

# Precipitin antibodies in natural fascioliasis (\*)

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## RÉSUMÉ

TANYA (V. N.), OGUNRINADE (A. F.). — Anticorps précipitants dans la fasciolose naturelle. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (1) : 26-29.

Du sérum de bovins infestés naturellement par *Fasciola gigantica* a été analysé en vue d'y déterminer la présence d'anticorps par le test de double immunodiffusion (DID).

Des anticorps précipitants ont été décelés dans le sérum de 74 p. 100 des cas reconnus positifs. Toutefois, il n'a pas été possible d'établir une relation significative entre le titre sérologique et le degré d'infestation par la douve.

**Mots clés :** Fasciolose naturelle - *Fasciola gigantica* - Anticorps - Double immunodiffusion.

## SUMMARY

TANYA (V. N.), OGUNRINADE (A. F.). Precipitin antibodies in natural fascioliasis. *Rev. Elev. Méd. vét. Pays trop.*, 1984, 37 (1) : 26-29.

Sera of cattle with natural *Fasciola gigantica* infections were tested for the presence of precipitin antibodies to this parasite by the double immunodiffusion (DID) test.

Immunoprecipitins were detected in 74 p. 100 of known positives. However, no significant correlation was observed between the serological titre and the degree of fluke infestation.

**Key words :** Natural fascioliasis - *Fasciola gigantica* - Antibodies - Double immunodiffusion.

## INTRODUCTION

Serological tests are particularly useful for the diagnosis of prepatent fascioliasis in domestic animals and humans where standard coprological examination may yield false negatives due to the absence of fluke ova in faeces. The early diagnosis of many diseases is important in veterinary medicine in order that early institution of chemotherapy or culling so as to save the animal or its carcass may be recommended. Serological diagnosis may also be useful in epizootiological surveys on incidence and prevalence of fascioliasis. Further more, chemotherapeutic successes could be evaluated by serological means.

Several techniques have been described for the detection of humoral response in fascioliasis. These include the double immunodiffusion test (precipitin reaction), complement fixation test (CFT), indirect fluorescent antibody (IFA) technique, counter electrophoresis (CEP), fluorescent antibody test (FAT) and variants of these (9). However, these authors found that most of these tests were either too complex for routine use or were not specific enough. Therefore, there is the need for a simple and rapid test for field diagnosis of fascioliasis. Unfortunately, most of the existing tests have not been tested in the field.

The double immunodiffusion (DID) test appeared to be the simplest of these tests (5). Therefore, we have assessed the sensitivity of the DID test for the diagnosis of fascioliasis in field-infected cattle.

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## MATERIALS AND METHODS

### Tests samples

19 serum samples were used as test samples. These were all obtained from cattle naturally infected with *Fasciola gigantica*.

The samples were collected from cattle slaughtered at the Ibadan Municipal abattoir between December, 1981 and January, 1982. 10 serum samples were also collected from non-infected cattle and used as controls.

Serum was obtained from blood collected by cardiac puncture immediately after slaughter and evisceration. Serum was collected from the clotted blood after centrifugation at 1 500 g for 10 min. All the test samples were stored (refrigerated) at 4 °C prior to the test.

### Antigen preparation

A somatic antigen was prepared from *F. gigantica* by the method of OGUNRINADE (5). Adult flukes were homogenised in a suspension with 10 ml 1 M phosphate buffered saline (PH 7.3). The crude antigen so prepared was labelled adult worm antigen (AWA).

Since OGUNRINADE (5) detected excretory antigen in the bile of infected cattle, bile obtained from this source was also used on one occasion as the antigen.

A count was also made of all the flukes in each infected animal from which test serum was obtained.

### Double immunodiffusion (DID) test

The DID test was performed as described by TIGGELE and OVER (9). The test was done in plates containing 1 p. 100 Noble agar (Difco Laboratories, Detroit) in 0.04 M barbital buffer (PH 8.6) and 0.2 p. 100 sodium azide added at a dilution of 1 in 1 000.

5 mm diameter wells were cut in the agar plates. The central well was filled with the antigen while the peripheral wells were filled with doubling dilutions of sera or bile.

The reaction was allowed to proceed at room temperature for 24 hours before the results were read.

## RESULTS

The DID test detected 73.7 p. 100 of all infected cattle sera at neat titres. Single precipitin lines were formed between AWA and sera. Bile from infected cattle reacted with AWA and also with infected cattle sera. The serological titre of infected cattle sera ranged between 1 in 4 and 1 in 32.

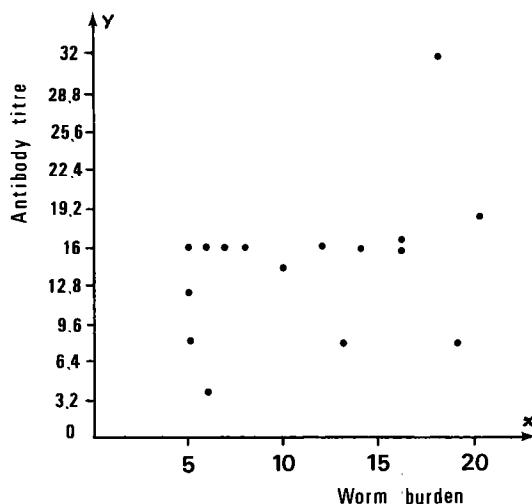


Fig. 1. — Correlation between precipitin antibody and worm burden.

There was no significant correlation between the antibody titre and the worm burden ( $r = 0.319$ ,  $P > 0.1$ ).

Some of the sera from non-infected cattle gave positive reaction when bile was used as antigen.

## DISCUSSION

The results of the DID test showed that precipitin antibodies against *F. gigantica* could be demonstrated in cattle naturally infected with these parasites.

The test detected 73.7 p. 100 of all infected cattle sera. BLANCOU, BOUCHET and DAYNES (1) and ICHIHARA, SUSUMI and KURAMOTO (4) reported 16-36 p. 100 and 90 p. 100 respectively. However, OGUNRINADE (5) detected only 54 p. 100 positives in naturally infected cattle. It is likely that the conditions of storage of both antigens and sera may be responsible for these differences in the results obtained. The clinical histories of the naturally infected cattle which provided the test samples for these studies were also not

TABLE 1—Results of the DID tests and worm burden of each animal from which the test serum was collected

Serum Number	Serial Dilution						Number of flukes recovered
	Neat	1:2	1:4	1:8	1:16	1:32	
Cattle 1	+	+	+	-	-	-	6
2	+	+	+	+	-	-	19
3	+	+	+	+	-	-	5
4	-	-	-	-	-	-	8
5	+	+	+	+	-	-	13
6	+	+	+	+	+	-	8
7	+	+	+	+	+	-	7
8	+	+	+	+	+	-	12
9	+	+	+	+	+	-	16
10	-	-	-	-	-	-	7
11	-	-	-	-	-	-	5
12	-	-	-	-	-	-	16
13	+	+	+	+	+	-	14
14	+	+	+	+	+	-	5
15	+	+	+	+	+	-	12
16	+	+	+	+	+	+	18
17	+	+	+	+	+	-	6
18	+	+	+	+	+	-	8
19	-	-	-	-	-	-	7
Infected bile	+	+	+	-	-	-	5

+ = positive reaction and - = negative reaction.

known and these could be responsible for the differences in the results of these authors.

The bile of infected cattle contained both antigen and antibody since it gave precipitin reactions with the antigen and the sera of infected cattle. The presence of excretory antigens in bile may be due to the replacement of fluke surface *glycocalyx* which is reported to occur as a means of evading host immune attack by flukes (3). The presence of antibodies in bile confirms the assertion of OGUNRINADE (5) that the bile duct is not an immunologically privileged site for flukes. The latter author reported that biliary antibodies against flukes are secreted from serum and are not synthesised locally.

SINCLAIR and KENDALL (8) showed a highly significant correlation between the number of flukes recovered and the total precipitin reaction in each individual rabbit. Like ICHIHARA *et al.* (4), SEWELL (7) and OGUNRINADE (5) we did not find any significant correlation between the serological titre and the degree of fluke infestation in cattle. This can be

due to the secretion of antibody into bile. Thus the serum IgG titres bear no relation to the inducing flukes. ROSS (6), DOYLE (2) and OGUNRINADE (5) found that there is considerable loss of established flukes in chronic bovine fascioliasis and this may also be a reason for the lack of a significant correlation.

The bile from infected cattle surprisingly reacted with some of the sera from non-infected cattle which were used as controls.

It may be that these animals which were used as controls because of the absence of flukes in the bile ducts had prepatent fascioliasis or they could have recently been cured of a long term infection and still had specific IgG in their sera. Another possibility is that the animals could have had cross reacting helminth infection. OGUNRINADE (5) found the DID test to be specific for fascioliasis in animals with intercurrent infections of paramphistomes and schistosomes. However, the possibility of other cross reacting helminths requires some investigation.

Although the exact role of humoral antibodies in fascioliasis is still not known, their detection

by the DID test is of diagnostic value. The test can give more accurate results if a highly purified antigen which does not cross react with other

helminths is used. Therefore, such an antigen needs to be developed before the test can be reliably applied for field diagnosis of fascioliasis.

#### RESUMEN

TANYA (V. N.), OGUNRINADE (A. F.). Anticuerpos precipitantes en la distomatosis natural. *Rev. Elev. Méd. vét. Pays trop.*, 1984, **37** (1) : 26-29.

Se analizó el suero de bovinos infestados naturalmente por *Fasciola gigantica* para determinar la presencia de anticuerpos por la prueba de la doble inmunodifusión.

Se determinaron anticuerpos precipitantes en el suero de 74 p. 100 de los casos positivos. Sin embargo, no fué posible establecer una relación significativa entre el título serológico y el nivel de infestación por la duela.

*Palabras claves* : Distomatosis natural - *Fasciola gigantica* - Anticuerpos - Doble inmunodifusión.

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