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Original article

Influence of gastrointestinal trichostrongylidosis on ram fertility

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Abstract

A study on the influence of gastrointestinal trichostrongyles on ram fertility was performed. Two groups of semen donor rams (Sarda breed) were utilized; one was experimentally infected with *Haemonchus contortus*, *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* and the other was used as a control group. In all animals, coprological, haematological and reproductive parameters were studied. The results suggest that the parasites had a limited effect with some changes in phosphorus, cholesterol and chlorine levels. In our experience the parasitic burden produced no significant change on ram fertility.

Key words: ram, gastrointestinal trichostrongyles, fertility

Introduction

The presence of parasites is able to reduce productive performance in terms of milk, meat, wool and weight gain. These reductions are seemingly easily observed by the breeder but are very difficult to demonstrate in the field with a scientific approach (Ambrosi 1995). However, these interferences for female sheep or for lambs were validated (Ambrosi 1995, Gatongi et al. 1997, Thompson et al. 2000, Fthenakis et al. 2001), information on rams was lacking; in fact in males the pathogenic effects are mainly referred to a direct action on the reproductive system. Studies focused on the effects of antiparasitic drugs that cause a transitory depression of fertility (Kayum et al. 1992, Kumar et al. 1992, Raisinghani 1992) suggest avoiding the treatment of camels during the mating season (Al-Quarawi et al. 2004). However, specific surveys on the indirect influence of parasites on the reproductive system of rams were lacking. Indeed this kind of interference on reproductive system may be more complex and variable as in *Fasciola hepatica* and *Schistosoma* spp. invasions that may affect ram fertility via enzymes, such as enolase (Tsantarliotou et al. 2008).

Few studies highlight the importance of some vitamins (A, E, C) and minerals (Se, Zn, I) in the function of the reproductive system. Helminths infection may reduce absorption and, consequently, levels of vitamins or minerals in the organism. In the case of

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vitamin A deficiency in rams, plasmin synthesis during spermatogenesis is reduced, probably due to the regulation of gene expression, although other, indirect mechanisms cannot be excluded (Zervos et al. 2005). The aim of our study was to investigate on the consequences of gastrointestinal trichostrongyles invasion ram fertility expressed as semen quality in sheep of Sarda breed, present in Italy in a number of 3 million heads (ISTAT data 2008), and never investigated in this respect. This study may be of greater interest nowadays as modern sheep breeding has high hygiene levels that reduce the opportunity to observe clinical evidence of gastrointestinal trichostrongylosis, mainly where the practice of artificial insemination and/or the use of highly selected rams may heavily influence the future economic yield of this kind of zootechnical enterprise.

Materials and Methods

Animals

Twenty rams of "Sarda breed", all of the same origin and within the age range of 2.5-3 years were used. All rams were negative for gastrointestinal parasites and were stabulated in "Centro Genetico dell'Istituto Zootecnico-Caseario di Bonassai", Sardinia, Italy. The rams used for this purpose could not be younger as they were intended for semen collection. The rams need adequate body conditions and have to be trained to use the dummy and artificial vagina, as in similar experiment (Tanyildizi and Türk 2004) even using the electroejaculation (Aire et al. 2001). Before enrollment to the study, all rams were out to pasture, stabled during the night, with irregular administration of commercial concentrates; once a year an anthelminthic drug (benzimidazole) was administered. Thirty and fifteen days (d-30 and d-15) before the challenge, all animals were submitted to single coprological examination by Baermann technique to search for lung parasites (MAFF 1986), as well as by sedimentation and levitation (MAFF 1986) to check for gastrointestinal roundworm, flatworm and coccidian infections; this technique is more sensitive than McMaster technique (Taylor et al. 2007). Only a weak presence of strongyle-type eggs was observed. The rams were treated with subcutaneous administration of ivermectin (200 mcg/Kg) at d-30 and then permanently stabled. To avoid infection by natural parasites the diet was composed of hay (from land without flocks out to pasture) and commercial concentrate. During the period of the trial, the litter and faeces were removed every day. Inclusion criteria were represented by good nutritional status and the absence of clinical signs of disease. Two homogeneous groups, infected (I) and control (C), composed of 10 rams each have been randomly formed. Each individual included was identified by a protocol number (1 to 20). The homogeneity of starting weights and haematological parameters was tested by the t-test for independent samples. Ten rams at day 0, were infected twice per week for 2 months and 10 rams were kept uninfected and considered as control group. The two groups were maintained in separate boxes and stabled under identical conditions.

Parasites

Each oral infection, was composed of *Haemonchus contortus* (2000 L_3), *Trichostrongylus colubriformis* (2000 L_3) and *Teladorsagia circumcincta* (3500 L_3). According to the Institute National de la Recherche Agronomique (INRA) of Nouzilly the inoculum should simulate a natural infection acquired during the pasture. The nematodes choice was made considering parasitic species with higher frequences observed in Sardinia (Scala et al. 1993). In detail, the larvae of *H. contortus* were isolated in the west of France. The larvae of *T. colubriformis* were isolated in Weibridge (UK). The larvae of *T.* circumcincta were isolated in the south-west of France. All three strains were separately cultivated by lamb invasion in the INRA experimental station and sent to Sardinia. The dosing were prepared one day before infection and maintained at room temperature.

Animal weight

Each ram was weighted 5 times a month starting 2 weeks before the first invasion.

Coprological examination

Faecal samples for quantitative coprological examination were collected weekly for 16 times starting twenty days after the first invasion, considered as prepatent period (Taylor et al. 2007). The samples were collected from rectum and faecal egg count was estimated using a modified McMaster technique (MAFF 1986) with a sensitivity of 15 eggs per gram (EPG) using NaCl flotation solution (density 1.2).

Blood samples

The blood samples were taken monthly for 5 times starting 2 weeks before the first invasion. In detail, blood cells counts (RBC, WBC, Hb, MCV, MCH, MCHC, Plt) were obteined and in the serum Ca, P, Mg, Cl, Fe, Na, K levels were asseyed (Zedda et al. 1996).

Semen evaluation

The semen was collected weekly 20 times starting 2 weeks before invasion. Semen was collected once a day between 8:00 and 10:00 A.M. by artificial vagina and after the volumen evaluation, the ejaculates were placed immediately in a water-bath at 30°C and the percentage of motile sperms was estimated under phase-contrast microscope at 38°C on a hot plate, while the optical density (the sperm concentration) was evaluated using a calibrated spectrophotometer. The motility rate was evaluated by estimating the proportion of motile and non-motile spermatozoa, while the vitality score was based on the individual spermatozoa movements, using an arbitrary scale ranging from 0 to 5, as described by Evans and Maxwell (1987). Chemistry/enzymatic exams (GOT, GPT, LDH, CK, urea ALP, total cholesterol, triglycerides, GGT, amylase, cholinesterase, total proteins, Ca, P, Na, K, Cl, Mg, Fe) on seminal plasma samples were evaluated, after centrifugation at 1,500 rpm for 15 minutes, in a BM/HITACHI 747 autoanalyzer.

Statistical analysis

The distribution of all variable were tested for normality using the Kolmogorov-Smirnov test (Hawkins 2005). None of variables was significantly different from a normal distribution and consequently parametric tests were chosen. Mean differences of semen and seminal plasma between infected and control animals were evaluated with the one-way analysis of variance (ANOVA) test. Mean differences of haematological parameters in relation to each sampling in infected and control animals was evaluated by means of ANOVA, GLM (general linear model) procedure for repeated measures. The software used was SPSS for Windows, version 15.0 (Hawkins 2005).

Results

Animal weight

Rams started with a mean weight of 74.72 kg (SD ± 8.75). No significant differences were observed between the mean weight of the two groups ($p > 0.05$).

Coprological examination

After the first treatment (d-30) the C group was constantly negative during all observation period. Coprological tests results, during all sampling weeks, are shown in Fig. 1.

The response of the rams to the invasion appears to be different but constant in time; with some animals (2, 4, 7) showing a high number of shedded eggs and others (1, 9, 10) showing a very low level of egg dropping (Table 1). The rams showed different peaks of the eggs emission; in some animals it occurred very early (1, 3, 7), in one intermediately (5) and in others it was concentrated in a cluster of three weeks between the 12th and the 14th sampling (Table 1).

The eggs shedding, expressed as a geometrical mean in the infected animals, remained constant also if the infection proceeded until the 5th week. The end of the natural endogenous cycle was confirmed by a dramatic fall in the 15th week (Table 2). Rams 2, 4, 7 are responsible for the wide changes in standard deviation values.

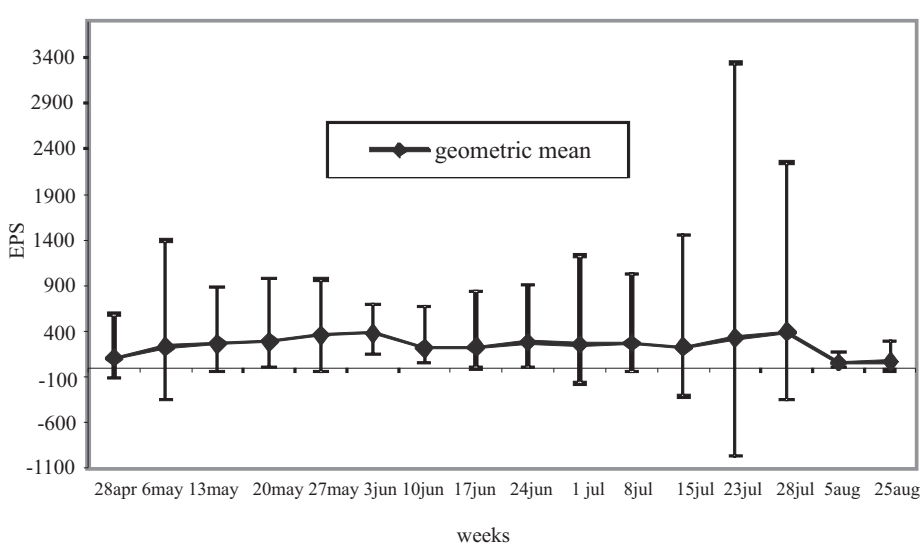

Fig. 1. Strongyle type eggs per gram of faeces (EPS) values during different sampling weeks, geometric mean and standard deviation in infected rams.

Table 1. Strongyle type eggs per gram of faeces (EPG) values in ten infected animals during 16 sampling weeks.

Rams	Sampling Weeks															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	15	60	210	345	465	285	105	240	135	60	60	90	30	45	15	–
2	255	645	510	1125	705	780	720	1185	1050	1020	840	2670	1965	1365	135	–
3	75	255	315	300	315	630	480	135	405	165	180	210	255	155	–	30
4	1185	2925	1590	1515	1755	780	915	960	1305	2160	1560	1680	7020	4185	150	420
5	135	105	270	255	165	270	405	480	225	225	330	105	240	300	60	–
6	105	150	105	150	150	105	105	60	105	135	90	75	45	150	30	–
7	315	600	720	420	405	660	570	825	615	810	1080	585	1470	1980	240	240
8	255	300	375	705	540	135	30	75	–	–	75	105	270	420	90	75
9	30	30	45	15	–	–	30	30	45	75	–	60	60	75	–	15
10	30	180	105	150	105	615	300	195	225	135	240	165	540	825	15	30

Table 2. Mean of haematological parameters and weights of rams during different sampling days and statistical significance (p) between infected (I) and control (C) groups.

	Sampling days										
	-2		3		8		12		16		p
Parameters	C	I	C	I	C	I	C	I	C	I	
Weight	77.2	71.63	77.91	70.78	78.34	69.88	79.79	73.26	76.27	72.12	0.101
GB	7.06	6.22	7.40	9.44	9.59	10.09	9.62	9.82	14.10	11.55	0.926
GR	10.46	11.16	10.14	10.93	9.80	9.68	9.99	10.33	10.80	9.78	0.849
Hb	11.68	11.46	12.25	12.59	12.05	11.38	11.18	10.46	11.10	10.60	0.423
Hat	36.8	37.85	38.16	38.96	35.40	34.64	38.33	36.69	40.72	34.71	0.257
MCV	35.43	33.95	37.78	35.68	37.85	35.56	38.47	36.23	37.86	35.73	0.028
MCH	11.94	10.47	12.09	11.54	12.51	12.48	11.23	10.33	10.30	10.07	0.235
MCHC	33.56	30.73	32.07	32.33	33.08	35.39	29.18	28.49	27.35	28.48	0.983
Plt	201.78	191.80	111.90	141.60	119.10	136.70	112.60	125.6	222.20	268.60	0.432
MPV	4.54	4.51	4.68	4.82	4.44	4.92					0.114
Ca	9.77	9.99	8.88	9.22	9.21	9.27	9.33	9.54	10.04	10.36	0.135
P	5.84	5.92	5.95	6.34	5.71	5.57	5.98	5.75	6.07	5.80	0.920
Mg	2.27	2.17	2.19	2.02	2.21	1.86	2.04	1.86	2.24	2.25	0.066
Cl	95.42	98.86	98.28	96.73	103.49	95.42	93.93	94.51	103.33	101.20	0.513
Fe	139.00	166.90	153.10	151.00	129.30	126.50	129.90	143.70	144.60	137.00	0.442
Na	137.10	138.20	141.56	142.16	141.96	141.68	143.16	141.46	141.08	141.90	0.727
K	5.24	5.22	5.14	5.26	4.72	4.46	4.46	4.32	4.60	4.32	0.067

Blood samples and semen evaluation

Statistical analysis for blood cell count and blood chemistry did not revealed differences between the two groups (Table 3). The semen values of concentration, mobility and vitality of spermatozoa appeared to be significantly higher in I group. Seminal plasma phosphorus (P) showed very low values in I group compared to the C group ($P<0.005$). On the contrary P levels remained normal in blood sera in both groups. Seminal plasma chlorine (Cl) level appeared to be significantly higher ($P<0.001$) in infected animals. Total cholesterol showed higher values in C group ($P< 0.003$) (Table 4).

Table 3. Mean of semen parameters in rams, standard deviation and statistical significance (p) between infected (I) and control (C) groups.

Parameters	Group	Mean	Standard dev	p
Volumen	C	1.69	,27	,848
	I	1.71	,21	
Concentration	C	3.40	,88	,005
	I	4.04	,39	
Spermatozoa	C	5.69	1.59	,013
	I	6.89	1.30	
MOT5'*	C	3.08	,28	,000
	I	3.37	,13	
VIT5'**	C	55%	,8	,000
	I	64%	4	
MOT120'***	C	2.85	,32	,000
	I	3.23	,21	
VIT120'****	C	,42	,07	,000
	I	,52	,06	

* Sperm motility after 5 minutes; **Sperm vitality after 5 minutes
*** Sperm motility after 2 hours; **Sperm vitality after 2 hours

Table 4. Mean of seminal plasma minerals and cholesterol values; standard deviation and statistical significance (p) between infected (I) and control (C) groups.

Parameters	Group	Mean	Standard dev	p
Ca	C	22.05	32.07	,441
	I	14.05	1.99	
P	C	24.73	12.32	,005
	I	11.82	3.01	
Na	C	81.80	11.49	,056
	I	91.40	9.45	
K	C	21.84	5.19	,265
	I	19.73	2.57	
Cl	C	27.40	3.13	,001
	I	35.20	5.63	
Mg	C	7.44	2.70	,033
	I	5.43	,54	
Fe	C	13.30	2.45	,071
	I	20.10	10.92	
Cholesterol	C	48.90	14.59	,001
	I	30.20	5.37	

Discussion

Our experimental infection and a parasite burden considered as a heavy infection (Ambrosi l995) showed an effect of the parasites only on the qualitative characteristics of the ejaculate. However, our trial showed a parasitological situation similar to the natural experience on sheep of Sarda breed with mean values up to 400 EPG, during a year, also without specific treatment (Scala et al. 1993). This consideration lead us to suppose some degree of natural (genetic) resistance of this breed to gastrointestinal nematodes invasions.

The phosphorus level in the seminal plasma in infected group was significantly reduced. This reduction could be due to *T. colubriformis* pathogenic action (Poppi et al. 1985), but this conflicts with the haematological data that remained normal. P has important implication in many biological processes other than reproduction: bone metabolism, protein synthesis, transfer of lipids and amino acids, cell grooving and differentiation, energy production. As consequence, the apparent discrepancy in our data may reflect a fascinating hypothesis of competition between essential biological host processes in which the choice of the organism was to survive on the cost of reproduction. Nevertheless it is strange that the sperm vitality between the two groups does not show any significant differences; indeed this feature should be negatively linked to the P levels in the different semen fractions (Abdel-Rahman et al. 2000).

Another interference of the parasite burden is concerning the total cholesterol. This steroid is an important component of spermatozoa wall and its reduction may affect the fertility. Sperm membrane cholesterol efflux contributes to one such novel signalling mechanism that controls sperm capacitation, and the details of this effect are now beginning to be understood at the molecular level (Travis and Kopf 2002).

Difficult to understand the higher level of Cl in the infested rams suggests the role of this mineral in cell wall permeability and the absence of differences between groups in semen evaluation. The limited pathogenic effect of the parasites could be due to the fact that the used rams were adults and probably had previous contacts with gastrointestinal parasites and previously got a limited number of treatments acquiring resistance (Jacquiet 2001). To obtain semen donors we need trained animals at least 8-month-old and it is particularly difficult, also in stabled animals, to avoid any contact with parasites. In our case, we may suspect the interference of some immunologic memory. This hypothesis is strengthened by the EPG distribution in infested animals (Fig. 1) that seems to simulate aggregate clusters. We observed not a true resistance but a specific kind of adjustment named "resilience" understood as a capacity of the animal to control the pathogenic effect of parasites (Gruner 2002). This hypothesis should be confirmed also by the observed absence of anaemia and leucocytosis and the semen data appear to be normal. Our results could also indicate that parasite levels, similar to natural invasion, do not really affect the semen characteristic of the rams and only heavy and acute infections

may generate severe symptoms also in reproductive system in Sardinian rams. These results, probably expected, are of a high importance, because such phenomena were never experimentally demonstrated. The lack of information (also of physiological data) supported by scientific evidences of an ever suspected interference with male fertility does not allow us to draw more conclusions but stimulates for further investigations.

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