THE SEROLOGICAL EXAMINATION OF A STRAIN OF *TRICHOMONAS FOETUS* ISOLATED IN QUEENSLAND

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SUMMARY.

A strain of Trichomonas foetus **isolated in southern Queensland was compared with the two known serological types, Belfast and Manley, by the agglutination test and was found to be serologically similar to the Belfast type.**

INTRODUCTION.

The Belfast strain of *Trichomonas foetus* was first isolated by Kerr and Robertson (1941) in Northern Ireland and was found by Pierce (1949) to be well distributed in Wales and south and central England. The Manley strain of *T. foetus,* first isolated by Mahmoud (1944) from an outbreak at Manley in Cheshire, England, was confined to Wales and the central-west of England (Pierce 1949). These are the only known serological types.

Bovine trichomoniasis was first diagnosed in Queensland in 1950 (Sutherland, Simmons and Bell 1953). Since then the disease has been found in four other herds.

The serological comparison of a Queensland strain with the Manley and Belfast strains is recorded in this paper.

METHODS.

Strains.

The Belfast and Manley strains were kindly supplied by Dr. A. E. Pierce, Institute of Animal Physiology, Cambridge.

The Queensland strain, F228, was isolated from an experimental heifer that had been served 11 days previously by an infected bull. Vaginal mucus from this heifer was inoculated into modified Plastridge's medium (Sutherland *et al.* 1953) and the strain was maintained by subculturing twice weekly.

Media.

Three different media were used—modified Plastridge's medium, Douglas broth, and glucose broth on inspissated horse serum slope (see Appendix). In the last-mentioned medium, 2·5% glucose was incorporated for growth of the Belfast strain, whereas 1·5% glucose was found more suitable for cultivating the Manley strain.

Preparation of Antisera.

The suspensions for inoculation were prepared as follows:—

Belfast and Manley antigens were prepared from cultures in glucose broth on horse serum slopes. After incubation at 32°C. for 2–3 days the growth in glucose broth was harvested, centrifuged and washed. The suspension of these organisms in sterile normal saline was used as the inoculum.

Strain F228 grew well only in modified Plastridge's medium. As the agglutination was to be done with antigen containing traces of bovine serum, it was considered undesirable to inoculate rabbits with organisms in this medium, as antibodies to bovine serum would be produced. Bovine serum was therefore eliminated by passage through mice as follows: 2 ml. of a 7-day culture in modified Plastridge's medium was injected intraperitoneally into a mouse. Three days after inoculation, the mouse was killed, the peritoneal fluid harvested and diluted with three parts of saline, and this suspension inoculated into four more mice. The combined harvest of peritoneal fluid from these mice was further passaged into 12 mice. The pooled peritoneal fluid from these mice was centrifuged, and the deposit of organisms washed and re-suspended in saline to use as the inoculum.

Antiserum against each of the three strains was prepared in rabbits. Six intravenous injections were given over a period of 18 days, commencing with 0·5 ml. and increasing by 0·5 ml. to 2·5 ml. for each of the final two injections. The first injection was made with a living suspension. For subsequent injections the suspension was preserved with 0·5% formalin and kept at 4°C.

The rabbits were bled three times at intervals of two days commencing two days after the last injection, and the three lots of serum from each rabbit were pooled.

Agglutination Test.

This test was done by the method of Pierce (1947), using the readings for the different degrees of agglutination that were shown in his photomicrographs. This system of reading was also used by Kerr and Robertson (1941). The reactions range from a preagglutination zone characterised by immobilisation, which is represented by ++++++, through decreasing degrees down to slight agglutination, represented by (+).

To obtain well-grown cultures for the test, the Belfast strain was grown in Douglas medium, the Manley strain in 1·5% glucose broth on an inspissated horse serum slope and strain F228 in Douglas medium inoculated with growth from modified Plastridge's medium. These antigens were tested against antisera in double dilutions ranging from 1/24 to 1/3072. All the tests were done in the one day by the one person in order to eliminate inconsistencies in interpretation of results.

RESULTS.

The results are presented in Tables 1, 2 and 3. Belfast, Manley and F228 antigens were agglutinated by their homologous antisera to high titres, but gave low titres or no agglutination with normal rabbit serum.

The F228 suspension was agglutinated to a high titre by Belfast antiserum but only to a low titre by Manley antiserum (Table 1).

A slight cross reaction occurred between Manley antigen and Belfast antiserium. Manley antigen was not agglutinated by F228 antiserum (Table 2).

Table 1.

AGGLUTINATION OF F 228 ANTIGEN BY SERA PREPARED AGAINST BELFAST, MANLEY, AND F228 STRAINS OF *T. foetus*.

Antiserum.	Dilution of Serum.							
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072
F228	++++	+++	+++	++(+)	+	(+)	(+)	—
Belfast	+++	++(+)	+(+)	+	+	(+)	—	—
Manley	+	(+)	—	—	—	—	—	—
Control	—	—	—	—	—	—	—	—

Table 2.

AGGLUTINATION OF MANLEY ANTIGEN BY SERA PREPARED AGAINST BELFAST, MANLEY, AND F228 STRAINS OF *T. foetus*.

Antiserum.	Dilution of Serum.							
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072
F228	—	—	—	—	—	—	—	—
Belfast	+ (+)	+	(+)	—	—	—	—	—
Manley	++++	++(+)	++	++	+	(+)	—	—
Control	—	—	—	—	—	—	—	—

Table 3.

AGGLUTINATION OF BELFAST ANTIGEN BY SERA PREPARED AGAINST BELFAST, MANLEY, AND F228 STRAINS OF *T. foetus*.

Antiserum.	Dilutions of Serum.							
	1/24	1/48	1/96	1/192	1/384	1/768	1/1536	1/3072
F228	+++	++	++	++	+(+)	+	(+)	—
Belfast ..	++++	+++(+)	++(+)	++(+)	++(+)	++	+(+)	(+)
Manley ..	+(+)	+	+	+	(+)	—	—	—
Control ..	(+)	(+)	—	—	—	—	—	—

Belfast antigen was agglutinated by F228 antiserum to a high titre but gave only a slight cross reaction with Manley antiserum (Table 3).

DISCUSSION.

The results show that the strain of *T. foetus* isolated in Queensland is antigenically similar to the Belfast strain.

Four of the five herds in which trichomoniasis has been diagnosed in Queensland exchanged cattle directly and indirectly with the herd from which strain F228 was isolated. It is reasonable to assume that the infections in

these four herds were due to one antigenic type. The strain isolated from the fifth herd died before typing could be done. Until more strains have been isolated and typed it would be wise to use both Manley and Belfast types in the agglutination test for diagnosis of trichomoniasis in Queensland.

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APPENDIX.

Preparation of Glucose Broth on Inspissated Horse Serum.

Nutrient broth was prepared as follows:—

60 gm. Difco meat extract, 60 gm. sodium chloride and 120 gm. Bacto peptone were dissolved by heating in 12 litres of distilled water. The pH was adjusted to 7·2 with 1N sodium hydroxide, and 0·0125% calcium chloride was then added. 1·5% glucose was added to the medium for the Manley strain and 2·5% to the medium for the Belfast strain. The pH was adjusted to 7·2–7·4 and the broth autoclaved at 15 lb. for 15 minutes.

The broth was dispensed in 8 ml. quantities into 1 oz. McCartney bottles containing an inspissated horse serum slope. The slope was prepared by pipetting 5 ml. horse serum into the bottle and inspissating for one hour at 80–85°C. on each of three consecutive days. Paraffin oil to a depth of ¼ inch was layered onto the surface of the broth, the medium steamed for 20 minutes and then autoclaved at 15 lb. for 15 minutes.

Preparation of Douglas Medium.

The formula was obtained from the Animal Research Station, Wallaceville, New Zealand.

The medium consists of a digest broth covering an inspissated bovine serum slope.

The broth was prepared as follows:—

Ox heart, free from fat, was minced and mixed with tap water in proportion of 1 lb. meat to 1 litre water. The mixture was stirred and pH adjusted to 8·5 with 20% potassium hydroxide. After heating to 75°C., the mixture was cooled to 50°C., then, 1% Allen and Hanbury's Liquor Trypsin Co. added. The pH was restored to 8·5 with 20% potassium hydroxide, and digestion allowed for 1½ hours keeping the pH constant. At the end of this period a further 1% Liquor Trypsin Co. was added, and digestion continued for a further 1½ hours. The pH was adjusted to 5·5 with concentrated

hydrochloric acid, and the digest heated gently until boiling. The hot digest was filtered through gauze and filter paper, and to the filtrate was added 0·25% sodium chloride and 0·0125% calcium chloride. When dissolved, the pH was adjusted to 8·2 with 20% potassium hydroxide. After cooling, the digest was filtered through filter paper, 2·5% glucose added and pH adjusted to 7·2–7·4 with normal hydrochloric acid.

The slope was prepared by pipetting 5 ml. bovine serum into a 1 oz. McCartney bottle and inspissating at 80–85°C. for one hour on each of three consecutive days.

To prepare the medium, 10 ml. of broth was added to each bottle containing the slope. Liquid paraffin to a depth of ¼ inch was layered onto the surface of the broth. The medium was steamed for 20 minutes, then autoclaved at 15 lb. per square inch for 15 minutes. The pressure was reduced gradually to prevent violent boiling of the fluid portion of the medium.

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