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Corynebacterium pyogenes and bovine abortion

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SUMMARY

In the examination of bovine fetal material it was found that there was a significant increase in the proportion of mixed infections identified as the time between abortion and the collection of the samples increased. Examination of paired serum samples from abortions from which only *Corynebacterium pyogenes* was isolated revealed evidence of active infection in two-thirds, suggesting that *C. pyogenes* may have been acting as a primary abortifacient in these cases.

INTRODUCTION

The association of *Corynebacterium pyogenes* infection with bovine abortion is well known. The literature has been reviewed (Hinton, 1972). Abortion has been produced experimentally in both cattle (Reisinger, 1928; Johnson & Graham, 1945; Kolar, 1963) and sheep (Sørum, 1953; Smith, Reynolds, Clark & Milbury, 1971). The role of *C. pyogenes* as an abortifacient is not clearly understood though a possible pathogenesis has been suggested (Hinton, 1972).

Kolar (1963) isolated *C. pyogenes* from the fetus in 204 cases of abortion and he noted that the prognosis in these cases was often poor. Retention of the fetal membrane, either with or without purulent metritis occurred in 60 % and 45 % of cases respectively and many of these cows were subsequently sterile. A quarter of cows developed either fever, anorexia, metritis, pneumonia, mastitis or arthritis after abortion and were sent for emergency slaughter. In only a fifth of cases were there no apparent secondary complications.

Lovell (1939) found that though the serum of apparently normal cattle contained low titres of antibodies to *C. pyogenes* haemolysin (toxin) the titres were frequently raised in cases of mastitis or suppuration associated with *C. pyogenes* infection and were also raised in the only abortion examined. On the other hand in post-parturient endometritis, a condition in which *C. pyogenes* is usually considered to be a localized secondary and ascending infection, the antihaemolysin titres are rarely raised much above Lovell's normal titres (Dawson, 1951).

In view of Kolar's disturbing report it would seem that abortion associated with *C. pyogenes* infection merits further study. This paper gives the circumstances in which *C. pyogenes* was isolated from 100 such cases and also gives the results of examining sera using a serum antihaemolysin test.

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MATERIALS AND METHODS

Abortion cases

The 100 cases from which *C. pyogenes* was isolated were identified during the routine examination of bovine abortion material at the Carmarthen Veterinary Investigation Centre. *C. pyogenes* was isolated from either fetal membrane (placenta), fetal stomach or swab of vaginal mucus. The strains of *C. pyogenes* were identified on the basis of their colonial and morphological characteristics and their ability to produce beta-haemolysis and to liquify solid serum.

Paired serum samples were examined from 21 of these cases for antibodies to *C. pyogenes* haemolysin. In addition paired sera from 20 abortions which showed no evidence of *C. pyogenes* infection were screened by way of controls.

Haemolysin production

Haemolysin was produced by growing a subculture of *C. pyogenes* strain NCTC 5224 in litmus milk (Oxoid Ltd.) for 48 hr. at 37° C. The haemolysin (toxin) in the whey was titrated against 2 % rabbit erythrocytes (r.b.c.) and the volume adjusted to 50 MHD/ml. with CFT dilutent (Oxoid Ltd).

Serum haemolysin neutralization test

The serum was diluted in 0·5 ml. of the haemolysin solution and incubated for 1 hr. at 37° C.; 0·5 ml. 2 % rabbit r.b.c. in CFT dilutent were added and after a further 2 hr. incubation the wells were examined for haemolysis by placing the perspex trays over a diffuse light-source. The final dilutions ranged between 1/10 and 1/20480.

Both samples of a pair were tested together using the same batch of reagents. The serum antihaemolysin titre was taken as the reciprocal of that dilution of serum which when incubated with 25 MHD of haemolysin for 1 hr. prevented 50 % haemolysis. A significant change in titre has been taken to be at least fourfold either up or down.

RESULTS

The isolation of C. pyogenes *from bovine abortions*

The results obtained from the 100 cases is listed in Table 1. These show that as the time between the abortion and the collection of the specimens increases so does the chance that a mixed infection will be identified. This difference is significant ($P < 0{\cdot}001$).

The serum haemolysin neutralization test

The results of the serum haemolysin neutralization test in the 21 cases of abortion associated with *C. pyogenes* infection are listed in Table 2.

The cases can be divided into four groups on the basis of the serological response. In Group I (10 cases) there was a fourfold or more rise in serum antihaemolysin titre while in the three cases in Group II the titres fell by at least eightfold.

In the two cases in Group III there were high titres at both samplings while in

Table 1. *The isolation of* Corynebacterium pyogenes *and other pathogens from* 100 *bovine abortions which yielded* C. pyogenes *on culture*

Days after abortion*	No. of cases	Material cultured (No. positive for *C. pyogenes*/No. examined)			Cases with mixed infection			
		Fetus	Fetal membrane	Vaginal mucus	*Br. abortus*	Mycotic	*S. dublin*	Total (%)
1	31	8/8	24/24	7/9	0	1	0	1 (3)
2	25	1/3	24/24	3/3	2**	4	0	6 (24)
3–7	35	1/1	26/26	11/12	7	3	2	12 (34)
8–20	9	—	3/3	6/6	0	2	1	3 (33)
Total	100	10/12	77/77	27/30	9	10	3	22 (22)

* Day 1 = first 24 hr. after abortion.
** In one of these cases a mycotic infection was also demonstrated.

Table 2. *The antihaemolysin titre in* 21 *cases of bovine abortion associated with* Corynebacterium pyogenes *infection*

Group	Sample yielding *C. pyogenes* on culture at first examination*			Day of sampling**		Antihaemolysin titre		Change in titre		Other pathogens isolated
	FM	FS	VM	1st	2nd	1st	2nd			
I	+			1	25	40	320	+	8	
	+			1	14	80	320	+	4	
			+	1	14	160	640	+	4	
	+	+		1	20	1280	5120	+	4	
	+	+		1	23	1280	10240	+	8	
	+			2	15	10	80	+	8	
	+	−		2	21	160	5120	+	32	
	+			3	17	40	5120	+	128	
	+			3	19	640	2560	+	4	
	+		−	5	17	160	1280	+	8	
II	+			1	15	2560	40	−	64	
	+	+		4	17	10240	320	−	32	
	+			7	15	1280	160	−	8	*S. dublin*
III	+			5	19	5120	2560	−	2	
	+			5	20	1280	640	−	2	
IV	+			2	11	40	40		0	
	+			2	14	80	80		0	*A. fumigatus*
	+			4	17	10	10		0	*A. fumigatus*
			+	4	15	40	40		0	
			+	4	12	80	80		0	
	+			6	14	80	80		0	

* FM = fetal membrane. FS = fetal stomach. VM = vaginal mucus.
** Day 1 = first 24 hr. after abortion.

the six cases in Group IV there was no evidence of active infection with the titres remaining unchanged at $\leqslant 80$.

In 18 of the 20 control cases the titres were $\leqslant 80$ at both samplings and these showed no significant change. One of the other cases was classified in Group I

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because the titre rose from 40 to 160 while the last had titre of 640 at both samplings and was classed as Group III. The difference in the distribution of 'infected' and 'control' cases in the four groups is highly significant ($P < 0{\cdot}001$).

DISCUSSION

The examination of paired serum samples revealed that in two-thirds of cases in which *C. pyogenes* was the only significant bacterial or fungal isolate there was a significant change in anti-haemolysin titre. This indicates that the abortion and the infection were probably specifically associated and consequently *C. pyogenes* could have been acting as a primary abortifacient. However, it is appreciated that the interpretation of this serological test must be approached with caution because *C. pyogenes* is a common pyogenic organism of cattle and that the specificity of the test has not been fully assessed.

In addition, there was evidence that *C. pyogenes* may also act as a secondary invader in that the proportion of mixed infections increased significantly as the time between the abortion and the collection of the specimens increased, and because *C. pyogenes* may be isolated without serological evidence of infection, as occurred in four cases in Group IV.

Obviously further work needs to be done on this condition so that reliable diagnostic criteria can be established and the implications of Kolar's (1963) observations can be evaluated on a much wider scale.

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