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# Detection and quantitation of humoral immunity to *Paramphistomum microbothrium* in gut mucosa of cattle

**OGUNRINADE (A. F.), ABATAN (M. O.).** Détection et quantification de l'immunité humorale à *Paramphistomum microbothrium* dans la muqueuse intestinale des bovins. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (3) : 275-277.

**A partir du sérum et des extraits de rumen et de duodénum prélevés sur du bétail naturellement infesté par *P. microbothrium* et sur des animaux témoins, les immunoglobulines-A (IgA) et les immunoglobulines-$G_1$ ($IgG_1$), totales et spécifiques, ont été détectées et quantifiés. Par le test d'immunofluorescence indirecte, l'immunoglobuline-$G_1$ spécifique a été détectée dans les sécrétions du rumen un plus grand nombre de fois que l'immunoglobuline-A. On peut en conclure que l'immunoglobuline-$G_1$ fait partie de la réaction immunitaire d'expulsion des paramphistomes du rumen des bovins.** *Mots clés* : Bovin - *Paramphistomum microbothrium* - Immunité - Nigeria.

## INTRODUCTION

The gastrointestinal tract is the portal of entry and the predilection site of many parasitic helminths. These parasites induce local and systematic immunity which are detectible in sera, gut extracts or faeces of infected animals (6, 7).

*P. microbothrium*, a trematode helminth restricted in its development to the duodenal and ruminal mucosa of cattle (1), appears ideal for investigating local immunity to the gastrointestinal tract of cattle and was examined for this purpose.

## MATERIAL AND METHODS

### Cattle

These were all adult White Fulani cattle slaughtered at the Ibadan municipal abattoir. Overall, 20 animals aged between 5-7 years were used as subjects. Ten of these harboured heavy (5-10,000 worms), single infection with *P. microbothrium* while the rest were throroughly screened and found negative for *P. microbothrium* and related trematodes of the gastrointestinal tract and associated circulatory system. The latter were used as controls. However, nearly all the cattle harboured light infections with strongyles.

### Collection of specimens

All samples were collected *post mortem*. Serum was obtained from cardiac blood following syringe aspiration. Ruminal liquor was obtained by gentle squeezing of the ruminal mucosa (after removal of ingesta) and the fluid extract was collected in a bijou bottle. The collected ruminal liquor was sieved and further clarified by centrifugation at 1,500 revolutions per minute for 30 min. Duodenal extract was similarly obtained from both groups of cattle, after ingesta was removed by scraping the mucosa surface with a glass slide. The scraped mucus was dissolved in an equal volume of phosphate buffered saline (pH 7.2) and clarified by centrifugation. Normally, fresh samples of ruminal and duodenal extracts were used in the assays but these were occasionally stored frozen at - 20 °C with 1 p. 100 sodium azide as preservative prior to use.

### Measurement of total and specific antibody

The concentrations of immunoglobulins A and $G_1$ in the samples were quantified by the single radial immunodiffusion technique using commercial kits (Miles, U.K.). Briefly, 50 of each test serum and standard of known concentration were pipetted into a central well of 1 p. 100 Agar containing antibodies against immunoglobulin A and $G_1$.

Following radial diffusion at room temperature for 48 hours, readings of diameters of each ring of the test sample and standards were taken and the concentration of immunoglobulin in each sample were estimated for a semi-log linear plot of the diameter versus the concentration of the known standards.

Indirect Fluorescent Antibody Test (IFAT) was done on 4 µm cryocut sections of *P. microbothrium* as descri-

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*TABLE I Mean concentration of Immunoglobulin-A and Immunoglobulin-$G_1$ in serum, ruminal and duodenal extract of cattle.*

| Immunoglobulin Class | Animal Groups | Mean concentration of immunoglobulins (mg/ml ± standard deviation) | | |
|---|---|---|---|---|
| | | Serum | Duodenal | Ruminal |
| IgA | Infected<br>Control | 4.0 ± 0.5<br>3.8 ± 0.8 | 3.0 ± 0.3<br>3.2 ± 0.14 | trace*<br>trace |
| $IgG_1$ | Infected<br>Control | 18.0 ± 2.4<br>20.0 ± 1.8 | 3.5 ± 0.3<br>3.2 ± 0.4 | 2.1 ± 0.3<br>0.5 ± 0.2 |

* less than 0.3 mg/ml.

bed by OGUNRINADE (5). The indirect fluorescent antibody test was performed using the cryocut sections of the worm as antigen and serial dilutions of the test and control sera as antibody. Fluorescent-labelled anti rabbit bovine IgA or $IgG_1$ was used as conjugate. The preparation was viewed under UV light microscopy.

## RESULTS

The results of the measurements of total immunoglobulin in the test samples are presented in table I. Using a student t-test, there was no significant difference in total immunoglobulin-A in serum, duodenal or ruminal extracts between infected and control cattle ($P > 0.05$). However, a significantly high concentration of immunoglobulin-$G_1$ was detected in ruminal liquor of infected cattle ($P < 0.05$). Similarly, specific antibody was detected in ruminal liquor in a greater number of cases especially when immunoglobulin-$G_1$ was determined (Table II).

## DISCUSSION

Systemic immune response in trematode infections such as *F. gigantica* is chiefly of immunoglobulin-$G_1$ specificity (4). However, local antibody response in the gut mucosa of cattle consists mainly of immunoglobulin A and $G_1$ classes (2). Immunoglobulin-$G_1$ is known to play a special role in exocrine secretions of cattle and a selective transport of this immunoglobulin occurs in the gut mucosa of cattle (3). In *H. contortus* infections, both immunoglobulin A and $G_1$ have been associated with local immunity in the gut mucosa of sheep (6). This result shows that immunoglobulin-$G_1$ may also play a role at the local immunity in cattle in paramphistome infections. Since infection with *P. microbothrium* is self-limiting (1), it seems that immunoglobulin-$G_1$ may play a major role in immune expulsion of paramphistome infections in cattle.

*TABLE II Detection of specific antibody by IFAT.*

| Cattle | Extract | No. Examined | No. Reacting at > 1/16 | |
|---|---|---|---|---|
| | | | anti-IgA-FITC | anti-$IgG_1$-FITC |
| Infected | S<br>R<br>D | 10<br>10<br>10 | 2<br>1<br>1 | 2<br>8<br>2 |
| Control | S<br>R<br>D | 10<br>10<br>10 | 1<br>—<br>— | 1<br>2<br>— |

S = Serum ; R = Ruminal ; D = Duodenal Extract.

## CONCLUSION

Specific immunity to *P. microbothrium* infections in the rumen and duodenal mucosa of cattle is of immunoglobulin-$G_1$ specificity. It is suggested that immunoglobulin-$G_1$ may play a major role in immune expulsion of *P. microbothrium* in cattle.

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**Total and specific Immunoglobulin-A (IgA) and Immunoglobulin-$G_1$ ($IgG_1$) were detected and quantified in serum, ruminal and duodenal extracts of cattle naturally infected with *P. microbothrium* and controls. Using the Indirect Fluorescent Antibody Test (IFAT), specific immunoglobulin-$G_1$ was detectible in ruminal liquor in a greater number of cases than immunoglobulin-A. We concluded that immunoglobulin-$G_1$ may be involved in immune expulsion of paramphistomes from the rumen of cattle.** *Key words* : Cattle - *Paramphistomum microbothrium* - Immunity - Nigeria.

**OGUNRINADE (A. F.).** detección y cuantificación de la inmunidad humoral para con *Paramphistomum microbothrium* en la mucosa intestinal de bovinos. *Rev. Elev. Méd. vét. Pays trop.*, 1987, **40** (3) : 275-277.

**Se evidenciaron y se cuantificaron las inmunoglobulinas-A (IgA) y -G1 (IgG1) a partir de sueros y de muestras de la panza y del duodeno tomadas en bovinos naturalmente infestados. Por medio de la pueihba de inmunofluorescencia indirecta se evidenció la inmunoglobulina-G1 especifica más frecuentemente que la inmunoglobulina-A en las secreciones de la panza. De esto se puede concluir que la inmunoglobulina-G1 hace parte de la reacción inmunitaria de expulsión de los paramfistomas de la panza de los bovinos.** *Palabras claves* : Bovino - *Paramphistomum microbothrium* - Inmunidad - Nigeria.

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