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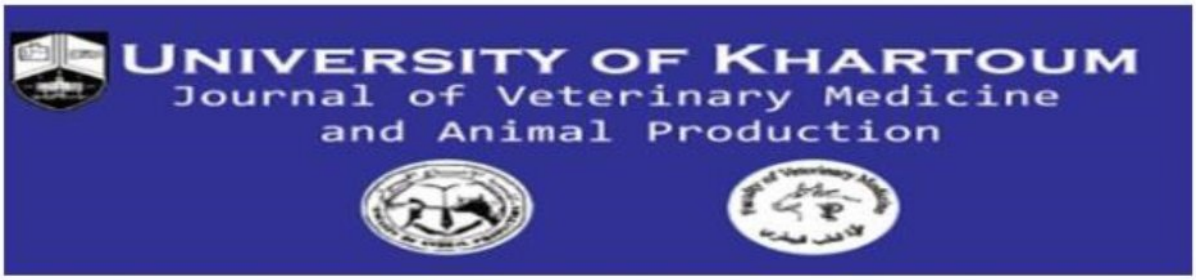

Effect of *Theileria lestoquardi* infection on some reproductive performance of experimentally infected Desert sheep in the Sudan

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Abstract

This study was aimed to investigate the effect of Theileria lestoquardi on the reproductive performance of Sudanese desert ewes. Thirty ewes were divided into three groups (A, B and C) of 10 each subjected to estrous synchronization and hand mating. Group A was infected before mating, group B was infected 2-3 months after conception and group C remained as uninfected control. Conception rate was found to be 11.1% in the first mating, and increased to 33.3% and 44.4% in the second and third mating for group A, GC reached 90%. In GB, three ewes aborted 20 days post infection. In GA the gestation period was two weeks shorter, body weight of the newly born lambs was reduced 30% and the mortality rate was 50%. All infected group revealed a delay in the first progesterone rise after delivery. Generally the infection of Theileria lestoquardi was highly affects the reproductive performance.

Key words: Theileria lestoquardi, sheep, ovarian activity, progesterone, conception.

المستخلص

هدفت هذه الدراسة للتحقيق من تاثير الاصابة بطفيل الثيليريا لستوكاردى على الاداء التناسلى الانتاجى للنعاج الصحراوية السودانية. تمت الدراسة على ثلاثين نعجة صحراوية سودانية والتى بدورها قسمت الى ثلاث مجموعات كل مجموعة تحتوى على 10 نعاج. تمت عدوى المجموعة ا بطفيل الثيليريا لسيوكاردى قبل التزاوج وتمت عدوى المجموعة ب 2-3 شهور بعد الاخصاب. اما المجموعة ج فقد بقيت بدون اصابة للمقارنة. تم تنسيق الشبق للمجموعة ا ثم تم السماح لها بالتزاوج. وجد ان معدل الاخصاب فى المجموعة يمثل 11% ثم ازداد الى 33.3% و44.4% فى التزاوج الثانى والثالث. ومن ناحية اخرى فان معدل الاخصاب وصل الى 90% فى المجموعة ج. فى المجموعة ب حدث اجهاض لثلاث نعاج بعد يومين من العدوى. اما فترة الحمل فقد نقصت اسبوعان فى المجموعة ا. وجد ان تاثير العدوى على متوسط اوزان الحملان المولودة حديثا قد تدنى بنسبة 30% وان نسبة الموت قد وصلت 50% فى المجموعة ا. كشفت الدراسة على ان هناك تاخر فى افرازهرمون البروجستيرون بعد الولادة فى المجموعة ا. عكست الدراسة تاثير الثايليريا ليستوكاردى على وقت البلوغ فى الحملان المصابة.

Introduction

Tick-born diseases of small ruminant are of highly economic importance in many countries. Malignant theileriosis of sheep and goats caused by *Theileria lestoquardi* is considered among the most important of these diseases and constituted an obstacle to the industry in countries like the Sudan (Bakheit, 2006) where sheep comprised about 36% of the total livestock population of 45 million animals (Anonymous, 2000). The continuing increase in the human population worldwide resulted in an increases demand for animal proteins and milk, however, major constrains to livestock development are related to poor animal health, nutrition and genetic potential. Poor animal health is often a consequence of infectious diseases which attracted the attention of workers to the importance of tick-borne diseases especially piroplasmosis and other economic diseases. The animal owners are now aware of the economical consequences of the diseases and their great threat to their herds, hence; affect their prospects in increasing animal production. Little work is done on the effects of infections on the reproductive performance of sheep.

The aim of this study was to investigate the effect of experimental infection with *Theileria lestoquardi* on reproductive performance of Sudanese desert sheep.

Materials and Methods

Experimental animals

Thirty Sudanese desert ewes and three rams (2-3 years old) were purchased from local market around Khartoum after confirming that they were free of ticks, parasitic infections and Brucella. They were kept for five weeks at the Department of Radioisotopes, Central Veterinary Research Laboratories as an adaptation period to the new

environment. They have been examined for all clinical detectable diseases such as brucellosis, trichomoniasis and any reproductive disorders or abnormalities. All animals were dosed with broad spectrum anti-helmenthics and anti-coccidial drugs and fed on green forage (Abu 70) supplemented with a manufacturer composition diet (daily ration). The animals were randomly divided into three groups (A, B and C) of ten ewes each and one ram and housed separately at the Department of Radioisotopes barns of 20 m^2 each with free access to feed and water. Group B was synchronized (as will be described later) and allowed freely with the ram and their conception was recorded throughout three months till confirmation of pregnancy with progesterone detection.

Infection with *Theileria lestoquardi*

Flat nymphs of Hyalomma anatolicum, reared at the Department of Tick and Tick-borne Diseases, Khartoum Sudan, were allowed to feed on ram naturally infected with Theileria lestoquardi "brought from Atbara- Northern Sudan" to pick up the infection using ear bags feeding technique according to the method described by Bailey (1960). Some of the emerging flat adults were crushed and examined microscopically for detection of infection, then 50 infected flat adult ticks were applied to feed again on each of experimental ewes. Ewes of group A were experimentally infected with Theileria lestoquardi before mating. Pregnant ewes in Group B were experimentally infected with the same strain of T. lestoquardi after confirmation of being pregnant (1-3 months), whereas, ewes in group C remained as control.

Establishment of infection was monitored daily by measuring rectal temperature and examination of Geimsa stained smears till the presence of piroblasms and shizonts in the lymphocytes according to Kolmer et al. (1951).

Oestrous synchronization

Oestrous synchronization was performed to the infected ewes of group A, and the control group C by insertion of progesterone releasing intravaginal device (CIDR) that contains 0.3 gm slow release progesterone (Inter Ag, Hamilton, Netherlands). The CIDR remained in situ for 14 days as suggested by Ritar et al. (1984). At time of CIDR withdrawal, the ewes received an intramuscular injection of 400-500 IU of pregnant mare serum gonadotrophin (PMSG - intervrt, UK). Heat signs were detected 24 - 72 hours following PMSG and ewes that showed heat signs were individually mated by introducing to the rams. Conceived ewes were recorded and un-conceived ones were re- mated on second oestrus or even the third and forth oestrus later.

Ram reproductive evaluation

The rams were selected on the basis of their sexual desire (libido) and they were also tested for their semen quality in the Animal Production Research Center at Kuku-Khartoum.

Sample collection

Serum samples were collected weekly from individual ewes of the three groups throughout the experimental period. The collected samples were used for the measurements of the serum progesterone level using standard method of progesterone- enzyme immune-assay (EIA) by the commercial kits (EIA- Progesterone, Immunometrics Co. Ltd, UK).

Results

Conception rate

Table 1 shows the conception rate in the

infected ewes of group A, B and the control group C. Nine ewes (90%) in group C conceived from the first service, while in group A, only one ewe (11.11%) conceived to the first mating. Three more ewes conceived in the second mating, and 4 in the 3rd mating. However, the ewe that did not conceive after the 4th mating got pregnant 5 months later. The one that did not conceive throughout the experimental duration in group C showed a persistent rise in progesterone concentration (ie persistent cl).

Table 1: Effect of infection of ewes with *Theileria lestoquardi* on conception rate

Animal Group	No. of animals	Conception rate				Not conceived
		1st service	2nd service	3rd service	>4th service	
Group A	9	1 (11.11%)	3(33.33%)	4 (44.44%)	1 (11.11%)	_
Group B	10	(90%) 9	_	_		10%
Group C	10	(90%) 9	_	_		10% 1

Group A: ewes infected with *T. lestoquardi* before conception

Group B: ewes infected with *T. lestoquardi* after conception

Group C: control uninfected ewes

Gestation length

The gestation length of the ewes in the control group C was found to be 159 ±7.15 days ranging between 151-168 day which is significantly longer ($P < 0.05$) than that in the infected group A and group B which were found to be 144.2 ± 4.76 and 153 ± 4.5 days, respectively (Table 2).

Table 2: Effect of infection of ewes with *Theileria lestoquardi* on lambing rate and gestation length (mean days ±SD)

Animal group	No of delivered ewes	Gestation length in days (mean ± SD)	Range
Group A	7	144.2 ± 4.76 [b]	138-150
Group B	4	153.25± 4.50 [a]	148-157
Group C	9	159.00 ± 7.15 [a]	151-168
P- value	-----	0.003	------

Values with different superscript letters within same column were significantly different at P<0.05

Group A: ewes infected with *T. lestoquardi* before conception

Group B: ewes infected with *T. lestoquardi* after conception

Group C: control uninfected ewes.

Birth weights of lambs

The newly born lambs in group C were apparently healthy and their mean birth weight was found to be 2.62 ± 0.233 kg, they were significantly heavier (P<0.05) than those in infected group A, with mean birth weight of 1.78 ± 0.795 kg, and those in infected group B with a mean birth weight of 2.4 ± 0.38 kg (Table 3).

Table 3: Effect of infection of ewes with *Theileria lestoquardi* on lamb birth weight (kg)

Animal group	Body weight in Kg (mean± SD)	Range
Group A-lambs (9)	1.78 ± 0.795 [b]	1 – 3
Group B-lambs (5)	2.4 ± 0.380 [a]	2 – 3
Group C-lambs (14)	2.62 ± 0.233 [a]	2.25 -3
P-value	0.004	____

Values with different superscript letters within same column were significantly different at P<0.05

Group A: ewes infected with *T. lestoquardi* before conception
Group B: ewes infected with *T. lestoquardi* after conception
Group C: control uninfected ewes

Litter size, abortions and lambs survival rates

The litter size, abortions and lambs survival rates data are represented in Table (4). The nine ewes in group C gave birth to 14 healthy lambs; five of them gave twins with 100% survival rate. Two ewes in group A died during the execution of the experiment, one ewe delivered triple lambs and each of the remaining 6 ewes gave birth to a single lamb. However, lambs in the infected group A (4/9 lambs) died either immediately after birth or shortly after with a mortality rate of 45%. On the other hand, in infected group B, one ewe delivered twins, three ewes gave single births, three ewes aborted in the 2nd month, and one died before delivery. The total number of born lams in this group was only 5 and 2 of them (40%) died immediately after birth.

Table 4: Effect of infection of ewes with *Theileria lestoquardi on* number of delivered ewes, abortions, litter size and their survival rates

Animal groups	No. of delivered animals	No. of abortions	No. of lambs	Twining	triplets	Lamb survival rate
Group A	7 (77%)	0	9	------	1	55%
Group B	4 (50%)	3	5	1	------	60%
Group C	9 (90%)	0	14	5	------	100%

Group A: ewes infected with *T. lestoquardi* before conception

Group B: ewes infected with *T. lestoquardi* after conception

Group C: control uninfected ewes.

Resumption of ovarian activity

Ovarian activity of ewes was considered when blood P4 level increased to more than 3.0 mmol/L. Monitoring of progesterone profile after delivery showed that ewes of group C resumed their ovarian activity at 21.33±8 days as shown by days to first rise in progesterone level after lambing (Table 5). The PPAI is therefore shorter ($P<0.05$) in GC than that of both the infected group A (59.67±42.79 days) and B (40.25±28.63 days).

Table 5: Effect of infection of ewes with *Theileria lestoquardi* on days from lambing to first progesterone rise (P4)

Animal group	Days from lambing to 1st progesterone rise (M ± SD)	Range
Group A	59.67 ± 42.79[a]	21-90
Group B	40.25±28.63[a]	12-73
Group C	21.33 ±8.06[b]	9-32
P-value	0.032	------

Values with different superscript letters within same column were significantly different at P<0.05

Discussion

In this study the experimental infection of ewes with *T. lestoquardi* was successfully obtained for execution of the study on the reproductive performance. Synchronized ewes came to heat normally after 24-48 hours post PMSG injection and the non conceived ewes showed heat signs again at 15-21 days later. This observation is in agreement with that of Las Cruces (2000) who reported that normal oestrous cycle for ewes is approximately 17 days, and may vary from 14 to 19 days and the heat period usually lasts for 30 to 35 hours. The conception rate to first service in Group A (11%) is significantly (P<0.05) lower than in the control group, this may be highly related to the infection with *T. lestoquardi*. Generally ewes had a high fertility rate at a good pasture condition and management system (Las Cruces, 2000). However, other factors could be considered such as breed, date of mating, and other management factors.

In this study, the mean gestation length of the infected ewes (144.2 ±4.76 days) is shorter than that of the control ones (159 ±7.15 days). This may be due to the effect of *T. lestoquardi* infection that might not keep pregnancy to its normal end. However, the gestation length for most breeds of Sudanese goats as reported by ELNaim (1979) ranges from 143-153 days. Musa *et al.* (2005) reported that the mean gestation length for West African ewes was 150.44 ± 2.64 days ranging between 145-156 days, whereas, Las Cruces (2000) reported gestation length of 147 to152 days which was consistent with our results. Concerning lambs birth weights, the results showed that the control ewes gave lambs with birth weights ranging from 2.3 to 3.0 kg (2.59 kg) which is significantly (P<0.05) higher than that of the infected ones which ranged from 1.0 to 3.0 kg (1.87 kg). This loss in birth weight along with shorter gestation length may be attributed to the stress of pregnancy and the recruitment of the infection with *T. lestoquardi*. Musa *et al.* (2005) in their study on the production performance of West African sheep in Sudan found that the overall birth weight of lambs was 2.90 ± 0.50kg. Fthenakis *et al.* (2001) reported significant effect of sarcoptic mange infection on the total lambs born per ewe (1.2 and 1.6 for infected and control group, respectively). This supported the present finding that the ewes in group C gave more twins than the infected ones of group A. Moreover,

Mukasa *et al.* (2002) found that litter size in tropical sheep ranges from 1.0 to 1.5 indicating that twinning rate in sheep ranges from 0 and 50%.

The newly born lambs from the infected ewes (with mean birth weight of 1.78) died within 24 hours post partum. This finding was in agreement with that of Disclaimer (2008) who concluded that lamb birth weight was the greatest factor influencing lamb survival. Moreover, positive relationship between birth weight and survival rate was also reported by Markos (2006) and Mtenga *et al.* (2006). Similarly, the probability of mortality was greater for triplets than for twin born lambs and greater by 25% for males compared with females (Judith and Collins, 2005). This was closely related to the present finding that triple lambs from infected ewes died within a few hours from birth, whereas the survival rate was 100% in the control group. However, Hossain *et al.* (2003) concluded that size, weight and health status of ewes may play an important factor which affect birth weight and survival of lambs.

The present study showed that *T. lestoquardi* caused abortions in early pregnant ewes whereas; mid-pregnant or late pregnant ewes were not affected and gave lambs that were clinically normal. Although abortion is an uncommon cause of reproductive failure in sheep, and it may be due to infections as toxoplasmosis, bacteriosis, salmonellosis, listeriosis, brucellosis and ovine pestivirus (Las Cruces, 2000).

Delayed post partum activity in the two infected groups of ewes compared to the control ewes may probably be attributed to the weakness of the ewes due to the infection. However, Walkden-Brown and Bocquier (2000) observed in Burundian meat goats a delay in post-partum resumption of cyclicity in dry season due to poor quality and low availability of feed.

Conclusions

Although sheep are highly fertile animals, the present study may highlight the effect of *T. lestoquardi* as an adverse effect on the reproductive potential of Desert sheep in the Sudan. Infection with *T. lestoquardi* may contribute significantly to the reproductive wastage in sheep population, which economically more important in countries where intensive system of lambing management is used however, more studies are needed.

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