ALTERATIONS IN THE SEMEN OF DOGS EXPERIMENTALLY INFECTED WITH BRUCELLA CANIS

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SUMMARY: Semen alterations of 2 mongrel, adult dogs, experimentally infected with Brucella canis (ES 11/78), were studied, during a period fo 12 weeks. Volume, motility, concentration and spermatic pathology were observed. The latter was estimated by two methods: the William's stain and the glutaraldehyde 0.2% in differential interference contrast microscopy. Semen volume didn't show significant alteration, while spermatic concentration showed a moderated oscilation, specially, a decrease in the 7th week after inoculation. It was also observed a reduction in the sperm motility. Among the morphologic alterations of spermatozoa, midle piece defects, coiled tails, proximal and distal droplets, head agglutination, and also the presence of inflamatory cells, such as neutrophiles and macrophages, were noted.

UNITERMS: Brucella canis* ; Dogs* ; Semen*

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INTRODUCTION

Scrotal dermatitis, epididymitis, prostatitis, and, less frequently, orchitis are the clinical forms of brucelic infection in males (CARMICHAEL & KENNEY[3], 1968; MOORE & KAKUK[9], 1969; GLEISER et alii[6], 1971; CARMICHAEL & GEORGE[2], 1976; GEORGE et alii[5], 1979). If there is a testicular injury, it seems that it is a consequence of the direct action of **Brucella canis** in the testes which determines some disturbances in spermatogenesis, such as the production of a qualitavely modified semen, leading frequently to sterility (CARMICHAEL & GEORGE[2], 1976; GEORGE et alii[5], 1979).

According to CARMICHAEL & KENNEY[4] (1970), the bacteremia, in dogs infected with **Brucella canis**, begins 1 to 3 weeks after the exposition to the infectious agent persisting, sometimes, for many months. Their effects on the seminal picture are clinically observed through sterility. (MOORE & KAKUK[9], 1969; CARMICHAEL & GEORGE[2], 1976; GEORGE et alii[5], 1979)

The purpose of this study is to characterize possible seminal alterations, during a period of 12 weeks, in male dogs experimentally infected with **Brucella canis.**

MATERIAL AND METHODS

Four mongrel, adult and healthy dogs were utilized in this study; they were selected both clinically, and by laboratory tests (hemogram and urinalyses). The animals were free of **Brucella canis**, indeed, they were not reagent not only to the plate agglutination* (PA) but also to the tube agglutination test (TA), being also negative to blood culture, according to methods previously described.. (LARSSON & COSTA[8], 1980)

The dogs randomly divided into two groups (control and experimental) were identified by numbers: 222, 224, 225 and 226. Before starting the experiment, the animals were immunized against Distemper and Canine Infections Hepatitis, and also received an anti-helmintic therapy (Mebendazole: 30mg/kg/day for 3 days).

The dogs were kept, individually, for a period of 30 days, before inoculation. After this time, they were reexamined through serologic tests (PA and TA) and blood culture. Dogs number 225 and 226 were orally inoculated with a suspension of **Brucella canis** sample ES 11/78.. (LARSSON[7], 1979)

After inoculation, and during a period of 12 weeks, all dogs were examined, weekly; for this, serologic tests (PA and TA), hemoculture and spermogram were performed.

The semen, collected by massage of the penis, was immediately after evaluated for physicochemical characteristics, i. e., volume, motility, and sperm cell concentration.

The spermatic morphology (BLOM[1], 1973) was evaluated by the William's stain (becoming possible the visualization of spermatozoa head defects, through optic microscopy), and the glutaraldehyde 0.2% wet preparation (detailing the structures of acrosomes, midle pieces and

spermatozoa tails, through differencial contrast phase microscopy – DIC). In both methods, 200 cells were considered.

At the end of the experimental period the animals were euthanized and necropsied; thus, fragments of lymph nodes, spleen, epididymes, prostate and testes were obtained, exposed to H.E. stain and submmited to histopathology.

RESULTS

The control dogs didn't show serological and bacteriological evidences of **Brucella canis** infection. Further histopathology nor spermogram revealled alterations in these animals.

The hemocultures of the experimental dogs were also negative; nevertheless they became seropositive being the agglutinins detectable by PA and TA. Thus, it was possible to detect antibodies against **Brucella canis** (titer 100 or more), in both males, three weeks after inoculation. These titers persisted until the 7th and 8th week after inoculation in dogs 225 and 226, respectively.

The histopathology of experimentally infected dogs showed prominent follicular hiperplasia of both spleen and lymphy nodes; epididymitis and prostatitis were also detected.

Semen picture of the inoculated males are shown in Tab. 1, 2 and 3.

The spermatic concentration of males 225 and 226 varied from 320,000 to 960,000/mm^3 and from 320,000 to 1120,000/mm^3, respectively. Furthermore, in relation to sperm motility, the smallest value obtained was 30%, in both animals, being the highest values 80% and 70%, for males 225 and 226, respectively.

Concerning the sperm pathology of the experimentally infected dogs it was possible to observe swollen midpieces, being this alteration very frequent, i.e., 65% in male 225 and 25% in the other one (226). Among the tail defects, proximal and distal droplets were noted; (Fig. 2) 41,5% and 48,5% in males 225 and 226, respectively. In addition, it was also found head-to-head agglutination (Fig. 1) and a considerable number of inflamatory cells, such as neutrophiles and macrophages.

TABLE 1 – Characteristics of the semen of male dogs experimentally infected with **Brucella canis** (ES 11/78), São Paulo, 1982.

Animals	Time (Weeks)	Characteristics		
		Vol/ml	Motility %	Concentration mm^3
225	1	1.0	70	480,000
	2	0.8	80	880,000
	3	1.0	60	960,000
	4	0.5	60	475,000
	5	0.5	60	433,300
	6	0.5	30	408,000
	7	1.0	60	320,000
	8	1.5	70	832,000
	9	1.5	60	450,400
	10	3.0	40	496,000
	11	1.0	50	340,000
	12	1.5	70	320,000
	X	1.15	59.16	532,900
226	1	2.0	70	720,000
	2	2.0	70	1120,000
	3	2.0	70	1040,000
	4	1.5	30	840,000
	5	2.5	70	540,000
	6	1.0	60	480,000
	7	3.0	60	120,000
	8	4.0	70	520,000
	9	2.5	70	383,000
	10	3.0	70	340,000
	11	3.0	70	304,800
	12	2.0	70	424,000
	X	2.37	65	569,310

TABLE 2 – Spermatic pathology detected through the William's stain method, in male dogs experimentally infected with **Brucella canis** (ES 11/78), São Paulo, 1982.

Animals	Time (Weeks)	William's stain					
		Normal	Narrow at the base	Pearshaped head Abnormal head Abnormal free head	Underveloped double forms	Giant Short head Broad head	Small normal heads Abaxial. I.
225	1	183	3	1	–	6	2
	2	193	–	–	–	5	2
	3	195	–	–	–	1	4
	4	195	1	–	–	4	–
	5	200	–	–	–	–	–
	6	199	–	–	–	–	1
	7	198	–	–	–	1	1
	8	196	–	–	–	1	3
	9	196	–	–	–	2	2
	10	195	–	–	2	1	2
	11	190	–	–	–	5	5
	12	198	–	1	–	1	–
	$\overline{X}$	194.8	0.33	0.17	0.17	2.25	1.83
226	1	195	–	–	–	2	3
	2	192	–	–	–	7	1
	3	196	–	1	–	2	1
	4	199	–	–	–	1	–
	5	199	–	–	–	1	–
	6	199	–	–	–	1	–
	7	199	–	–	–	1	–
	8	199	–	–	–	–	1
	9	199	–	–	–	1	–
	10	194	–	2	–	3	1
	11	193	2	3	–	2	–
	12	198	–	–	–	1	1
	$\overline{X}$	196.8	0.16	0.5	–	1.83	0.67

TABLE 3 – Spermatic pathology detected through glutaraldehyde 0.2% wet preparation, in male dogs experimentally infected with **Brucella canis** (ES 11/78), São Paulo, 1982.

Animals	Time (Weeks)	Glutaraldehyde				
		Normal	Acrosomal defects Pouch formation Detached normal heads	Midle piece defects	Bent and coiled tails Proximal droplets	Distal droplets
225	1	189	1	4	4	2
	2	200	–	–	–	–
	3	200	–	–	–	–
	4	195	5	–	–	–
	5	175	–	3	–	22
	6	190	–	1	5	4
	7	188	–	1	4	7
	8	194	–	1	–	5
	9	196	1	–	–	3
	10	188	–	1	4	7
	11	183	6	1	4	8
	12	193	–	1	3	3
	$\bar{X}$	190.9	1.08	1.08	2.0	5.08
226	1	171	1	12	10	6
	2	187	–	7	2	4
	3	179	3	5	10	3
	4	196	–	1	1	2
	5	198	–	1	–	1
	6	194	–	–	6	–
	7	195	–	1	2	2
	8	190	–	1	9	–
	9	191	–	1	3	5
	10	177	1	13	3	6
	11	184	–	7	6	3
	12	193	–	–	1	6
	$\bar{X}$	187.9	0.42	4.08	4.42	3.17

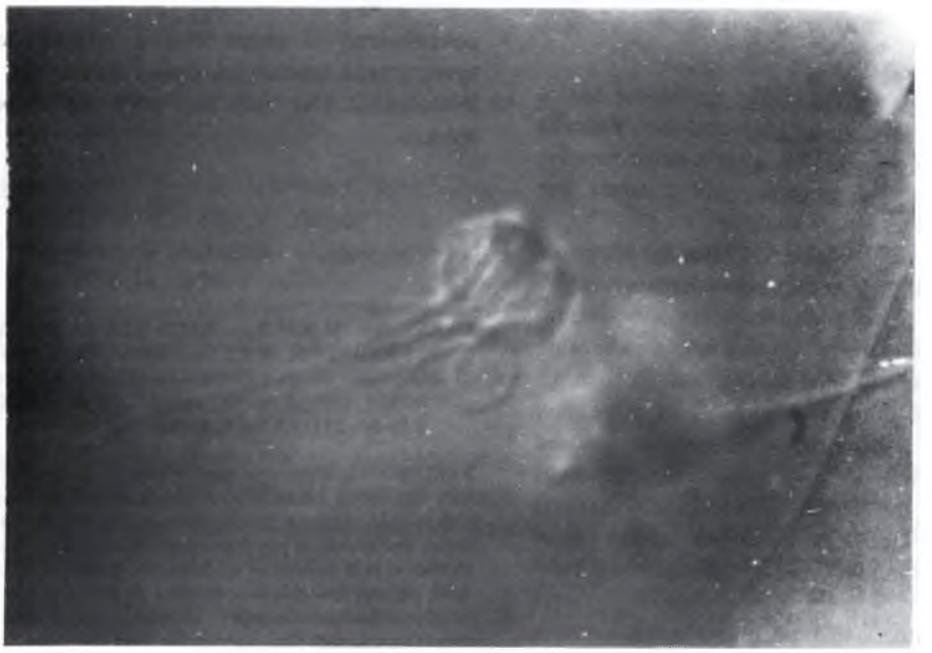

FIGURE 1 – Spermatozoa with macrofages
aument of 1,250x

FIGURE 2 – Spermatozoa with citoplasmatic droplets
and spermatozoa with coiled tail
aument of 1,250x

DISCUSSION

Epididymitis, with swelling of the epididyme tail, is one of the earlier alterations in dogs infected with **Brucella canis** (CARMICHAEL & GEORGE[2], 1976); consequently, an increased percentage of abnormal spermatozoa are observed in the ejaculate of sick animals.

GEORGE et alii[5] (1979) observed that the sperm of experimentally infected dogs presented inflamatory cells, specially neutrophiles 5 to 6 weeks after inoculation. According to these authors the sperm abnormalities became more prominent 8 weeks after the inoculation; further, 15 weeks after, they noted the major percentages of swollen midpieces, coiled tails, inflamatory cells, macrophages and head-to-head agglutination of spermatozoa; these findings were also observed in the present study. In this respect, SERIKAWA et alii[10] (1981) described sperm head agglutinations in the urine of males, from 11th to 19th weeks after inoculation.

Injuries of spermatozoa may occur in brucelic orchitis and epididymitis, a fact that leads to a decrease in their motility, 8 weeks after the inoculation (GEORGE et alii[5], 1979). In our study, this fact was observed, only in the 6th, 10th and 11th weeks after inoculation for male 225 and in the 4th week after inoculation for male 226.

In a similar study, GEORGE et alii[5] (1979) observed a significant reduction in the volume of ejaculate, 5 weeks after inoculation; in addition, it was noted that when stimulated, the inoculated dogs presented a painful ejaculation; although ejaculation sometimes failled, the animals usually maintained their normal libido. Similar findings were observed in our study, exception made to the decrease in semen's volume.

It was not possible to find in the literature data concerning to spermatic concentration of dogs infected with **Brucella canis**; in this aspect our results were irregular; nevertheless, it seems that a relative decrease of the spermatozoa number/ml does occur in the 7th week after inoculation; this fact was observed in both inoculated animals.

LARSSON, M.H.M.A.; BARNABÉ, V.H.; VISINTIN, J.A.; FERNANDES, W.R.; GUERRA, J.L.; COSTA, E.O. Alterações do quadro espermático de cães infectados experimentalmente com **Brucella canis**. **Rev.Fac.Med.vet.Zootec.Univ. S.Paulo**, 21(1):57-63, 1984.

RESUMO: Estudou-se o quadro espermático de 2 cães adultos, sem raça definida, infectados experimentalmente com **Brucella canis** (ES 11/78), durante um período de 12 semanas. Observaram-se volume, motilidade e patologia espermática. Esta foi avaliada por dois métodos: pela coloração de William e pela técnica do glutaraldeído a 0,2% em microscopia de contraste de interferência diferencial. O volume seminal não mostrou alteração significante, enquanto que a concentração espermática revelou uma oscilação moderada, especialmente uma diminuição na 7a. semana após a inoculação. Observou-se, também, uma redução na motilidade espermática. Dentre as alterações morfológicas dos espermatozóides, observaram-se defeitos de peça intermediária, cauda enrolada, gotas citoplasmáticas proximal e distal, aglutinação de cabeça, assim como a presença de células inflamatórias, tais como neutrófilos e macrófagos.

UNITERMOS: **Brucella canis***; Sêmen* ; Cães*

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