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EVALUATION OF INDIRECT ELISA IN DIAGNOSIS OF NATURAL OVINE CYSTICERCIOSIS AND HAEMONCHOSIS

K. SULTAN[1]*, A.Y. DESOUKY[1], N.M. ELBAHY[2], M.A. ELSIEFY[1]

[1]*Department of Parasitology, Faculty of Veterinary Medicine, Kafrelsheikh University, 33516, Kafrelsheikh, Egypt*
[2]*Department of Parasitology, Faculty of Veterinary Medicine, Menufyia University, Egypt*

*E-mail: Khsultan149@hotmail.com

ABSTRACT: *This study aimed to evaluate the sensitivity and specificity of indirect ELISA in diagnosis of natural infection of sheep with Cysticercus tenuicollis and Haemonchus contortus the most prevalent parasitic helminths in Egyptian sheep. By using non-purified crude antigens derived from the whole cyst of C .tenuicollis and adults H.contortus in the indirect ELISA assay; the results showed that both antigens sensitivity were 90%, 87.5% and the specificity were 60% and 75% respectively. These data proves the suitability of ELISA in diagnosis of such infections in living animals and the necessitation of using purified antigens rather than non-purified to increase the accuracy of the assay.*

SHORT COMUNICATION

Key words: *ELISA, Ovine, Cysticercus, Haemonchus*

INTRODUCTION

Sheep considers one of the most promising animals to achieve the aims of animal products supplies for the human being Haenlein and Abdellatif (2003). The infection with larval stage of *Taenia hydatigena (i.e. Cysticercus tenuicollis)* is considered one of the most wide spreading parasitic diseases infecting sheep all over the world causing considerable economic losses Abidi et al. (1989). In the other hand, *Haemonchus contortus* is regarded as the most important gastrointestinal nematodes infecting sheep in tropical and subtropical countries Sissay et al., (2007). In Egypt, *C. tenuicollis* and *H. contortus* infection in sheep were recorded with high prevalent Sultan et al., (2010).

For accurate identification of the digestive tract nematodes, the most wide spread method is fecal examination which includes fecal egg count and fecal larval culture; these methods requires experience, time-consuming and have doubtful results Eysker and Ploeger (2000) and moreover the ordinary diagnostic procedures for Ovine cysticercosis pre-mortem are useless El-Massry, (1988). While, the utilize of serological tests such as Enzyme Linked Immunosorbent assay (ELISA) are more sensitive and specific than the conventional methods of diagnosis of parasitic infections Ndao (2009).

This study was designed to evaluate the sensitivity and specificity of indirect ELISA in diagnosis of *C. tenuicollis* and *H. contortus* in sheep.

MATERIALS AND METHODS

During postmortem examination of sheep slaughtered in El-Mahalla El-Kubra abattoir, *C. tenuicollis and* adult *H. contortus* were collected, washed, examined and identified to species level according to available identification keys. Also, blood samples were individually collected in clean screw Falcon tubes, centrifuged at 3000 rpm/5 minutes to obtain clear sera, which were ambulated, labeled and preserved at -20 ̊C until use.

Cysticercus tenuicollis whole cyst prepared according to Elmassry (1999), while adult *Haemonchus contortus* crude antigens prepared according to Kandil (1994). The protein content of each was determined according to modified Lowry's assay (1951). Prepared antigens were ambulated and stored at -20 ºC until used. In order to figure the optimum dilutions of both serum and antigen checkerboard titration. *H.contortus* crude somatic antigen and *C. tenuicollis* whole cyst antigen) diluted at their optimal concentration, ELISA plate wells filled with 100 µl of the antigen, incubated, washed , blocked by 1% bovine serum albumin, and re-incubated, re-washed, sera diluted (in ratio 1: 100) were added, re-incubated, washed, adding of conjugate (i.e. Anti-sheep IgG whole molecule Alkaline Phosphatase, produced by Sigma® (used as instruction of the manufacturer), incubated wells, washed, Substrate (i.e. P- nitrophenyl phosphate produced by Sigma® and used as instruction of the manufacture), incubated, reaction appears with yellow coloration, stopped by addition of 1N NaoH 50 µl/ well and measured using ELISA reader (star Fax 303+, 12 well strips) at absorbance 405 nm.

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RESULTS AND DISCUSION

Sera collected from naturally infected sheep with *C.tenuicollis* (n=30) and free from infection (n= 10) were tested by indirect ELISA with whole cyst antigen concentration equals to 40 µg/ ml, the cutoff value (which calculated as double fold of mean of the negative sera) for *C.teniucollis* whole cyst antigen was 0.293 and 27 of 30 sera obtained from naturally infected sheep, this means 90% sensitivity. Four sera samples derived from apparently non-infected sheep gave positive reaction, so sensitivity was 60%.

While, *H. contortus* adult crude somatic antigen used with concentration equals to 40 µg/ ml; the cutoff value, was 0.326. Seven out of 8 derived from naturally infected sheep that were harboring *H.contortus* gave positive reaction (87.5% sensitivity), whereas 2 sera samples derived from sheep free from *H.contortus* in their abomasum gave positive reaction, so sensitivity was 75%.

Using of immunological assays as a tool for diagnosis of helminths infection seems promising tools. The results of *C.teniucollis* whole cyst antigen agreed with results of El-Massry (1999). The results of *H.contortus* adult crude somatic antigen agreed with results obtained by Handrilix (1990) and Schallig (1994). The considerable low level of sensitivity and specificity of both used antigens may be attributed to the antigens which used was crude non-purified, non-characterized antigen. Indicating that ELISA assay is rapid, easy and sensitive assay can be used in diagnosis of infections especially helminths infections, but must consider that its results depends mainly on the type of antigen which used and sera which used as a control positive and/or negative, in few words to obtain the best results, should use specific, purified antigen with positive control hyper-immune sera prepared in suitable lab animal and the negative control sera preferred to be sera of another host rather than animal species in the research.

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