Indian Journal of Animal Sciences **83** (7): 688–692, July 2013/Article

Effect of experimental *Trypanosoma evansi* on fertility in Black Bengal Buck

SAKTIPADA PRADHAN[1], SEULI SAHA ROY[2] and SUNIT KUMAR MUKHOPADHYAYA[3]

West Bengal University of Animal and Fishery Sciences, 37- K.B. Sarani, Belgachia, Kolkata, West Bengal 700 037 India

Received: 16 May 2012; Accepted: 19 January 2013

ABSTRACT

Healthy black Bengal bucks (12), about 12 month-old, of similar body weights (8.66±0.49) were acclimatized for 3 weeks, and then divided into 2 groups – infected group (1; n=6) and non-infected control group (C; n=6). In the *Trypanosoma evansi* infected bucks mean semen volume (0.86±0.10 ml) and mean spermatozoal concentration (0.92±0.09 $\times 10^6$/mm^3) decreased significantly. Mean percentage of spermatozoal abnormalities (7.5±0.57) and mean percentage of dead spermatozoal count (32.60±3.50) increased significantly on 12th week post infection in infected buck. In control group mean semen volume and mean spermatozoal concentration slightly increased. Mean percentage of dead spermatozoa and spermatozoal abnormalities was invariable and no significant change was observed in control group throughout the experiment period. The tissue section of testes showed diffuse degeneration of spermatogonia and sertolicells, devoid of spermatozoa and proliferation of fibrous connective tissue in intertubular spaces. The testicular blood vessels in some places were infiltrated with inflammatory cells. The present study indicated antifertility effect of *T. evansi* in Black Bengal bucks.

Key words: Black Bengal buck, Fertility,Goat, Spermatozoa, *Trypanosoma evansi*

Trypanosomiasis caused by *Trypanosoma evansi*, a serious disease of horses, donkeys and dogs, is responsible for high mortality, whereas the disease typically runs a chronic course in other domestic animals (Losos 1980). Its outbreaks occurred in horses, buffaloes, cattle and goats in Philippines and India (Manuel 1998, Reid 2002, Kumar *et al*. 2012) resulting in economic loses due to mortality and morbidity (Payne *et al*. 1990, Partoutomo *et al*. 1995, Tuntasuvan and Luckins 1998). Appropriate control measures of *T. evansi* in buffaloes, cattle, horses, goats/sheep and pigs could prevent the loss of about US $88, $84, $151, $7, $114 per animal/ year, respectively, in Philippines (Dobson *et al.* 2009). The diagnosis of trypanosomiasis in field condition is still unsatisfactory, due to lack of rapid, reliable diagnostic methods and limited information on clinical manifestations and clinical pathology. Testicular lesion in rams occurs due to infection of *Trypanosoma vivax* (Akpavie and Ikede 1987), *Trypanosoma evansi* (Ikede 1979) and *Trypanosoma congolense* (Kaaya and Oduor-Okelo 1980). The Black Bengal goat is important for its adaptability, fertility, high productivity and softness of skin. The skin of Black Bengal goats is of superior quality for leather goods and is in great demand both in domestic and foreign markets (Acharya 1982). Their meat is famous for its tenderness, flavour and leanness (Chowdhury and Faruque 2004). Since there is a need of detailed information in trypanosomosis on semen quality and fertility in Black Bengal goats, hence an attempt was made to investigate effect of *T. evansi* on fertility parameter of Black Bengal buck.

Present address: [1]Assistant Professor (drsaktipadapradhan @yahoo.co.in), [3] Associate Professor (skmpath@gmail.com), Department of Veterinary Pathology. [2]Associate Professor (vetseuli@gmail.com), Department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, R.K. Nagar, Tripura (West) 799008.

MATERIALS AND METHODS

Selection and management of bucks: Healthy Black Bengal bucks (12), 12-month –old, almost of similar body weights (8.66±0.49) procured commercially were housed in fly proof and tick free concrete pen. The animals were then treated with albendazole and amprolium against helminthes and coccidiosis, respectively, and also sprayed with deltamethrin to rid them of external parasites. They were fed with fresh grass and *ad lib.* fresh drinking water daily. The pen was cleaned properly twice daily. A footbath was placed in the entrance of the pen and used by personnel entering and leaving the pen. Three weeks acclimatization period was allotted before the animals were considered for experiments. After that 12 selected bucks were divided into 2 groups – infected group (1: I_1 to I_6) and non-infected control group (C: C_1 to C_6).

T. evansi *isolates and experimental infection:* T. evansi was isolated from a clinical case of cattle of a local dairy

farm and was maintained in Swiss mice. The concentration of *T. evansi* in blood sample of the infected mice was determined by using a Neubauer haemocytometer and then diluted in Alsever's solution. The bucks (I_1 to I_6) were inoculated intravenously (I/V) with 1 ml of Alsever's solution containing 1.0×10^6 live *T. evansi*. The experiment was carried out for 12 weeks after giving infection and body weight were taken weekly during this period. The bucks were observed carefully twice daily for clinical symptoms. From third day onwards at 24 h intervals blood samples were collected from ear tip of all the goats and tested by wet film method of blood smear and also by mice inoculation test.

Examination of semen quality: Semen was collected from each goat throughout the experimental period at weekly intervals and collected in graduated semen collection tube to measure the volume (ml). Dead and live, morphological abnormalities of spermatozoa were observed by Eosin – Nigrosin stain (Blom *et al*. 1950) and method of Salisbury and Mercier (1945). Concentration of spermatozoa ($\times10^6$/ mm^3) was determined by haemocytometer (Salisbury *et al.* 1943).

Pathomorphological study of testes: All the experimental animals were sacrificed after termination of experiments (12 weeks) and gross pathological lesions of testes were recorded. Formalized fixed testes were used for pathological studies. The tissue blocks (2 m^2) were fixed in 10% neutral buffered formalin and embedded in paraffin wax. The tissue sections about 5mm thickness were stained by Mayer's Haematoxylin and Eosin (HE) methods.

Statistical analysis: The percentage data were angularly transformed and analyzed using appropriate statistical techniques as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The Black Bengal bucks were experimentally infected (1.0×10^6, I/V) with local strain of *T. evansi* isolated from an acute clinical case of cattle. Ngeranwa *et al.* (1993) revealed that small East African goats were susceptible to intravenous injection with about 100 000 *T. evansi*. But Otieno and Gachanja (1976) observed no clinical symptoms in small East African goats after subcutaneous injection with about 200 000 *T. evansi*. These results suggested that intravenous route of infection is more desirable than the subcutaneous route to induce a clinical symptom, however, several other factors are also responsible for severity of disease. All infected bucks showed pale mucous membrane, anemia, fever, weakness, rough hair coat, loss of appetite, emaciation and progressive weight loss. These results corroborated with the findings of Reid (2002). Out of 6 infected animals 4 bucks showed enlarged testicules. Rectal temperature of infected goats varied from 100.8°F to 106.6°F. The body temperature of control group of animal ranged from 99.4°F to 102.8°F. The pathology of haemophilic trypanosomosis may vary depending upon the species, breed and strain of

Table 1. Semen quality and quantity of experimentally *T. evansi* infected Black Bengal Buck

Group	Pre. Ino	Post inoculation period										
		1st wk	2nd wk	3rd wk	4th wk	5th wk	6th wk	7th wk	8th wk	9th wk	10th wk	11th wk	12th wk
						Semen volume (ml) (mean±SE)							
Infected	1.21±0.12	1.20±0.21	1.19±0.21	1.19±0.20	1.05±0.19	1.02±0.21	1.00±0.23	0.96±0.18	0.94±0.17	0.93±0.18	0.90±0.16	0.88±0.19	0.86*±0.10
Control	1.10±0.21	1.11±0.20	1.09±0.21	1.14±0.19	1.17±0.21	1.19±0.22	1.22±0.20	1.23±0.23	1.26±0.27	1.30±0.28	1.34±0.26	1.35±0.27	1.38±0.19
						Spermatozoal count($\times10^6$/mm^3) (mean±SE)							
Infected	1.52±0.19	1.51±0.17	1.49±0.19	1.45±0.21	1.40±0.23	1.36±0.22	1.30±0.19	1.25±0.20	1.21±0.16	1.10±0.15	1.01±0.13	1.01±0.15	0.92*±0.09
Control	1.47±0.25	1.46±0.23	1.46±0.22	1.48±0.24	1.47±0.25	1.49±0.30	1.48±0.31	1.50±0.28	1.51±0.30	1.51±0.29	1.52±0.27	1.54±0.25	1.53±0.20
						Spermatozoal abnormalities (%) (mean±SE)							
Infected	4.61±0.90	4.59±0.50	4.59±0.57	4.68±0.40	4.72±0.45	4.90±0.39	5.11±0.25	5.25±0.35	5.73±0.48	5.94±0.57	6.23±0.52	6.81*±0.27	7.5*±0.57
Control	5.10±0.49	5.11±0.56	5.12±0.49	5.15±0.42	5.14±0.32	5.08±0.34	5.05±0.39	5.12±0.45	5.18±0.50	5.22±0.49	5.22±0.57	5.17±0.59	5.25±0.59
						Dead spermatozoa (%) (mean±SE)							
Infected	16.10±4.10	16.20±2.1	16.34±2.30	17.90±2.10	19.21±2.90	21.50±3.20	22.36±3.12	23.00±3.21	23.60±2.70	24.50±2.80	25.30±2.62	29.10*±2.90	32.60*±3.50
Control	15.50±2.40	15.61±2.02	16.21±2.10	17.50±2.41	16.50±2.62	17.10±2.71	16.95±2.85	17.01±2.91	17.86±2.50	16.51±2.70	17.25±3.00	17.60±2.40	18.10±2.53

Infected group (n=6), Control group (n=6) P≤0.05 (*)

Tryapanosoma spp (Reid 2002). According to Holmes *et al.* (2000) course of disease may depends on infective dose, stage of infection and environmental factors such as nutritional level. Ventura *et al*. (2002) showed variation within *T. evansi* isolated from different geographical area by polymerase chain reaction (PCR). Sharma *et al.* (2000) reveled that *T. evansi* infection increased susceptibility to gastrointestinal infection in animal. Onah *et al*. (1998) and Singla *et al*. (2010) described that *T. evansi* reduced the response to vaccination due to its immunosuppressive nature.

The mean semen volume in bucks was 1.21±0.12 ml just before inoculation of *T. evansi.* Volume of semen gradually decreased in infected group (Table 1). In the 12th week of infection lowest volume (0.86±0.10 ml) of semen ejaculated from infected bucks. In the control group semen volume gradually increased throughout experimental period and in 12th week of experiments they ejaculated maximum mean volume (1.38±0.19 ml) of semen. During this study period it was observed that mean semen volume of infected goats decreased significantly (P≤0.05) on 12th week of experiment in comparison to pre-inoculation mean semen volume of infected goat as well as control group on that week. In the present study decline in semen volume in *T. evansi* infected goat may be due to diffuse degeneration of spermatogonia and sertolicells.

Just before inoculation infected group of animals showed mean 1.52±0.19 (× 10^6/mm^3) spermatozoal counts, but spermatozoal count gradually decreased with the progressiveness of infection, and lowest mean 0.92±0.09 (×10^6/mm^3) spermatozoal count was observed on 12th week of infection. On the other hand, in control group mean spermatozoal concentration slightly increased throughout the experimental period and highest concentration of spermatozoa (1.53±0.20, ×10^6/mm^3) was recorded on 12th week of experiment. It was also recorded that mean spermatozoal concentration of infected buck significantly (P≤0.05) decreased on 12th week post infection in comparison to pre-inoculated buck and control group of the particular week (Table 1). Decline in mean spermatozoal concentration may be due to obliteration of many spermatozoal tubes. Losos and Ikede (1970) observed similar types of results in albino rat, rabbit and sheep.

Changes of mean percentage of spermatozoal abnormalities in infected bucks are presented in Table 1. The infected group showed highest mean percentage of spermatozoal abnormalities on 12th week of infection. In control group there was no significant change in mean percentage of spermatozoal abnormalities. It was clear that mean percentage of spermatozoal abnormalities increased significantly (P≤0.05) on 11th and 12th week in comparison to pre-inoculation mean percentage of abnormal sperm as well as control group of those weeks. In present study detached tail, bent midpiece, short tail, bent tail, tail coiled over itself, mega head, presence of cytoplasmic droplet in neck, midpiece and terminal portion of tail were recorded.

The infected group of buck showed lowest mean percentage of dead spermatozoa on 1st and 12th week of infection, respectively. On 12th week of experiment control group of animal showed 18.10±2.53 mean percentages of dead spermatozoa. In control group mean percentage of dead spermatozoa was invariable and no significant change was observed throughout the experimental period. Mean percentage of dead spermatozoal count (Table 1) significantly (P≤0.05) decreased on 11th and 12th week post inoculation, in comparison to pre-inoculation mean percentage of dead spermatozoal count and control group of respective weeks. Significant increase in dead spermatozoa in infected group might be due to depletion of cellular nutrients and oxygen as *T. evansi* requires these for its movement, multiplication and growth. De Villa *et al.* (1991) and Ngeranwa *et al.* (1991) observed that fertility of small East African buck was reduced due to *T. evansi* infection. Ikede *et al.* (1983) and Chandra *et al.* (1999) also decreased fertility in horses and rabbits, respectively. Anosa and Isuou (1980) revealed that *T. vivax* was responsible for infertility in male sheep and goat. The presence of *T. evansi* was reported in the cauda epididymal fluid of infected male rats showing the possibility of sexual transmission of the parasite (Singla *et al.* 2003).

All infected bucks showed pale and anaemic carcass, serohaemorrhagic fluid in the thoracic, pericardial and peritoneal cavities, splenomegaly and enlargement of lymph nodes. Only 2 infected bucks showed haemorrhages in kidneys and small intestine. Out of 6 infected bucks 4 showed scrotal oedema, whereas, in control group there was no change in gross pathology of carcass. The tissue section of testes showed diffused degeneration of spermatogonia and sertoli cells. There was also proliferation of fibrous connective tissue in intertubular spaces. The tissue section of testes also revealed that there was slight to severe interstitial lymphoid and macrophage reaction with the devoid of spermatozoa. The testicular blood vessels in some places were infiltrated with inflammatory cells particularly with mononuclear cells (Fig. 1). Kaaya and Oduor-Okelo

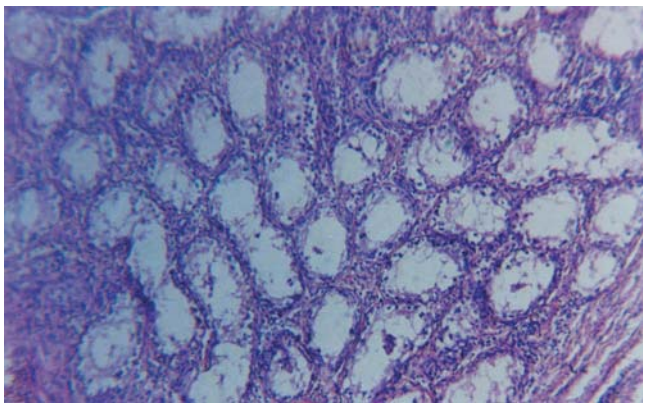

Fig. 1. Section of testes showing diffuse degeneration of spermatids and sertoli cells, and infiltration of mononuclear cells.

(1980) observed degeneration of spermatogonia and sertolicells, proliferation of fibrous connective tissue in intertubular spaces, infiltration of inflammatory cells in *Trypanosoma congolense* infected goat. Anosa and Isoun (1980) detected testicular degeneration in *Trypanosoma vivax* infected goats. According to Dargantes *et al.* (2005) together with aspermia, direct trypanosomal damage, interstitial infiltrates, microthrombi and hyperthermia was mainly responsible for infertility in *T. evansi* infected male goat.

In absence of data of similar nature, the present study could not be compared. Above experiments provided a clear idea on the clinical symptoms, semen quality, quantity and histopathological changes of testes of *T. evansi* infected Black Bengal bucks and also an indication for diagnosis of infertility in male, which indirectly can assist to prevent the economic loss of the entire society.

REFERENCES

Acharya R M. 1982. *Sheep and Goat Breeds of India.* Animal Production and Health Paper 30 (A). Publ. by FAO, Rome. 190 pp.

Akpavie S O and Ikede B O. 1987. Ejaculate characteristics of sheep infected with *T. brucei* and *T. vivax* and changes caused by treatment with Berenil. *Research in Veterinary Science* **42**: 1–6.

Anosa V O and Isoun T T. 1980. Further observations on the testicular pathology in *Trypanosoma vivax* infection of sheep and goats. *Research in Veterinary Science* **28**(2): 151–60.

Blom E. 1950. A simple rapid staining method for the determination between live and dead sperm cells by means of eosin and nigrosin. *Nodic Veterinary Medicine* **2**: 58–61.

Chandra D, Tripathi B N, Srivastava R V N and Singh R. 1999. Pathology of experimental *Trypanosoma evansi* infection in rabbits. *Indian Journal of Veterinary Pathology* **23**: 44–46.

Chowdhury S A and Faruque S. 2004. Meat production characteristics of Black Bengal goat. *Asian-Australasian Journal of Animal Sciences* **17**: 848–56.

Dargantes A P, Camepbell R S F, Copeman D B and Reid S A. 2005. Experimental *Trypanosoma evansi* infection in the goat. II. Pathology. *Journal of Comparative Pathology* **133**(4): 267–76.

De Villa R M, Eduardo S I and Dennig H K. 1991. Clinico-pathologic and hematologic observations in goats experimentally infected with *Trypanosoma evansi* (Manila strain). *Philippine Journal of Veterinary and Animal Sciences* **17**: 131– 42.

Dobson R J, Dargantes A P, Mercado R T and Reid S A. 2009. Models for *Trypanosoma evansi* (surra), its control and economic impact on small-hold livestock owners in the Philippines. *International Journal for Parasitology* **39**(10): 1115–23.

Holmes P H, Katunga-Rwakishaya E, Benison J J, Wassink P K and Parkins J J. 2000. Impact of nutrition on the pathophysiology of bovine trypanosomiasis. *Parasitology* **120**: S73–S85.

Ikede B O. 1979. Genital lesions in experimental chronic *Trypanosoma brucei* infection in rams. *Research in Veterinary Science* **26**: 145–51.

Ikede B O, Fatimah I, Sharifuddin W and Bongso T A. 1983. Clinical and pathological features of natural *Trypanosoma evansi* infection in ponies in West Malaysia. *Tropical Veterinarian* **1**: 151–53.

Kaaya G P and Oduor-Okelo D. 1980. The effects of *Trypanosoma congolense* infection on the testis and epididymis of the goats. *Bulletin of Animal Health and Production in Africa* **28**(1): 1–5.

Kumar H, Gupta M P, Sidhu P K, Mahajan V, Bal M S, Kaur K, Ashuma, Verma S and Singla L D.2012. An outbreak of acute *Trypanosoma evansi* infection in crossbred cattle in Punjab, India. *Journal of Applied Animal Research* **40**: 256–259.

Losos G J and Ikede B O. 1970. Pathology of experimental trypanosomiasis in albino rat, rabbit and sheep. Priliminary report. *Canadian Journal of Comparative Medicine* **34**: 209–12.

Losos G J. 1980. Diseases caused by *Trypanosoma evansi*, a review. *Veterinary Research Communications* **4**: 165–81.

Manuel R A. 1998. Sporadic outbreaks of surra in the Philippines and its economic impact. *Journal of Protozoology Research* **8**: 131–38.

Ngeranwa J J N, Gathumbi P K, Mutiga E R and Agumbah G J O. 1993. Pathogenesis of *Trypanosoma (brucei) evansi* in small East African goats. *Research in Veterinary Science* **54**: 283–89.

Ngeranwa J J N, Mutiga E R, Agumbah G J O, Gathumbi P K and Munyua W K. 1991. The effects of experimental *Trypanosoma (Trypanozoon) (brucei) evansi* infection on the fertility of male goats. *Veterinary Research Communications* **15**: 301–8.

Onah D N, Hopkins J and Luckins A G. 1998. Increase in CD5CB cells and depression of immune responses in sheep infected with *Trypanosoma evansi*. *Veterinary Immunology and Immunopathology* **63**: 209–22.

Otieno P S and Gachanja J W. 1976. Studies of experimental *Trypanosoma evansi* infection in goats. *Bulletin of Animal Health and Production in Africa* **24**: 295–97.

Partoutomo S, Soleh M, Politedy F, Day A, Wilson A J and Copeman D B. 1995. A study on the pathogenesis of *Trypanosoma evansi* in buffaloes, Holstein-Friesian and Ongole cattle. *Jurnal Ilmu Ternak dan Veteriner* **1**: 41–48.

Payne R C, Sukanto I P, Graydon R, Sarosa H and Jusif S H. 1990. An outbreak of trypanosomiasis caused by *Trypanosoma evansi* on the island of Madura. *Tropical Medicine and Parasitology* **41**: 445–46.

Reid S A. 2002. *Trypanosoma evansi* control and containment in Australia. *Trends in Parasitology* **18**: 219–24.

Salisbury G W, VanDemark N L and Lodge J R. 1943. *Physiology of Reproduction and Artificial Insemination of Cattle.* 2nd edn, 401– 27 pp. CBS Publishers and Distributors.

Salisbury G W and Mercier E. 1945. The reliability of estimates of the proportion of morphologically abnormal spermatozoa in bull semen. *Journal of Animal Science* **4**: 174–78.

Sharma D K, Chauhan P P and Agrawal R D. 2000. Interaction between *Trypanosoma evansi* and *Haemonchus contortus* in goats. *Veterinary Parasitology* **92**: 261–67.

Singla L D, Juyal P D and Sharma N S. 2010. Immune responses to haemorrhagic septicaemia (HS) vaccination in *Trypanosoma evansi* infected buffalo calves. *Tropical Animal Health and Production* **42**(4): 589–95.

Singla N, Parshad V R and Singla L D. 2003. Potential of *Trypanosoma evansi* as a biocide of rodent pests. *Mice, Rats and People: Rodent Biology and Management.* pp.43–46. (Eds) Singleton G R, Hinds L A, Krebs C J and Spatt D. ACIAR monograph, Canberra, Australia.

Snedecor G W and Cochran W G. 1994. *Statistical Methods.* 8th edn. Affiliated East-West Press, New Delhi, India.

Tuntasuvan D and Luckins A G. 1998. Status of surra in livestock in Thailand. *Journal of Protozoology Research* **8**: 162–70.

Ventura R M, Takeda G F, Silva R A, Nunes V L B, Buck G A and Teixeira M M G. 2002. Genetic relatedness among *Trypanosoma evansi* stocks by random amplification of polymorphic DNA and evaluation of a synamorphic DNA fragment for species-specific diagnosis. *International Journal for Parasitology* **32**: 53–63.