SERODIAGNOSIS OF DERMATOPHILOSIS IV. ANTIGENIC SELECTION FOR OPTIMAL ANTIBODY RESPONSE DETERMINATION IN *DERMATOPHILUS CONGOLENSIS* INFECTED ANIMALS BY COUNTERIMMUNOELECTROPHORESIS

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ABTRACT

Four different antigenic extracts of *Dermatophilus congolensis* were evaluated for their suitability for counterimmunoelectrophoresis (CIE) technique in detecting antibodies to *D. congolensis*. Thirty (30) sera collected during an outbreak of dermatophilosis in a herd of Santa Gertrudes cattle were tested. Twenty-eight (28) of the sera reacted to at least one of the antigenic extracts while 14 of them reacted to all the four extracts. Twenty (20) sera reacted to the two extracellular extracts (ammonium sulphate extracts, DcAS and extracellular polysaccharide, DcPS) while 18 sera reacted to the two whole cell-associated antigens (Triton X-100 extract of the cell walls, DcTX and cytoplasmic antigens, DcCP). Twenty-eight sera (93.33%) reacted to DcAS followed by 25 (83.33%) sera which reacted to DcCP, 21 (70.0%) to DcTX and lastly 20 (66.67%) to DcPS. Twenty-four (80.0%) sera reacted to both DcAS and DcCP; 18 (60.0%) and 15 (50.0%) of the sera reacted to both DcPS and DcTX antigens and DcPS and DcCP antigens respectively. Of the four extracts, only DcAS and DcCP detected multiple antibodies and these antigenic extracts were found to have the highest antibody detection rates. This observation recommends the extracellular and cytoplasmic antigens for seroepidemiological studies of dermatophilosis.

INTRODUCTION

Dermatophilus congolensis, the aetiological agent of dermatophilosis, is fairly homogenous in its antigenic constitution and all isolates so far characterized are classified as belonging to the same strain (1). In spite of the extensive studies of humoral immunity to dermatophilosis, the antigenic components of *D. congolensis* have still not been well-defined. Makinde and Wilkie (2) and Makinde (3) observed that antibody to extracellular antigens were abundant in acute and/or mild active dermatophilosis infection while in chronic infections

higher levels of antibody were found against whole cell-associated antigens of *D. congolensis*. These observations therefore indicate that the choice of antigen would determine the level of antibody detectable as well as the severity of infection which in turn is directly related to the level of stimulation by different components of the causative organism.

Thus, proper evaluation of fulminating dermatophilosis in a herd of cattle during an outbreak will require the use of specific antigenic components of *D. congolensis* that are likely to give the most realistic indications of the different phases of the disease in the host irrespective of the serological test used. This would in turn advise on the appropriate chemotherapeutic measures to take to control the outbreak. In this study counterimmunoelectrophoresis which has been found to be a useful technique in the serological diagnosis of dermatophilosis (4) was utilized to evaluate the antigenic preparations from the extracellular products of *D. congolensis* and compare them with its whole cell extracts in an outbreak of dermatophilosis.

MATERIALS AND METHODS

Sera

Thirty (30) animals with dermatophilosis infection in a herd of Santa Gertrudes cattle were bled via the jugular veins and sera subsequently separated from the blood were stored frozen at -20°C.

Antigens

D. congolensis isolated from a scab from one of the infected animals was subcultured in trypticase soyabroth as described by Makinde (3). Four extracts were subsequently prepared from the broth culture to serve as sources of antigens.

The ammonium sulphate extract of the culture supernatants (DcAS) and cytoplasmic antigens (DcCP) were both prepared as described by Makinde and Ojo (5). Extracellular polysaccharide of *D. congolensis* was prepared by the method described by Rosan and Hammond (6). Briefly, three volumes of acetone were added to the *D. congolensis* broth culture filtrate and the precipitate formed was allowed to settle at 4°C for 48 hours before collection by centrifugation at 10,000 g for 30 minutes. The precipitate was suspended in 10% trichloroacetic acid first for 4 hours at room temperature and later for 24 hours at 4°C. The supernatant from the precipitate formed was collected by centrifugation at 10,000 g for 10 minutes to which an equal volume of acetone was added. The precipitate formed was allowed to settle at 4°C for 24 hours, collected by centrifugation and dissolved in distilled water. The solution obtained was dialyzed against distilled water at 4°C for 72 hours with three water changes. The thick slimy dialysate was lyophilized and designated as DcPS.

Detergent extraction of *D. congolensis* cell walls was carried out as described by Hearn and Mackenzie (7). Cell wall pellet obtained from whole cell homogenate prepared as described by Makinde and Ojo (5) was suspended in 100ml of 0.5M NH_4HCO_3 at pH 8.0 containing 0.5% (vol/vol) of Triton X-100 (Scintillation Grade, Packard Instrument Co. IL, USA) and extracted with stirring for 2 hours at 30°C. The supernatant was collected by centrifugation at 10,000 g for 30 minutes and saved. The pellet obtained was subjected to another round of detergent extraction as described above with 50 ml of the bicarbonate and detergent solution and the supernatants collected. Both supernatants were pooled and lyophilized and designated as DcTX.

Counterimmunoelectrophoresis

The method described by Makinde and Majiyagbe (42) was adopted. At the end of the each electrophoresis run precipitation lines were checked for and readings were recorded.

RESULTS

Figure 1 summarises the antibody detection rates of the four antigenic extract of *D. congolensis* in CIE in an outbreak of dermatophilosis. They also emphasize the inter-relationship between the various extracts. The antibody detection rates was highest with DcAS (28 or 93.33%) followed by DcCP to (25 or 83.33%), DcTX (21 or 70.0%) and DcPS (20 or 66.67%). All the four extracts detected antibody in 14 (44.77%) of the sera while 24 out of 25 sera which reacted with DcCP also reacted positively with DcAS. All the 20 sera reactive with DcPS also reacted with DcAS. Eighteen and 15 sera which reacted with DcPS also reacted positively with DcCP and DcTX respectively.

The sensitivity and specificity of the various antigen when compared with one another are shown on Table I(a to h). DcAS and DcCP were found to be more sensitive than the others while DcPS was the most specific.

DISCUSSION

The differences in sensitivity between different serological tests and within a serological test reflect inherent variables distinctive to various procedures and the nature of antigens used (8). Thus the usefulness of any serological method will therefore depend on the relationship and relevance of the antigen chosen for the test as well as the specific antibody responsible for immunity against the organism being investigated. The choice of immunogenic components of *D. congolensis* for serological studies will likely present a clearer and more valuable picture of the disease than just any antigenic components. Also the more purified the antigen chosen for such studies the more definitive and specific, the results are likely to be.

Selective use of different antigenic preparations of *D. congolensis* has been carried out previously by various workers (2,4,5,9,10,11,12). Observations made in this study showed that different components of *D. congolensis* stimulate immune responses in infected animals to various degrees depending on their exposure to the hosts or on their antigenicity. It is also likely that multiple stimulation of these responses due to continuous reinfections or persistent infections in both acute and chronic cases most especially during an outbreak of dermatophilosis do occur. It was also observed that most of the affected animals reacted against the crude extracellular and cytoplasmic antigens, which are likely to contain the highest proportion of proteins and therefore are expected to be more antigenic than the purer detergent-extracted antigens which would normally contain low concentrations of different types of proteins in relation to carbohydrate (5,13,14).

This study further confirms previous observations on the importance of antigenic components of *D. congolensis* that should be used for serological studies of dermatophilosis. The CIE as used in this study also consolidates the assertions made by Makinde and Majiyagbe (4) on the choice of antigens for antibody detection by CIE while at the same time confirms the superiority of extracellular and cytoplasmic antigens of *D. congolensis* in passive haemagglutination studies as observed by Makinde and Ojo (5).

In conclusion, it is suggested that for a primary investigation of an outbreak of dermatophilosis both extracellular and cytoplasmic antigens of *D. congolensis* would be ideal for detecting antibody response. The CIE as a serological test has also been found to be suitable because of its sensitivity and specificity and also because it is a simple and cheap test to carry out in the tropics where large scale seroepidemiological surveys are still favoured.

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FIGURE 1: Inter-relationship between four different extracts of *D. congolensis* and their antibody detection rates in dermatophilosis by counter-immunoelectrophoresis CIE

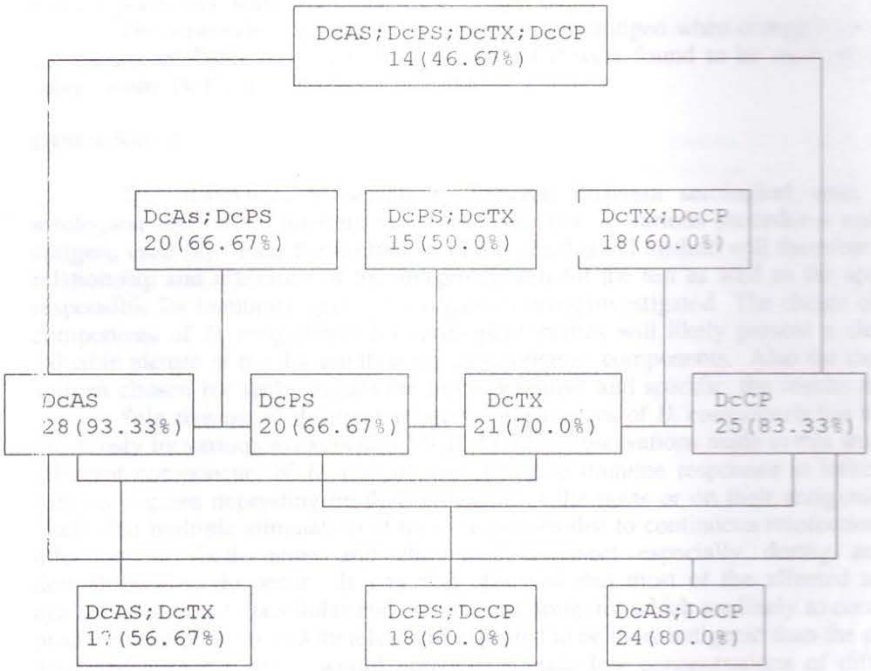

TABLE I(a): Comparison of DcPS, DcTX and DcCP with DcAS in the detection of *D. congolensis* antibody from 30 Serum samples by CIE.

DcAS		DcPS		DcTX		DcCP	
	Positive	Negative	Positive	Negative	Positive	Negative
Positive	20	8	19	9	24	4
Negative	0	2	2	0	1	1
Sensitivity		20/28(71.4%)		19/28(67.8)		24/28(85.7%)
Specificity		2/2(100%)		0/2(0%)		1/2(50%)
Positive Predictive value		20/20(100%)		19/21(90.5%)		24/25(96%)
Negative Predictive value		2/10(20%)		0/9(0%)		1/4(25%)

(b)

DcPS	DcTX		DcCP	
	Positive	Negative	Positive	Negative
Positive	15	5	18	2
Negative	6	4	7	3
Sensitivity		15/20(75%)		18/20(90.0%)
Specificity		4/10(40%)		3/7 (43.0%)
Positive Predictive value		15/21(71.4%)		18/25(72.0%)
Negative Predictive value		4/9(44.4%)		3/5 (60%)

(c)

DcAS	DcPS	
	Positive	Negative
Positive	20	8
Negative	0	2
Sensitivity		20/28(71.4%)
Specificity		2/2(100%)
Positive Predictive value		20/20(100%)
Negative Predictive value		2/10(20%)

(d)

DcAS	DcTX	
	Positive	Negative
Positive	19	9
Negative	2	0
Sensitivity		19/28(67.8)
Specificity		0/2(0%)
Positive Predictive value		19/21(90.5%)
Negative Predictive value		0/9(0%)

(e)

DcAS	DcCP	
	Positive	Negative

Positive	24	4
Negative	1	1
Sensitivity		24/28(85.7%)
Specificity		1/2(50%)
Positive Predictive value		24/25(96%)
Negative Predictive value		1/4(25%)

(f)

DcPS	DcTX	
	Positive	Negative
Positive	15	5
Negative	6	4
Sensitivity		15/20(75%)
Specificity		4/10(40%)
Positive Predictive value		15/21(71.4%)
Negative Predictive value		4/9(44.4%)

(g)

DcPS	DcCP	
	Positive	Negative
Positive	18	2
Negative	7	3
Sensitivity		18/20(90.0%)
Specificity		3/7 (43.0%)
Positive Predictive value		18/25(72.0%)
Negative Predictive value		3/5 (60%)

(h)

DcTX		DcCP
	Positive	Negative
Positive	18	2
Negative	7	3
Sensitivity		18/21(85.7%)
Specificity		2/9 (22.2%)
Positive Predictive value		18/25(72%)
Negative Predictive value		2/5 (40%)

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