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THE PERSISTENCE OF COLOSTRAL *ANAPLASMA* ANTIBODIES AND INCIDENCE OF *IN UTERO* TRANSMISSION OF *ANAPLASMA* INFECTIONS IN CALVES UNDER LABORATORY CONDITIONS

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ABSTRACT

POTGIETER, F. T. & VAN RENSBURG, L., 1987. The persistence of colostral *Anaplasma* antibodies and incidence of *in utero* transmission of *Anaplasma* infections in calves under laboratory conditions. *Onderstepoort Journal of Veterinary Research*, 54, 557–560 (1987).

Twenty-six calves, born from 25 *Anaplasma*-infected, intact and splenectomized cows, from a herd kept under strict tick-free laboratory conditions, were monitored for the presence of *Anaplasma* antibodies, using the rapid card agglutination test. Serum was collected at birth, weekly for 12 weeks, and then monthly for approximately 6 months. Specific antibodies passively acquired could be detected in calf sera for an average period of 8 weeks after birth. Calves that remained positive for longer than 12 weeks were suspected of having contracted *in utero* infections. Infection of the calves was confirmed by splenectomy. It was concluded that 4 calves in Group I contracted *in utero* infections. Two of the dams were chronically infected, whilst the other 2 underwent acute primary reactions during the 1st and 2nd trimesters of gestation, respectively.

Subsequently all calves born from infected cows in this tick-free herd were serologically screened before being splenectomized at an average age of 8 months. Out of 50 cows, 8 *in utero* infected calves were identified serologically and this finding was confirmed through splenectomy or subinoculation of blood.

Both *Anaplasma centrale* and *Anaplasma marginale* were carried transplacentally. Splenectomized and intact cows, chronically infected or undergoing primary reactions during the 1st, 2nd or 3rd trimester of gestation, produced infected calves. A 15,6 % incidence of *in utero* transmitted infections were observed amongst 77 calves under these conditions. None of the 13 splenectomized cows, undergoing primary *A. centrale* infections during gestation, aborted. Clinical signs of disease were not observed in any of the 12 *in utero* infected calves prior to splenectomy.

The implications of these findings are discussed.

INTRODUCTION

The widespread occurrence of a variety of ticks and tick-borne diseases in the Republic of South Africa (RSA) places severe constraints on the availability of cattle which are fully susceptible to these diseases. Such animals are needed for the production of live blood vaccines, the preparation of antigen and for experimental purposes. For this reason a breeding herd, consisting of approximately 100 animals accommodated in stables, has been maintained under strict tick-free conditions in the Section of Protozoology at this Institute for many years. Very strict supervision and quarantine measures are imposed on this herd. Production of babesiosis and anaplasmosis vaccines enjoy priority under the highly structured management system briefly indicated below.

The calves produced are splenectomized at an average age of 8 months. When these animals reach a mass of ± 350 kg they are infected and bled to produce a bivalent live blood vaccine. They are first infected with *Babesia bovis*, bled, treated with euflavine[1] and rested before being infected with *Babesia bigemina*. They are then treated with imidocarb diproprionate[2] 5 mg/kg i.m. to sterilize both *Babesia* infections. Three months later they are tested biologically by subinoculation of blood to susceptible splenectomized calves before being used for anaplasmosis (*A. centrale*) vaccine production.

Owing to the scarcity of animals of this particular status, a certain number of heifers, needed for breeding, must be used to make anaplasmosis vaccine. In this way, many of the female animals are either already carriers of *A. centrale* by the time they conceive or have to be infected during gestation.

This particular management situation provided an unique opportunity to study the persistence of passively acquired antibodies in the sera of calves and the incidence of *in utero* transmission of *Anaplasma* infections under controlled laboratory conditions.

[1] Euflavine B Vet C, Centaur Labs, Johannesburg

[2] Imizol, Coopers, SA.

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MATERIALS AND METHODS

Experimental animals

Seventy-five cows and 77 of their calves, mainly *Bos taurus*, cross-bred animals, were used in this investigation. The cows were either born and reared under strict tick-free conditions at this institute or purchased from pre-selected farms on condition that they have been screened for infectious reproductive diseases, bovine enzootic leucosis, *Babesia, Theileria* and *Anaplasma* infections, using serological tests and the microscopic examination of blood smears.

Group I. Twenty-five cows and 26 of their calves were first studied as a separate group. Eleven of the 12 cows had mixed infections. They were inoculated with *A. centrale* vaccine and challenged with *A. marginale* as reported in a previous study (Potgieter & Van Rensburg, 1983). One cow was infected with *A. centrale* only (Table 2). The thirteen other cows were all splenectomized and infected with *A. centrale*.

Group II. Fifty cows and 51 of their calves, from the rest of the herd, were monitored for *in utero* transmission. Fourty-eight of these cows were splenectomized, ex *A. centrale* vaccine donors. The other two were intact cows which contracted natural field infections of *A. marginale* prior to their introduction into the herd.

Monitoring of pregnant cows and their calves

Rectal temperatures of all animals were recorded daily. Thin blood smears were prepared from the tip of the tail, fixed in methanol, stained in 10 % Giemsa for 35 min and examined regularly. Blood smears of chronically infected animals were examined weekly or fortnightly, but those of newly infected animals and splenectomized calves, daily. Regular fly control was practised with permethrin[3].

Blood was collected in 20 % ACD (citric acid, sodium citrate, dextrose) anticoagulant for subinoculation purposes in biological tests. Depending on the size of the

[3] Stomoxin, Coopers, SA

TABLE 1 Serology (CAT) and blood smear examinations of calves born from splenectomized cows showing chronic and acute *A. centrale* infections

Calf No.	Serology (CAT) Before colostrum	Serology (CAT) Seropositivity (weeks)	Splenectomy age (weeks)	*In utero* infection
469	ND[2]	10	22	–
487	–	10	40	–
488	–	9	40	–
499	ND	12	36	–
631	–	7	34	–
625	ND	24	33	*A. centrale*
588[1]	+	21	29	*A. centrale*
623	ND	6	ND	ND
624	ND	12	ND	ND
619	–	11	36	–
605	ND	8	33	–
630[1]	+	25	40	*A. centrale*
609	–	5	31	–
		Negative controls		
419	ND	–	22	–
423	ND	–	22	–
474	–	–	40	–
476	–	–	40	–
589	–	–	ND	ND

[1] *Anaplasma* organisms observed in routine blood smears prior to splenectomy

[2] ND = not done
+ = positive
– = negative

TABLE 2 Serology (CAT) and blood smear examinations of calves born from intact cows chronically infected with *A. centrale* and *A. marginale*

Calf No.	Serology (CAT) Before colostrum	Serology (CAT) Seropositivity (weeks)	Splenectomy age (weeks)	*In utero* infection
406	ND[3]	4	24	–
412[1]	ND	24	30	*A. marginale*
416	–	12	29	–
440	ND	11	34	–
441	ND	4	ND	ND
468	ND	5	44	–
483	ND	5	ND	ND
471	ND	9	43	–
473	ND	10	41	–
475	ND	10	32	–
490	ND	4	39	–
494	ND	4	ND	ND
592[2]	ND	8	29	–
		Negative controls		
417	ND	–	24	–
418	ND	–	24	–

[1] *Anaplasma* organisms observed in routine blood smears prior to splenectomy

[2] Dam infected with *A. centrale* only

[3] ND = not done

animal tested, 500–1 000 mℓ of blood was transfused to a susceptible splenectomized calf.

The card agglutination test (CAT) described by Amerault & Roby (1968) was used in kit form[4] to demonstrate the presence of *Anaplasma* antibodies in the serum of all the experimental animals.

Experimental procedure

Since the majority of the cows had been artificially inseminated, calving dates could be determined accurately, and this facilitated attempts to collect sera of newborn calves prior to colostrum intake. Serum samples were collected from 25 dams and their 26 calves (Group I) weekly for the first 12 weeks of the calves' lives and monthly thereafter for the duration of this investigation. All these calves, except 5 which were used elsewhere, were splenectomized between 22 and 43 weeks of age.

Seven non-infected cows and their calves in the breeding herd that shared the same facilities were monitored as negative controls during this investigation.

Subsequently all calves born from *Anaplasma*-infected dams in this herd were routinely screened serologically to identify cases of possible *in utero* infections. The 51 calves born from a total of 50 cows (Group II)

TABLE 3 *In utero* infections of calves born from splenectomized cows undergoing primary *A. centrale* reactions during gestation

Cow No.	Trimester	Max. parasitaemia %	*In utero* infection
326*	3	18	—
121	2	36	—
451	2	8	*A. centrale*
145	3	18	—
085	3	31	—
184	3	21	—
215	2	3	—
142	3	14	—
219	3	23	—
135	2	27	*A. centrale*
223	1	29	*A. centrale*
243	2	23	—
510	3	43	*A. centrale*

* Non-splenectomized

[4] Anaplasmosis card test, Hynson, Westcott & Dunning, Baltimore, Maryland, USA.

TABLE 4 Summary of the results obtained from all *in utero Anaplasma* infections during this investigation

Group of cows	Cow No.	Status of infection during gestation	Calf No.	Infection
Group I (n = 25)	161 (S)	Chronic	412 (S)	*A. marginale*
	135	Acute/2nd trimester	630 (S)	*A. centrale*
	085 (S)	Chronic	625 (S)	*A. centrale*
	223 (S)	Acute/1st trimester	588 (S)	*A. centrale*
Group II (n = 50)	363 (S)	Chronic	635 (S)	*A. centrale*
	338	Chronic	638 (S)	*A. marginale*
	451 (S)	Acute/2nd trimester	650 (S)	*A. centrale*
	216 (S)	Chronic	652*	*A. centrale*
	228 (S)	Chronic	659*	*A. centrale*
	272	Chronic	663 (S)	*A. marginale*
	510 (S)	Acute/3rd trimester	686*	*A. centrale*
	243 (S)	Chronic	690*	*A. centrale*

S = splenectomized
* Infection confirmed through subinoculation of blood

were tested with the CAT just prior to splenectomy at approximately 6 months of age. The calves that tested serologically positive were either splenectomized or biologically tested, as described above, to confirm the serological findings.

RESULTS

The results are presented in Tables 1–4.

Group I

Twenty-six calves from the 25 cows in Group I were monitored. Pre-colostral serum was collected from only 8 new-born calves. Two of these calves tested serologically positive (Tables 1 & 2) and it was later confirmed that both had contracted *in utero* infections. A total of 4 calves (15,4 %) out of the 26 that tested positive throughout this study were proved to be infected transplacentally (Tables 1, 2 & 4). Colostral antibodies were detected in the sera of all the 22 non-infected calves monitored. Antibodies were detected for as long as 12 weeks after birth, but some calves already tested negative after 5 weeks (Tables 1 & 2). On average the 22 calves tested positive for the first 8 weeks of their lives. Three of these sero-negative calves were not splenectomized, assumed to be negative, and used in other experiments.

Anaplasma organisms were observed in routine blood smears of 3 *in utero*-infected calves before they were splenectomized (Tables 1 & 2). The parasitaemias of 2 calves were low and comparable with those of chronically infected carrier animals. The maximum parasitaemia observed in the 3rd calf was 1,6 %. No febrile reactions or other clinical signs of anaplasmosis were seen in any of the *in utero*-infected calves prior to splenectomy.

To prevent possible mechanical transmission of *Anaplasma* infection by *Stomoxys calcitrans*, fly control was routinely practised. The 7 negative control cows and their calves were accommodated in the same stable complex and had direct social contact with the infected animals. These were considered 2 reasonable measures to monitor mechanical transmission. Overall, approximately 2/3 of the entire herd of 300 cattle had been splenectomized and were fully susceptible to anaplasmosis. They were very closely supervized as described previously, and no accidental or uncontrolled infections occurred before or during this investigation.

Group II

Eight out of 51 (15,7 %) calves produced by 50 infected cows in the rest of the herd contracted *in utero* infections (Table 4). Some of the calves, found to be sero-positive, were not splenectomized but were tested biologically, as indicated in Table 4.

Out of the 13 calves tested from cows that had undergone primary *A. centrale* reactions during different trimesters of gestation, 4 (30,8 %) contracted *in utero* infections, as indicated in Table 3. None of the cows aborted.

Eight (12,5 %) positive cases resulted from 64 cows in the herd with chronic infections (carriers). Cows 085 and 243 both underwent primary reactions during the 3rd trimester, but the calves were not infected (Table 3). However, after becoming carriers, their next calves were both infected (Table 4). These were the only cows of which 2 calves each were tested.

A total of 75 infected cows and their 77 calves were screened in this study. Twelve (15,6 %) of these calves contracted *in utero Anaplasma* infections.

DISCUSSION

Useful information was obtained during this investigation. Unlike most similar studies, a much larger number of animals, namely 75 infected dams and 77 of their calves, were monitored in this investigation in stables under tick-free conditions.

The information obtained regarding the persistence of detectable maternal antibody in calf serum must be taken into consideration if the CAT is used in epidemiological studies to determine the transmission rate of anaplasmosis in young calves.

Trueblood, Swift & Bear (1971), using the complement fixation test, was able to demonstrate *Anaplasma* antibodies in a foetus. It was interesting to note that in the present study pre-colostral sera of 2 *in utero*-infected calves tested positive. The CAT apparently detected the new-born calves' own *Anaplasma* antibodies.

Basing his arguments on earlier observations, Ristic (1960) concluded that *in utero* transmission is infrequent and apparently unimportant in the spread of anaplasmosis. This opinion is also shared by Paull, Parker, Wilson & Campbell (1980). However, the relatively high incidence of *in utero* infection in 15,6 % of the animals under the specific management conditions studied here indicates that this mode of transmission of the infection must be considered important, especially when the transmission rate of the infection is correlated with vector activity and/or mechanical transmission in epidemiological studies.

Transplacental transmission would also certainly have to be considered an important factor in anaplasmosis eradication schemes directed at vector control only.

As was to be expected, a much higher incidence of transplacental infections (30,8 %) occurred amongst calves born from cows undergoing acute primary *A. centrale* reactions, compared with the 12,8 % of carrier dams. It has been shown by earlier workers that *in utero* infections occurred when dams were infected in the 3rd

month of gestation (Dykstra, Roderick, Farley, McMahan & Splitter, 1948, cited by Ristic, 1960) or recovered from an acute infection during the middle of the 2nd trimester (Zaugg & Kuttler, 1984). Other studies indicate that experimental infection of cows during the 3rd trimester of gestation led to *in utero* infection, fetal death and abortions (Fowler & Swift, 1975). More recently Zaugg (1985) specifically investigated transplacental transmission as it relates to the stage of gestation, and found that foetal infection took place during the 2nd and 3rd trimesters. Swift & Paumer (1976) showed vertical transmission in the last trimester and they believed that acute infection during gestation may be a requirement for transplacental transmission. The results obtained in the current study indicate that *A. centrale* was transmitted *in utero* to calves of splenectomized dams that underwent acute reactions during all 3 trimesters of gestation. However, it was not determined at what stage of gestation foetal infection took place. It is interesting to note that 2 cows which underwent primary *Anaplasma* reactions during gestation delivered normal non-infected calves. However, both cows, as carriers of the infection, subsequently gave birth to *in utero*-infected calves.

The fact that none of the *in utero*-infected calves developed any noticeable clinical signs of anaplasmosis indicates that this phenomenon probably escapes observation and recognition as a significant mode of transmission of *Anaplasma* infections in the field.

The absence of abortions and neonatal anaplasmosis of *in utero*-infected calves seen in this study are contrary to most reported cases of *in utero* transmission of *Anaplasma*. Paull *et al.*, (1980) found 2 out of 283 calves with non-clinical infections, whereas most other reports deal with neonatal anaplasmosis (Wandera & Munyua, 1971; Bird, 1973; Paine & Miller, 1977; Norton, Parker & Forbes-Faulkner, 1983) and abortions (Fowler *et al.*, 1975; Correa, Correa & Gottschalk, 1978).

It is not clear how certain foetuses can cope with *Anaplasma* infection, whilst others are severely affected. Trueblood *et al.* (1971) made a tentative conclusion that a 100–140-day-old foetus is capable of producing anti-*Anaplasma* complement fixing antibodies. The exact mechanisms whereby the young, including the foetus, are less susceptible to clinical effects of infection have yet to be determined.

From an epidemiological point of view, it would be interesting to know how well these inapparant *in utero*-infected animals would be protected against natural field challenge. It is believed that a 15,6 % incidence of vertical transmission could play a significant role in the epidemiology of this disease.

The results obtained in this investigation have led us to avoid whenever possible, breeding with *Anaplasma*-infected cows in this particular herd. Several attempts to sterilize *Anaplasma* carriers with different treatment regimes of oxytetracycline as an alternative have so far produced inconsistent results in this laboratory (Potgieter, unpublished observations, 1984). The expense of biological test procedures to confirm successful sterilization has also limited our efforts in this regard.

Serological screening of calves prior to splenectomy, at approximately 6 months of age, now provides a cheap and effective method for the timely identification of *in utero*-infected calves.

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