

EXPERIMENTAL INFECTION OF CATTLE WITH TRITRICHOMONAS FOETUS var. BRISBANE

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SUMMARY

Inoculation of *T. foetus* var. *brisbane* by the vaginal route into five cows resulted in infection, and there was evidence of abortion having occurred in four of them. Attempts to infect seven bulls by a variety of techniques were successful in one bull only.

The pattern of infection of this serotype is similar to that previously described for other strains of *T. foetus*.

I. INTRODUCTION

A new serotype, *Tritrichomonas foetus* var. *brisbane*, was described by Elder (1964). The purpose of this investigation was to study the pathogenicity of this serotype in cows and bulls.

II. MATERIALS AND METHODS

Methods of inoculation and examination of animals were essentially those described by Simmons and Laws (1957). A 24 hr culture in modified Plastringe's medium was usually used for inoculation but occasionally the culture was centrifuged and the deposit resuspended in normal saline. Counts of organisms were made using a haemocytometer. Isolates used for inoculation and those recovered from experimental animals were serotyped by the method of Pierce (1947). Twelve animals previously shown to be free of trichomoniasis were used. Four 2-year-old Australian Illawarra Shorthorn (A.I.S.) bulls (1, 2, 3 and 5), one 8-year-old Hereford bull (4) and three A.I.S. cows (1, 2 and 3) were exposed to *T. foetus* var. *brisbane* subcultured between 82 and 227 times. Two 2-year-old A.I.S. bulls (6 and 7), an 18-month-old Hereford cow (4) and a 3-year-old Jersey crossbred cow (5), that had calved previously, were exposed to a strain of *T. foetus* var. *brisbane* that had not been subcultured.

TABLE 1
HISTORY OF ANIMALS EXPOSED TO *T. foetus* VAR. *brisbane*

Animal No.	Weeks after Start of Experiment*	Treatment	<i>T. foetus</i> Isolated*	Animal No.	Weeks after Start of Experiment*	Treatment	<i>T. foetus</i> Isolated*
Bull 1 ..	1	Sampled and yarded alone	Weeks 2 and 5	Cow 1 ..	1	Sampled and yarded alone	Weeks 5-16 inclusive, 31 and 40
	2	Inoculated intra prepuce, 7 million organisms			5	Inoculated intra vagina, 2 million organisms	
	20	Discarded from experiment			7	Yarded with bull 2	
Bull 2 ..	7	Sampled and yarded with cow 1 ..	Week 53	13	Probably pregnant		
	8	Served infected cow 1		15	Granular vaginal discharge, suspect abortion		
	13	Inoculated intra prepuce, 2 million organisms		28	Inoculated intra vagina, 1 million organisms		
	35	Yarded with and served cow 1		31	Inoculated intra uterus, 2 million organisms		
	40	Yarded with and served cow 1		35	Inoculated intra vagina, 4 million organisms; yarded with and served by bull 2		
	53	Inoculated intra prepuce, 4 million organisms		39	Separated from bull 2		
63	Discarded from experiment	40	Inoculated intra vagina, 4 million organisms				
Bull 3 ..	42	Sampled and yarded	Week 53	46	Pregnant 6 weeks		
	44	Served cow 2		63	Discarded from experiment		
	53	Inoculated intra prepuce, 4 million organisms		Cow 2 ..	39	Sampled and yarded	Weeks 40-50 inclusive
	56	Served cow 2			40	Inoculated intra uterus, 4 million organisms	
	96	Inoculated intra prepuce, 2 million organisms			44	Served by bull 3	
104	Discarded from experiment	50	Pregnancy test uncertain				
Bull 4 ..	65	Sampled and yarded	Weeks 68 and 69	55	Not pregnant		
	67	Inoculated intra prepuce, 3 million organisms		56	Served by bull 3		
	96	Inoculated intra prepuce, 2.4 million organisms		65	Pregnant 10 weeks		
	104	Discarded from experiment		70	Not pregnant		
				94	Inoculated intra uterus, 2 million organisms		
				104	Discarded from experiment		

Bull 5 ..	1	Sampled and yarded with cow 3	Week 15	Cow 3 ..	1	Sampled and yarded with bull 5 ..	Weeks 3-8 inclusive
	8	Served infected cow 3			3	Inoculated intra vagina, 2 million organisms	
	15	Inoculated intra prepuce, 4.8 million organisms			15	Discarded from experiment	
	22	Discarded from experiment					
Bull 6 ..	1	Sampled and yarded with cow 4, then inoculated intra prepuce 5 ml culture with 2 g sterile sand	Weeks 1-59 inclusive	Cow 4 ..	1	Sampled and yarded with bull 6, then inoculated 5 ml culture intra uterus	Weeks 1-10 inclusive and 12
	8	Served cow 4			8	Served by bull 6	
	11	Yarded with and served cow 5			12	Separated from bull 6	
	40	Yarded with cow 4			40	Again yarded with bull 6	
	59	Discarded from experiment			53	Not pregnant, discarded from experiment	
Bull 7 ..	3	Sampled and yarded with infected cow 4	Week 30	Cow 5 ..	9	Sampled and yarded	Weeks 11-16 inclusive
	28	Inoculated with preputial washings, from bull 6, mixed with sterile sand			11	Yarded with infected bull 6	
	46	Yarded with and served cow 5 immediately after service by infected bull 6			24	Pregnant 11½ weeks	
	59	Discarded from experiment			36	Not pregnant	
					46	Served by bulls 6 and 7	
					53	Discarded from experiment	

* A positive isolation for a particular week is recorded if one or more of the three samples taken during that week was positive, animals were not sampled less than 24 hr after exposure, all other samples were negative.

III. RESULTS

The results are summarized in Table 1.

All five cows became infected at first exposure and the initial infections persisted for 6 to 12 weeks. The four cows (1, 2, 4 and 5) re-exposed to infection showed a much reduced period of infection with a maximum of 2 weeks. Two cows (1 and 4) are considered to have aborted in early pregnancy and two others (2 and 5), pregnant 10 and 11½ weeks respectively, were non-pregnant shortly afterwards. No foetus was found. There were no notable changes in the characteristics of the vaginal mucus from infected cows, an exception being an interval following suspected abortion.

Bull 6 was successfully infected and carried the organism for the 59 weeks of the investigation. The organism was not isolated from the other six bulls for longer than 2 weeks after an exposure.

IV. DISCUSSION

T. foetus var. *brisbane* readily produced infection when the cows were first exposed and led to abortion or infertility. These results are similar to those obtained with other serotypes of *T. foetus* (Bartlett and Hammond 1945; Bartlett 1947; Simmons and Laws 1957). The duration of infection ranged from 6 to 12 weeks and was similar whether a recently isolated strain or a strain subcultured *in vitro* 82 or more times was used. Bartlett and Hammond (1945) and Murnane (1959) described similar periods of initial infection of 6–14 and 5–20 weeks respectively, while Simmons and Laws (1957) reported persistence of infection for 19 and 23 weeks. The failure of three heifers to carry the organisms for more than 2 weeks after re-exposure is in accord with observations using other serotypes (Bartlett 1947; Kerr and Robertson 1947; Simmons and Laws 1957; Murnane 1959).

Only one of seven bulls harboured the organism for any length of time and it may be significant that this bull was inoculated with a recently isolated strain. The persistence of infection in this bull was similar to that observed in bulls naturally infected with *T. foetus* var. *belfast* (Simmons and Laws 1957; Murnane 1959).

The difficulty experienced in infecting bulls either by direct inoculation or by placing them in contact with infected cows is similar to earlier reports (Hammond and Bartlett 1943; Murnane 1959; Gasparini, Vaghi, and Tardani 1963). However, Todorovic and McNutt (1967) successfully infected 4 out of 4 bulls using repeated inoculations of very large volumes of organisms (50 to 120 ml of culture containing up to 10^6 organisms/ml). In one bull the culture used had been maintained in Modified Plastridge's medium for more than 2 years by transferring it at 3- or 4-day intervals. McLoughlin (1968) successfully infected 11 bulls by massaging the scarified penis with culture while the animals were anaesthetized. There was no information given about the origin of the cultures or the dosage.

Murnane (1959) suggested that the poor infectivity could be due to the fact that strains had been passaged *in vitro* for long periods. This could be the case for bulls 1-5, but bull 7 did not become infected although it was mated with cow 4, which was infected with a recently isolated strain. Also, preputial washings containing motile trichomonads from bull 6 failed to infect bull 7. The addition of sterile sand or vaginal mucus to the inoculum did not result in infection in the bulls in which this was attempted. Hammond and Bartlett (1943) concluded that some bulls are resistant to infection by natural or artificial means and individual variations in resistance of bulls is probably more important than factors involving the source of infection.

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