EXAMINATION OF TWELVE STRAINS OF TRITRICHOMONAS FOETUS (RIEDMULLER) ISOLATED IN QUEENSLAND AND THE DESCRIPTION OF A NEW SEROTYPE, T. FOETUS VAR. BRISBANE

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SUMMARY

Examination of 12 strains of *Tritrichomonas foetus* isolated from bovine genital organs showed that 9 strains were serologically similar to *T. foetus* var. *belfast* and 3 strains possessed an antigen different from and a common antigen with *T. foetus* var. *manley*. These three strains did not react with antisera prepared against *T. foetus* var. *manley* unless the organisms were disintegrated before injection into the rabbit. It is proposed that they be called *T. foetus* var. *brisbane* (new serotype).

I. INTRODUCTION

Four morphological types of the family Trichomonadidae are described in cattle (Levine 1961). The genus *Tritrichomonas* Kofoid (1920) has three anterior and one posterior flagella and an undulating membrane. *Tritrichomonas foetus* (Riedmuller 1928)—synonyms *T. bovis, T. genitalis, T. bovinus, T. uterovaginalis vitulae* and *T. mazzanti*—has the following characteristics: a long undulating membrane which has a double contour of its marginal edge, a thick hyaline axostyle which extends beyond the cell, an annular ring, no regularity in the arrangement of its granules, measures from 15 to 22 μ in length, and is associated with infertility. *Tritrichomonas enteritis* (Christl 1954) has the following characteristics: a long undulating membrane with no double contour of the marginal edge, no annular ring, the axostyle is straight and slender and extends at the most one-quarter of the cell length beyond the cell, measures about 10 μ in length, has a regular arrangement of its granules, and was described from the caecum and colon. *Trichomonas pavlovi* (Levine 1961) has four anterior flagella compared with three in the genus *Tritrichomonas,* and was isolated from the intestine. *Monocercomonas ruminatium* (Braune 1913) (synonym *T. ruminatium*) has no undulating membrane and was isolated from the rumen.

Species of *Tritrichomonas* described in other animals are *T. fecalis* (humans), *T. suis* and *T. rotunda* (pigs), *T. eberthi* (poultry) and *T. equi* (horses), and other species have been described in rats, mice, guinea pigs, golden hamsters, wild rodents, monkeys and baboons (Levine 1961).

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Two serological types of *T. foetus* were described and shown to be serologically distinct by Kerr and Robertson (1945). *T. foetus* var. *belfast* was isolated by Kerr and Robertson (1941) in Northern Ireland and *T. foetus* var. *manley* by Mahmoud (1944) in England.

The first satisfactory serological test for trichomonads was developed by Robertson (1941), working with antisera from artificially immunized rabbits. Kerr and Robertson (1945) showed that specific agglutinating antibodies were present in serum from virgin heifers experimentally infected with *T. foetus* var. *belfast* and *T. foetus* var. *manley*. Pierce (1949) showed that all strains isolated and positive sera typed in Great Britain and Ireland could be associated with the *T. foetus* var. *belfast* or *T. foetus* var. *manley* antigenic types and that, in the limited number of strains isolated and positive sera typed, no other antigenic variety could be detected.

Sanborn (1955), using a microagglutination test, considered that two porcine strains of tritrichomonads were different from each other and from *T. foetus*. Robertson (1960) showed by direct agglutination and gel diffusion precipitation tests that the *T. foetus* var. *belfast* and *T. foetus* var. *manley* serotypes were readily distinguishable from each other and from two pig strains which were closely related but not identical, and closer to *T. foetus* var. *belfast* than to *T. foetus* var. *manley*. She thought that the serological distinctions did not justify separation of the pig and cow strains into species.

T. foetus var. *belfast* and *T. foetus* var. *manley* serotypes have been detected in New South Wales and Victoria (Mylrea 1962; I. Newsam, personal communication).

Bovine trichomoniasis was first diagnosed in Queensland in 1950 (Sutherland, Simmons, and Bell 1953). This strain was typed serologically as *T. foetus* var. *belfast* (Beames 1955). Since then, 11 outbreaks have been diagnosed. The serological typing of these strains is recorded in this paper.

II. METHODS

Strains.—The *T. foetus* var. *belfast* and *T. foetus* var. *manley* strains were obtained from the Commonwealth Scientific and Industrial Research Organization, Parkville, Victoria. The Queensland strains R, VS, BH, L, F, G, A and Q were isolated from vaginal mucous samples, OON from a uterus, D from a semen sample and B from the stomach contents of an aborted foetus. BULL is the strain F228 identified as *T. foetus* var. *belfast* (Beames 1955). All these samples were submitted for routine diagnosis to the Animal Research Institute, Yeerongpilly, except R, BH and G, which were obtained during an infertility survey, and OON, which was isolated at the Animal Health Station, Oonoonba, Townsville, during a survey of bovine uteri for pathogenic organisms. B strain came from an Australian Illawarra Shorthorn herd at Kingaroy, approximately 150 miles north-west of Brisbane. D strain came from a beef Shorthorn herd at Yelarbon, near the Queensland/New South Wales border, approximately 250 miles south-west

of Brisbane. OON strain came from a beef Shorthorn herd near Charters Towers, approximately 100 miles west of Townsville. All were inoculated into modified Plastridge's medium (Sutherland, Simmons, and Bell 1953) and maintained by subculturing once weekly.

Media.—Modified Plastridge's medium was used for growth of organisms, and glucose broth (Beames 1955) was used to dilute the antigen for the test.

Preparation of Antisera.—The suspensions for inoculation were prepared as follows:—

T. foetus var. *belfast* antigen was prepared from cultures in modified Plastridge's medium. After incubation at 37°C for 1–2 days, 5 ml of the culture was injected intraperitoneally into a mouse immediately after the mouse had received 30 mg Intramycetin (Parke Davis) in the right thigh (intramuscularly) and 0·5 mg cortisone acetate (Glaxo) in the left thigh (intramuscularly). Three days after inoculation the mouse was killed, the peritoneal fluid harvested, diluted to 15 ml with sterile saline, and 5 ml reinoculated into each of three mice, using the same procedure as for the first mouse. The combined harvest of peritoneal fluid from these mice was further passaged into six mice. The pooled peritoneal fluid from these mice was centrifuged, and the deposit of organisms washed, centrifuged and resuspended in 15 ml sterile saline to use as inoculum for a rabbit.

All other antigens were prepared by growing in modified Plastridge's medium containing nutrient broth instead of bovine serum and incubating them at 37°C for 2–3 days. The centrifuged deposit of organisms from these cultures was washed and resuspended in 15 ml sterile saline and used to inoculate a rabbit (live culture antigen).

The *T. foetus* var. *manley* antigen was also prepared, after purifying the culture by the method of Glaser and Coria (1935), by shaking the suspension in Ringer's solution on a Mickle machine with Ballotini beads No. 12. After shaking, the suspension was lightly centrifuged. A fresh suspension was prepared each week (disintegrated culture antigen).

Antiserum in rabbits was prepared as described by Beames (1955).

Agglutination Test.—The test was done by the method of Pierce (1947), using the readings for the different degrees of agglutination that were shown in his photomicrographs. The reactions range from a preagglutination zone characterized by immobilization, which is represented by +++++, through decreasing degrees down to slight agglutination, represented by (+).

All strains were grown in modified Plastridge's medium for 1–2 days at 37°C to make antigens. The antigens were tested against antisera in doubling dilutions ranging from 1/24 to 1/3072.

Absorption Tests.—A 1/10 dilution of serum was absorbed, using an equal volume of centrifuged deposit of culture in modified Plastridge's medium. These

were held for 2 hr in a 37°C water-bath, then centrifuged to remove the cells. A single absorption was usually sufficient, but a double absorption was carried out in some cases.

The agglutination test was carried out as above, using dilutions of sera 1/24, 1/48, 1/96 and 1/192. After absorption the serum was considered to be a 1/24 dilution.

Flagella Stain.—The method used was the May-Grünwald Giemsa stain for blood smears, without methanol fixation (Dacie 1958, p. 39).

III. RESULTS

Morphologically, BULL, BH, L, F, G, A, R, VS, Q, B, D and OON are similar. They possess (Figure 1) three anterior and one posterior flagella and an undulating membrane with a double contour of its marginal edge, and the axostyle extends beyond the cell. There is no particular arrangement of the chromatin granules and all strains are similar in size (average length 20·8 μ) to *T. foetus* var. *belfast* and *T. foetus* var. *manley*.

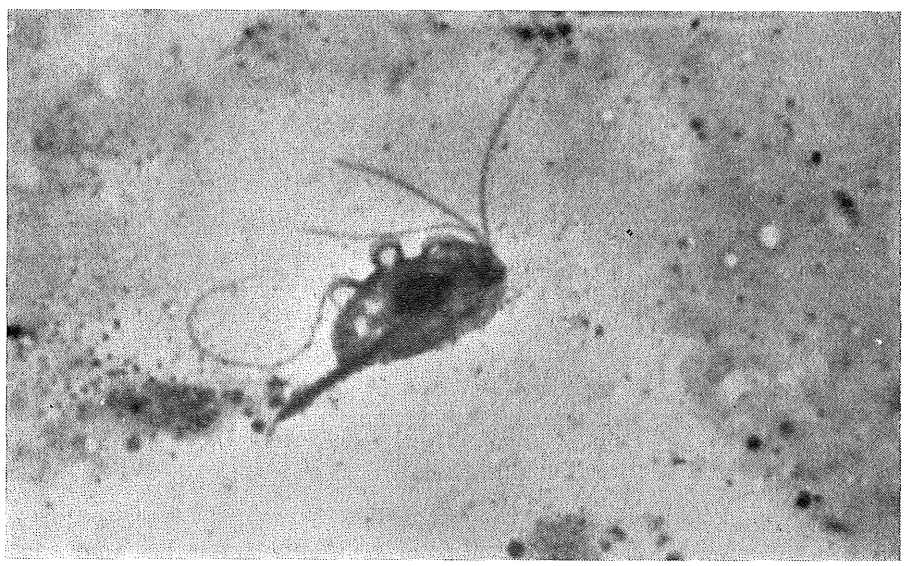

Fig. 1.—*Tritrichomonas foetus* var. *brisbane* (B strain).

Results of agglutination reactions of all strains with antisera prepared using live suspensions of organisms again *T. foetus* var. *belfast* and *T. foetus* var. *manley* are given in Tables 1 and 2. Ten strains (*T. foetus* var. *belfast,* BH, L, F, G, A, R, VS, BULL and Q) were agglutinated by belfast antiserum but not by manley antiserum. *T. foetus* var. *manley* itself was agglutinated by its own antiserum but not by belfast antiserum. Three strains (B, D and OON) were not agglutinated by either the belfast or the manley antiserum.

TABLE 1

RESULTS OF AGGLUTINATION REACTIONS OF STRAINS WITH SERUM PREPARED AGAINST *T foetus* VAR. *belfast* STRAIN

Strain	Dilutions of Belfast Antiserum								Rabbit Sera (Normal)	Saline
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072		
T. foetus var. *belfast*	+++++	++++	++++	++++	++++	+(+)	+	−	−	−
T. foetus var. *manley*	−	−	−	−	−	−	−	−	−	−
BH	++++	+++	+++	++(+)	++	+(+)	+	(+)	−	−
L	+++++	+++++	++++	++++	++++	+++	+	−	−	−
F	++++	++++	+++(+)	+++(+)	+(+)	+	(+)	(+)	−	−
G	++++	++++	+++	+++	+++	+(+)	(+)	(+)	−	−
A	+++++	++++	++++	+++	++	+	(+)	−	−	−
R	++++	++++	++++	+++(+)	+++(+)	+++	+(+)	−	−	−
VS	+++(+)	+++	+++(+)	+++(+)	+++	+	+	−	−	−
BULL	+++++	++++(+)	++++	++++	++++	+++	+	−	−	−
Q	++++	+++	+++	(+)	−	−	−	−	−	−
B	−	−	−	−	−	−	−	−	−	−
D	−	−	−	−	−	−	−	−	−	−
OON	(+)	−	−	−	−	−	−	−	−	−

TABLE 2

RESULTS OF AGGLUTINATION REACTIONS OF STRAINS WITH SERUM PREPARED AGAINST *T. foetus* VAR. *manley* STRAIN (LIVE CULTURE ANTIGEN)

Strain	Dilutions of Manley Antiserum								Rabbit Sera (Normal)	Saline
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072		
T. foetus var. *manley*	+++++	+++++	++++	++++	+++(+)	+	—	—	—	—
T. foetus var. *belfast*	—	—	—	—	—	—	—	—	—	—
BH	—	—	—	—	—	—	—	—	—	—
L	—	—	—	—	—	—	—	—	—	—
F	—	—	—	—	—	—	—	—	—	—
G	—	—	—	—	—	—	—	—	—	—
A	—	—	—	—	—	—	—	—	—	—
R	—	—	—	—	—	—	—	—	—	—
VS	+++(+)	+	—	—	—	—	—	—	—	—
BULL	+++	+++	+	—	—	—	—	—	—	—
Q	—	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—	—
D	—	—	—	—	—	—	—	—	—	—
OON	—	—	—	—	—	—	—	—	—	—

TABLE 3

RESULTS OF AGGLUTINATION REACTIONS OF STRAINS WITH SERUM PREPARED AGAINST B STRAIN

Strain	Dilutions of B Antiserum								Saline	Rabbit Serum Diluted 1/24
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072		
B	++++(+)	++++	+++(+)	+++	++	−	−	−	−	−
D	++++	++++	+++(+)	+++	−	−	−	−	−	−
OON	++++(+)	++++(+)	++++	++	+	−	−	−	−	−
T. foetus var. *belfast*	−	−	−	−	−	−	−	−	−	−
T. foetus var. *manley*	++++(+)	++++	+++	−	−	−	−	−	(+)	(+)

TABLE 4

RESULTS OF AGGLUTINATION REACTIONS OF STRAINS WITH SERUM PREPARED AGAINST D STRAIN

Strain	Dilutions of D Antiserum								Saline	Rabbit Serum Diluted 1/24
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072		
D	+++++	++++	+++	+	−	−	−	−	−	−
B	+++++	++++	+++	(+)	−	−	−	−	−	−
OON	++++	++	(+)	−	−	−	−	−	−	−
T. foetus var. *belfast*	−	−	−	−	−	−	−	−	−	−
T. foetus var. *manley*	+++	+++	++	+(+)	−	−	−	−	−	++

TABLE 5

RESULTS OF AGGLUTINATION REACTIONS OF STRAINS WITH SERUM PREPARED AGAINST OON STRAIN

Strain	Dilutions of OON Antiserum								Saline	Rabbit Serum Diluted 1/24
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072		
OON	+++++	++++	++(+)	+(+)	−	−	−	−	−	−
B	+++++	++++	+++	−	−	−	−	−	−	−
D	+++++	+++++	++(+)	+(+)	−	−	−	−	−	−
T. foetus var. *belfast*	−	−	−	−	−	−	−	−	−	−
T. foetus var. *manley*	+++++	++++	++	−	−	−	−	−	−	−

Results of agglutination reactions of B, D, OON, *T. foetus* var. *belfast* and *T. foetus* var. *manley* with sera prepared against B, D and OON are given in Tables 3–5. *T. foetus* var. *belfast* was not agglutinated by B, D or OON antisera, but the other strains, B, D, OON and *T. foetus* var. *manley,* were all agglutinated by B, D and OON antisera.

Table 6 gives the results of agglutination reactions of B, D, OON and *T. foetus* var. *manley* with B, D, OON and manley antisera after absorption with B, D, OON and *T. foetus* var. *manley*. B, D and OON give similar results and all absorb antibodies to B, D, OON and *T. foetus* var. *manley* in B, D and OON antisera but fail to absorb *T. foetus* var. *manley* antibodies from manley antiserum. *T. foetus* var. *manley* absorbs antibodies to itself in B, D, OON and manley antisera, but fails to absorb antibodies to B, D and OON in B, D and OON antisera.

TABLE 6

AGGLUTINATION RESULTS OF B, D, OON AND *T. foetus* VAR. *manley* STRAINS AGAINST B, D, OON AND MANLEY SERA ABSORBED BY B, D, OON AND *T. foetus* VAR. *manley* CULTURES

Antigen	Absorbing Strain			
	B	D	OON	Manley
	B Antiserum			
B	—	—	—	+
D	—	—	—	+
OON	—	—	—	+
T. foetus var. *manley*	—	—	—	—
	D Antiserum			
B	—	—	—	+
D	—	—	—	+
OON	—	—	—	+
T. foetus var. *manley*	—	—	—	—
	OON Antiserum			
B	—	—	—	+
D	—	—	—	+
OON	—	—	—	+
T. foetus var. *manley*	—	—	—	—
	*manley Antiserum**			
B	—	—	—	—
D	—	—	—	—
OON	—	—	—	—
T. foetus var. *manley*	+	+	+	—

* Results for absorption of manley antiserum were similar for antisera prepared with either a live culture antigen or disintegrated culture antigen.

\+ Indicates a reading of at least +++ at 1/48 dilution.

Table 7 shows the results of agglutination reactions of *T. foetus* var. *manley*, *T. foetus* var. *belfast*, B, D and OON with manley antiserum prepared by Dr. I. Newsam of C.S.I.R.O., using the same strain as in our experiments. *T. foetus* var. *manley* is agglutinated to a high titre, but *T. foetus* var. *belfast*. B, D and OON are not agglutinated.

Table 8 shows the results of agglutination reactions of *T. foetus* var. *manley*, *T. foetus* var. *belfast*, B, D and OON with manley antiserum prepared by using a disintegrated culture as antigen. *T. foetus* var. *manley*, B, D and OON are all agglutinated, *T. foetus* var. *belfast* is not agglutinated. Absorption of this antiserum by *T. foetus* var. *manley*, B, D and OON gave similar results to the antiserum prepared with a living antigen (Table 6).

IV. DISCUSSION

From Tables 1 and 2 it can be seen that BH, L, F, G, A, R, VS, BULL and Q are serologically similar to *T. foetus* var. *belfast*. BULL strain has been previously identified (Beames 1955). BH, L, F, G, A, R, VS and Q all came from bovine genital organs; they conform morphologically to the species *Tritrichomonas foetus* and serologically to *T. foetus* var. *belfast*.

B, D and OON appear to be identical both morphologically and serologically. They were isolated from a foetus, a semen sample and a uterus respectively, and it may be significant that none of them came from a vaginal sample. They are of similar size to the *T. foetus* var. *belfast* and *T. foetus* var. *manley* strains, have three anterior and one posterior flagella and an undulating membrane with a double contour of the marginal edge, the axostyle extends beyond the body and they have no particular arrangement of the chromatin granules. From a morphological point of view they are therefore strains of *Tritrichomonas foetus*. However, they did not react with sera prepared against *T. foetus* var. *belfast* or *T. foetus* var. *manley* prepared using whole trichomonads as antigen. But the strain of *T. foetus* var. *manley* was agglutinated by antisera prepared against the three of them, and they were agglutinated by sera prepared against *T. foetus* var. *manley*, using disintegrated cells as antigen. From the direct agglutination tests (Tables 1-5, 7 and 8) and the absorption tests (Table 6) it can be concluded that B, D, OON, and *T. foetus* var. *manley* have common antigens as well as different ones. The common antigens do not seem to be non-specific ones, as *T. foetus* var. *belfast* does not possess them. It would appear that antigens which *T. foetus* var. *manley* has in common with B, D and OON strains are weakly antigenic and are not the type-specific ones which distinguish it from *T. foetus* var. *belfast*.

Robertson (1960) investigated the antigens of *T. foetus* var. *belfast* and *manley* by the gel diffusion method. She considered that the antigenic makeup of trichomonads was complex, and consisted of at least five lines. She found that the polysaccharides of strains *T. foetus* var. *manley* and *T. foetus* var. *belfast*, as

TABLE 7

RESULTS OF AGGLUTINATION REACTIONS OF *T. foetus* VAR. *belfast*, *T. foetus* VAR. *manley*, B, D AND OON STRAINS WITH SERUM PREPARED AT C.S.I.R.O., MELBOURNE, AGAINST *T. foetus* VAR. *manley*

Strain	Dilutions of manley Antiserum							Rabbit Serum Diluted 1/24	Saline
	1/24	1/48	1/96	1/384	1/768	1/1536	1/3072		
T. foetus var. *manley*	+++++	+++++	+++++	++++(+)	+++	++(+)	(+)	—	—
T. foetus var. *belfast*	—	—	—	—	—	—	—	—	—
B	—	—	—	—	—	—	—	—	—
D	—	—	—	—	—	—	—	—	—
OON	—	—	—	—	—	—	—	—	—

TABLE 8

RESULTS OF AGGLUTINATION REACTIONS OF STRAINS WITH SERUM PREPARED AGAINST *T. foetus* VAR. *manley* WITH AN ANTIGEN PREPARED BY DISINTEGRATING THE CELLS

Strain	Dilutions of manley Antiserum						Rabbit Serum Diluted 1/24	Saline
	1/12	1/24	1/48	1/96	1/192	1/384		
	PA+E*	PA+E	PA+E	PA+E				
T. foetus var. *manley*	+++++	+++++	+++++	+++++	+++++	+++++	—	—
T. foetus var. *belfast*	—	—	—	—	—	—	—	—
B	+++++	+++++	+++++	++	—	—	—	—
D	+++++	+++++	+++++	+++	++	(+)	—	—
OON	+++++	+++++	+++	++	+	—	—	—

* The T. *foetus* var. *manley* antiserum prepared with disintegrated cells produced preagglutination and echelon formation of its homologous antigen.

extracted by diethylene glycol, were serologically specific for the strains from which were obtained, but lines other than those of the polysaccharide complex were shown by both strains and these were thought to be protein in nature.

There are only two reports of serological relationships between members of the genus *Tritrichomonas.* Sanborn (1955), using a microagglutination test, considered that the two porcine strains of trichomonads were different from each other and from *T. foetus.* It was not stated whether the strain of *T. foetus* used was the *belfast* or *manley* variety. Robertson (1960) showed by direct agglutination and gel precipitation tests that the *T. foetus* var. *belfast* and *T. foetus* var. *manley* strains were readily distinguishable from each other and from two pig strains, which were closely related and closer to *T. foetus* var. *belfast* than to *T. foetus* var. *manley.* As there is no reason to think that B, D and OON came from an animal species other than bovine, or that they are not strains of *T. foetus,* it is suggested that B, D and OON are new serotypes which form with *T. foetus* var. *manley,* a serogroup. These Queensland strains are designated *T. foetus* var. *brisbane* (new serotype).

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REFERENCES

BEAMES, R. M. (1955).—*Qd J. Agric. Sci.* 12:15-9.

BRAUNE, R. (1913).—*Arch. Protist.* 32:111-70.

CHRISTL, H. (1954).—*Z. Parisitenk.* 16:363-72.

DACIE, J. V. (1958).—"Practical Haematology". (J. and A. Churchill: London).

GLASER, R. W., and CORIA, N. A. (1935).—*Amer. J. Hyg.* 22:221-5.

KERR, W. R., and ROBERTSON, M. (1941).—*Brit. Vet. J.* 97:351-65.

KERR, W. R., and ROBERTSON, M. (1945).—*Vet. Rec.* 57:221-2.

KOFOID, C. A. (1920).—*Univ. Calif. Publ. Zool.* 20:145-68.

LEVINE, N. D. (1961).—"Protozoan Parasites of Domestic Animals and Man". (Burgess Publishing Co.: Minneapolis).

MAHMOUD, A. (1944).—*Ann. Trop. Med. Parasit.* 38:219-22.

MYLREA, P. J. (1962).—*Aust. Vet. J.* 38:15-20.

PIERCE, A. E. (1947).—*Lab. J.* 8:238-44.

PIERCE, A. E. (1949).—*Brit. Vet. J.* 105:286-94.

RIEDMULLER, L. (1928).—*Zbl. Bakt. 1 Orig.* 108:103-18

ROBERTSON, M. (1941).—*J. Path. Bact.* 53:391-402.

ROBERTSON, M. (1960).—*J. Hyg. Camb.* 58:207-13.

SANBORN, W. R. (1955).—*J. Parasit.* 41:295-8.

SUTHERLAND, A. K., SIMMONS, G. C., and BELL, A. T. (1953).—*Aust. Vet. J.* 29:67-9.

TRUSSELL, R. E., and MCNUTT, S. H. (1941).—*J. Infect. Dis.* 69:18-28.

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