CAPRINE BRUCELLOSIS: A QUALITATIVE COMPARISON OF THE SENSITIVITY OF THREE SERODIAGNOSTIC METHODS

BY

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Introduction

The serological diagnosis of **Brucella/abortus** infection in animals presents a number of problems (Davies, 1971). Numerous serological methods based on the biological and the physico-chemical properties of antibodies have been used (Morgan, 1970) but non of these tests has proved useful by itself, particularly in detecting animals that are incubating the disease or in distinguishing infected animals from vaccinated animals (Beh and Lascelles, 1973); thus a combination of tests is always recommended to arrive to a proper diagnosis (Davies, 1971; Beh and Lascelles, 1973).

Brucellosis in the Sudan has been diagnosed in cattle, sheep, goats and camels (El Nasri, 1960; Abdulla, 1966; Mustafa and Nur, 1968; Hussein and Saad, 1975) using serum agglutination test (SAT). A policy for vaccination against the disease has not been introduced in goats and that led to many public health and economical problems, although it is useful in excluding antibodies due to immunization from the serodiagnostic procedures. As SAT is used routinely for diagnosis, it is likely that the incidence of the disease has been underestimated in the Sudan, since the test is inconclusive or negative for chronically infected animals and less effective in detecting early infections (Morgan **et al,** 1978). The purpose of this investigation is to determine the incidence of brucellosis in Sudanese goats by three different serodiagnostic methods, using standard **Br.abortus** antigens, and to compare the sensitivity of the three procedures in detecting infected animals.

Materials and Methods

1. Animals used: Female goats only were used in this study. They were selected from 3 regions of the Sudan: Kassala, Sinnar and Khartoum. The goats were not vaccinated before, they ranged from 6 to 48 months of age.

2. **Collection of serum:** Blood was collected from each goat by venous puncture into sterile McCarteney bottle. The blood was allowed to stand for 1 h at room temperature, followed by centrifugation and separation of serum. Serum samples were transfered into sterile Bijou bottles and stored at -18° until used.

3. **Serological tests:** 302 goat serum samples were used in this investigation. Each sample was screened for Brucella antibodies using three tests: Rose Bengal plate test (RBPT), SAT and complement fixation test (CFT). Preparation of standard **Br.abortus** antigens and application of the tests were performed according to the methods of Morgan **et al** (1978). Positive samples by any method were retested to confirm the results.

4. **Interpretation of the results:** The results of RBPT were interpreted as either positive (+) or negative (−) as described by Davies (1971). Results of SAT were expressed as international units of antibody per ml (iu/ml). Goats sera containing 100 iu/ml and above were considered (+) while those containing between 50-100 iu/ml were doubtful ($\pm$) and values below 50iu/ml were (−) according to the recommendation of the joint FAO/WHO Expert Committee on caprine brucellosis (1971). Thus for SAT the results were recorded first as titres showing the degree of agglutination (numerator) and the serum dilution (denominator) before conversion into iu/ml following the procedures of Morgan **et al** (1978).

Results of CFT were interpreted as titres showing the degree of fixation (numerator) and the reciprocal of serum dilution (denominator) as indicated by Morgan **et al** (1978). Titres of 2/4 or more were considered positive by CFT while titres of 2/2 to < 2/4 were doubtful according to O'reily and Cunningham (1971).

Results

The overall incidence of brucellosis in goats as recorded by RBPT,SAT and CFT is shown in Table 1; details of titres of positive and doubtful samples is shown in Table 2. The number of goats positive by each test is expressed as a percentage of the total number tested (Table 1). Two goats were positive by RBPT, the same goats were also positive by SAT while five goats were positive with CFT. One doubtful sample and two negative samples by SAT were confirmed to be positive by CFT (Table 2). The overall incidence of the disease in goats was 0.65% when using either RBPTor SAT, but the incidence

rises to 2.2 % when CFT is used for diagnosis.

Discussion

This report is the outcome of three independent assessments of the incidence of Brucella infection in goats. The overall incidence of the disease is low: 0.65% and 2.2% as recorded by RBPT,SAT and CFT respectively. this may allow the areas from which the serum samples were collected to fall within the low prevalence regions with 3% or less infected animals, according to WHO/FAO reports (1953). Cross-immunity between **Br.abortus** and **Br.melitensis** does exist (joint FAO/WHO report, 1971); hence positive samples reported in this study may indicate infection with **Br.abortus** and or **Br.melitensis** although standard **Br.abortus** antigens were used in the serodiagnostic tests.

There are variations in the efficiency of the three tests used in picking up infected animals. The results of RBPT and SAT showed very close agreement, as both tests recorded an incidence of 0.65 %. This is not sufprising since RBPTis itself an agglutination test (Davies, 1971) and it was confirmed recently that RBPT and SAT detect the same class of antibody, which is predominantly 19 s Ig M(Allan **et al** 1976). In RBPT, however, the antigen is suspended in a lactate buffer of pH 3.65 (Morgan **et al** , 1978). This acidic buffer is believed to inhibit immunologically non-s pecific reactions (Rose & Roepke, 1957; Davies, 1971). In this way RBPT would automatically elim-inate "non specific reactions" associated with Ig M which in a normal tube SAT would show up as a suspect or positive titre (O'Reilly and Cunningham, 1971). That may provide an explanation for the two samples with agglutinin levels greater than 40 iu/ml with SAT (Table 2) and yet negative to the RBPT.

CFT on the other hand detects predominantly Ig G antibody (Allan **et al**, 1976) which is proved to be non-agglutinating (Beh and Lascelles, 1973). Infection with **Br. abortus**, even in the chronic phase, is known to stimulate predominantly the production of IgG, while vaccination titres are attributed mainly to IgM (Morgan, 1969; Elberg, 1973). From this it appears that CFT is more reliable in detecting infected animals, particularly chronic carriers, than RBPTand SAT. Therefore in a number of chronically infected cattle the SAT may revert to low or zero levels while CFT titres remain positive (O'Reilly and Cunningham, 1971). For this reason and because of it's greater specificity the CFT is rated higher than the SAT and RBPT in the diagnosis of brucellosis (Allan **et al**, 1976). It is not surprising in the present study that three positive samples by CFT were completely missed by RBPT and one of them was diagnosed as a doubtful sample by SAT. However, the SAT may be useful in detecting vaccinated animals, as the anti-body produced is predominantly IgM, and it is supposed to be superior for this purpose than RBPT (Davies, 1971). In a country, like the Sudan, where vaccination of goats against brucellosis has never been adopted SAT may not be of value. RBPT on the other hand detects infection earlier than does the SAT (Nicoletti,1967) and efficient in detecting cattle at the very early stages of infection than do the combined SAT/CFT (Davies, 1971), but at the same time it misses chronic carriers of the disease (Allan **et al**,1976).

Studies such as this one should lead to improved diagnostic accuracy in serological tests for animal brucellosis. It is evident from the results that CFT is the most accurate and sensitive diagnostic test for caprine brucellosis although it is complicated, time consuming and expensive to perform (Morgan **et al**,1978). It is also valid from the interpretation of the results that the incidence of animal brucellosis in this country (El Nasri, 1960; Abdulla, 1966; Mustafa and Nur, 1968; Hussein and Saad, 1975) has been much underestimated by depending solely on SAT as a diagnostic test. Our report may strongly recommend the routine use of CFT for the diagnosis of the disease, although we believe that its combination with RBPT, which is cheap and simple to perform (Davies, 1971), may be more satisfactory.

Summary

302 goat serum samples, collected from Kassala, Sinnar and Khartoum areas of the Sudan, were screened for Brucella antibodies using three serodiag-nostic tests: RBPT,SAT and CFT. The overall in-cidence of the disease in goats was 0.65 % when using either RBPT or SAT for diagnosis, but the incidence rises to 2.2 % when CFT is used. Results in-terpretation indicates that CFT is rated higher than the SAT and RBPT in the diagnosis of caprine brucellosis.

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Table 1:

The overall incidence of Brucella infection in goats (%) as shown by RBPT,SAT and CFT.

Serological test applied	No of goats tested	No positive	No doubtful	Percentage Positive
RBPT	302	2	—	0.65%
SAT	302	2	2	0.65%
CFT	302	5	—	2.2%

Table 2:

Details of titres of positive and doubtful samples.

Goat No	SAT Titre iu/ml		RBPT	CFT Titre
15	−4/20	41	−	+ 2/4
21	+2/80	123	+	+ 2/4
267	+3/160	287	+	+ 2/4
275	−2/10	15.5	−	+ 2/4
277	± 3/40	72	−	+ 2/4

References

— Abdulla, A.E.D. (1966). Sudan.J.Vet.Sci. & Anim. Husb. 7:28

— Allan, G.S. Chappel, R.J. williamson, P. and Mc Naught, D.J. (1976). J. Hyg., Camb. 76:287.

— Beh, K.J. and Lascelles, A.K. (1973). Res. Vet. Sci.14: 239.

— Davies, G, (1971). Vet. Rec. 88: 447.

— Elberg, S.S. (1973). Immunity to **Brucella** infection. Medicine, Baltimore 52: 339.

— El Nasri, M. (1960) vet. Rec. 72: 1200.

— Hussein, A.M. and Saad, M. A. (1975). Proc. the Vet. Conf., the Sudan Vet. Association, Khartoum.

— Joint FAO/WHO Expert committee on Brucellosis (1971). 5th report Geneva, (1970); Published by FAO and WHO,P 50.

— Morgan, W.J.B. (1969). Proc. Roy. Soc. Med. 62: 1050.

— Morgan, W.J.B. (1970). J. Dairy Res. 37: 303.

— Morgan, W.J.B; Mackinnon, D.J.; Gill,K.P.W.; Grower, S.G.M. and Norris, P.l.W. (1978). In "Brucellosis diagnosis standard laboratory technique", 2nd ed. Cent. Vet. Lab., Weybridge, surrey, England.

— Mustafa, A.A. and Nur, B.M. (1968). Proc. 3rd vet. Conf., the Sudan Vet. Association, Khartoum, P 319-327.

— Nicoletti, P. (1976). J.Am. Vet. Med. Ass. 151: 1778.

— O'Reilly, D. J. and Cunningham, B. (1971). Vet. Rec. 88: 590.

— Rose,J.E. and Roepke, M.H. (1957). Am. J. Vet. Res. 18: 550.

— WHO/FAO,Seminar on Zoonosis (1953). P. 74- 76.