Vet.Med.J.,Giza. Vol.46, No.2. (1998): 165-169

TESTING OF THE EGYPTIAN CATTLE AND WATER BUFFALOES (*BUBALUS BUBALIS*) FOR *PARAFILARIA BAVICOLA* USING ELISA

A. EL-GHAYSH* and G. ZAKRISSON**

* Department of Parasitology, Faculty of Veterinary Medicine, Cairo University, Egypt.
** Department of Parasitology, National Veterinary Institute, Uppsala, Sweden.

Received: 8/11/1997
Accepted : 9/3/1998

SUMMARY

A total of 411 sera (323 from cattle and 88 from water buffaloes) 2 to 15 years old were collected from different localities from Egypt (Cairo, Delta and Upper Egypt). The sera were tested by ELISA for *Parafilaria bovicola*. Monoclonal mouse anti-*Parafilaria bovicola* directed to 41 kDa protein (National Veterinary institute, Uppsala, Sweden) has been used. All the tested sera recorded negative results. The inhibition rate was recorded to be 25-30% in cattle and buffaloes from Cairo. While, in Delta and Upper Egypt it was recorded to be 14.9 to 21.3%. the older ages (10-15 years) recorded higher inhibition rate (28.3-30.8%). The standard deviation was evaluated at 95%.

INTRODUCTION

Parafilaria bovicola is one of Filaroidea which infects cattle and buffaloes in different countries. Its occurrence in the subcutaneous tissues and wandering lead to the appearance of haemmorrhagic nodules on the skin particularly in summer. At slaughter there are slimy bruising lesions on the subcutaneous surface of the carcasses. The carcasses usually are down graded, which cause a lot of economic losses in different countries.

The original description of the filarial worm *Parafilaria bovicola* was first recorded in 1934 by Tubangui (1934). De Jesus (1934) noted the bleeding nodules on the skin of the live animals due to. *P. bovicola* in Philippine. *P. bovicola* was recorded in cattle in Romania (Metianu, 1949), in Ruanda-burundi (Fain and Deramee, 1949), South africa (Pienaar and Van den Heever, 1964), Canada (Niilo, 1968 and Webster and Wikens, 1970) and Sweden (Nilsson, 1978). Water buffaloes were recorded to be infected with. *P. bovicola* in India (Patnaik and Pand, 1963). In 1974, Chauhan et al. recorded the occurrence of an immature Parafilariid worm in the eye of

buffalo (Bubalus bubalis) in India. They claimed that it was *P. bovicola*.

ELISA was developed by Sundquist et al. (1988) to test *P. bovicola* infection. They recorded that ELISA can detect the infection as early as 120 days after infection and it can be easily applied for large numbers to detect the immune status of a herd for *P. bovicola*.

Vector of *P. bovicola* are *Musca lusoria* and *Musca xanthomelas* (Nevill, 1975) and *Musca autumnalis* (Bech-Nielsen et al., 1982). Therefore, this investigation was initiated to examine cattle and water buffaloes for *P. bovicola* infestation in Egypt. Furthermore the identification of this parasite in Egypt will raise the question on which vector could transmit it. The presence of *P. bovicola* has not been studied before in Egypt. In this paper testing of sera from 323 cattle and 88 buffaloes from different localities from Egypt for *P. bovicola* using ELISA was adopted.

MATERIAL AND METHODS

1- The tested sera:

A total of 411 sera (323 from cattle and 88 from buffaloes) were collected from different localities in Egypt (Cairo, Delta and Upper Egypt). The age ranged from 2 to-15 years. The obtained samples from Cairo were collected from Cairo abattoir. While, those from Delta and Upper Egypt were collected from living animals. The samples were collected all months of the year. The collected blood was left at room temperature overnight to coagulate, then centrifuged at 3000 R separate the sera. The sera were kept at until used.

2- *P. bovicola* antigen:

The crude exoantigen was prepared acco Sundquist et al. (1988). Each ten *P. b* worms (from infected cattle in Swede incubated in 1 ml PBS for 60 minutes at 4° fluid was collected and centrifuged at 80(30 minutes and the supernatant was u exoantigen. The antigen was prepared Parasitology Department, National Vet Institute, Uppsala, Sweden. The used ant this investigation recorded no cross reacti other nematodes namely *Dictyocaulus vi Ostertagia ostertagi* and *Onchocerca* speci

3- ELISA procedure:

An available sites-ELISA described belo used. The exoantigen was diluted to a rate µg protein/ml in 0.1 M carbonate buffer, and adsorbed to micro-ELISA (Immunoplate II, Nunc, Copenhagen, Den overnight at +4° C. Serum samples and c positive and negative sera (National Vete Institute, Uppsala, Sweden) were diluted 1/ 0.1 M PBS, pH7.2 containing 0.05% Twe and 0.1 ml were added to each well after w with 0.01 M PBS with 0.05% Tween. Each sample was applied in 2 wells to get the result value. The plates were incubated f minutes at 37° C. Washing was carried ou Monoclonal mouse IgG anti Parafilaria bov (National Veterinay Institute, Uppsala Sw directed to the 41 kDa protein (Sundquist 1989) was diluted 1/600 together with r

antimouse IgG conjugated to HRPO (Dakopatts, Copenhagen, Denmark) diluted 1/1000 in 1 M PBS with 0.5% Tween 20 with 1% normal rabbit serum. Then the plates were incubated at 37° C for 60 minutes. The substrate solution consisted of recrystallized 5-amino-salicylic acid (Merk, Darmstadt, Germany) (pH 5.9) supplemented with 0.05% after H_2O_2 wahing the plates as mentioned before. The reaction was read after agitation at room temperature with a Titereck Multiscan spectrophotometer at 492 nm. Serum samples showing an inhibtion of the monoclonal antibody, less than 30% were considered to be sero-negative.

RESULTS

This investigation showed that all the tested sera were negative for *P. bovicola*. The inhibition rate was recorded to be 25 to 30% in cattle and buffaloes from Cairo. While, in Delta and Upper Egypt it was recorded to be 14.9 to 21.3%. The standard deviation was calculated at 95%. Table 1 showed that the higher inhibition rate was recorded in older ages from 10 to 15 years old (28.3%-30.8%) (Table 1).

DISCUSSION

There was no method of diagnosing the incidence of the *P. bovicola* in a herd, unless the bleeding

Table 1 Results of ELISA using *P. bovicola* antigen.

Animal	locality	Age	Sex	No. of samples .	Inhibition
Buffaloes	Cairo	10-15	F	35	28.3%
	Cairo	2	M	35	28.3 %
	upper Egypt	2-7	-	18	14.9%
Cattle	Cairo	-	-	31	23.4%
	Cairo	10-15	F	55	30.8%
	Cairo	2	M	24	25 .1 %
	Delta	2-7	F	133	15 .3 %
	upper Egypt	2-7	-	80	21.3 %

where No = the total number of the examined samples.

F= female and M= male.

and the inhibition % was calculated at 95 % S.D.

- = undetected

points in the live animal or the false bruising lesions on the carcasses after slaughter are found. The first bleeding points were only seen approximately 250 days after infestation. However, lesions of false bruising have been found in carcasses 130 days after infestation (Bech-Nielsen et al., 1982). ELISA could detect antibodies for *P. bovicola* 120 days after infection (Sundquist et al., 1988) i.e. half the time before the bleeding points were seen.

Using of monoclonal antibodies increased the sensitivity of the test. It was recorded to be more than 95% (Nevill et al., 1987).

P. bovicola was recorded in cattle and buffaloes in different countries (Chaunhan et al., 1974 and Nevill et al., 1987) however, Egypt was not included. The present study is performed to test sera of cattle and buffaloes from different localities in Egypt for *P. bovicola*. All the tested sera (411) gave negative result.

This study revealed the absence of specific *P. bovicola* antibodies in the tested sera which means the absence or the very low incidence of the parasite in Egypt. Probably, the high inhibition rate which was recorded in some samples was considered to be due to the presence of *Parafilaria sebticula* infection (Abd El-Hameed, 1987).

ACKNOWLEDGEMENTS

I would like to express my deep thanks to Prof. Dr. M. Kamal Selim for his valuable comments on the pre-print of this paper.

REFERENCES

Abd El-Hameed, Zeinab, K. (1987): Prelim morphological studies on the filarial worm *Onch armillata* in cattle and buffaloes M.V.Sc., Fac Veterinary Medicine, Cairo University, Egypt.

Bech-Nielsen, S., Bornstein, S., Christensson, D., Wa T., Zakrisson, G. and Chirico, J. (1982). *Para bovicola* (Tubangui 1934) in cattle: Epizootiology studies and experimental transmission of *Para bovicola* to cattle. Am. J. vet. Res., 43 (6): 948-95

Chauhan, P.P.S., Arora, G.S. and Ahluwalia, S.S. (19 note on the occurrence of an immature Parafilariid in the anterior chamber of eye of a buffalo (*B bubalis*). J. Helminth., 48: 289-291.

Dr Jesus, Z. (1934): Haemorrhagic filariasis in cattle by a new species of *Parafilaria*. Philip. J. S 125-132.

Fain, A. and Deramee, O. (1949): Les belminthes pa des bovides Astrida (Raunda-burndi). Annls. P Hum. Comp., 24: 207-214.

Marcoullis, G. and Grasbeck (1976): Prelim identification and characterization of antigen e from *Onchocerca volvulus*. Trop. Med. Parasito 314-322.

Metianu, T. (1949): Considerations sur la Parafil hemorragique des bovins. *Parafilaria bovico* Roumanie. Annls. Parasit. Hum. Comp., 24: 54-59

Nevill E.M. (1975): Preliminary report on the transm of *Parafilaria bovicola* in South Africa. Onderstep Vet. Res., 42 (1): 41-48.

Nevill, E.M., Wilkins, C.A. and Zakrisson, G. (1987 control of *Parafilaria bovicola* transmission in Africa. Onderstepoort J. Vet. res. 54: 547-550.

Niilo, L. (1968): Bovine haemorrhagic filariasis in cattle imported into Canada. Can. Vet. J., 9-132-137.

Nilsson, N.G. (1978): *Parafilaria bovicola*-rapport fran en arbetsgrupp (in Swedish). Sven. Veterinartidning, 30:785-787.

Patnaik, M.M. and Pande, B.P. (1983): A note on parafilariasis in Buffalo Bos (*Bubalis bubalus*) J. Helminth. 37 (4): 343-348.

Pienaar, J.G. and Van Den Heever, L.W. (1964): *Parafilaria bovicola* (Tubangui, 1934) in cattle in the Republic of South Africa., J. S. Afr. Vet. Med. Ass., 35: 181-184.

Sundquist, B., Zakrisson, G., Bech-Nielsen, S. and Bianco, A.E. (1988): Preparation and evaluation of specificity of *Parafilaria bovicola* antigen for detection of specific antibodies by ELISA. Vet. Parasitol., 28: 233-235.

Sundquist, B., Bech-Nielson, S. and Zakrisson, G. (1989). Characterization and purification of *Parafilaria bovicola* antigens by Chromatofocusing to enhance specificity in serodiagnosis. Vet. Parasitol., 33: 309-318.

Tubangui, M.A. (1934); Nematodes in the collection of the Philippine Bureau, of Science, II: Filarioidea. Philip. J. Sci., 55: 115-124.

Webster, W.A. and Wilkens, D.B. (1970): The recovery of *Parafilaria bovicola* Tubangui, 1934 from an imported Charolais bull. Can. Vet. J., 11: 13-14.