenesis of infertility in such cases may be connected with damage to the blood-ovary-barrier leading to formation of antibodies to the zonae pellucidae which had been associated with infertility (Wolgemuth et al., 1984, Maresh et al, 1990, Hazeguwal et al, 1995, Kolle et al., 1996).  

The animals that were negative for trypanosomes when tested but were seropositive for ZP antibodies might have been previously infected since even after successful treatment of animals, parasites usually disappear but infertility may not disappear in Ndama cows 3½ to 16 months after infection (Ige and Amodu, 1975). The correlation of seropositivity for ZP antibodies with trypanosomosis infection in this study allows the speculation that trypanosomosis infection could have caused enough damage to the ovaries in the affected animals to the point of exposure of the ZP proteins, secreted by the oocytes as well as granulose cells (Wolgemuth et al, 1984, Moresh et al, 1990), to the immune system The
possibility of ovarian structural damage in trypanosome infection which is enzootic in Nigeria causing infertility should therefore be considered in infertility investigation in farm and domestic animals.

Further studies on the histopathology and electron microscopy of the ovary during the course of trypanosome infection will throw further light to this suspected pathogenesis of infertility.

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REFERENCES


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Trypanosomosis and Zona Pellucida antibodies in Goats