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USING INDIRECT ELISA FOR THE SERODIAGNOSIS OF OVINE MONIEZIOSIS IN GIZA, EGYPT

By

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ABSTRACT

Monieziosis is one of the most important parasitic diseases of sheep;causing great economical losses in milk, meat, wool, death, infertility and costs of treatment. The aim of this study is to evaluate Indirect ELISA (iELISA) as a method of sero-diagnosis for *Moniezia expansa* infestation among sheep at Giza governorate. Two hundred eighty three sheep were examined for the infestation with *M. expansa* by feacal examination using concentration floatation techniques to detect *M. expansa* eggs and examination of intestines of slaughtered sheep to detect adult worms of *M. expansa.* The somatic antigen used to screen antibodies in the sera of the examined sheep was prepared from the adult worm. IELISA was applied on total of 92 sheep serum samples. It was found that prevalence of *M. expansa* among the examined sheep as 28.3% (80/283) by faecal examination. The sensitivity, specificity and accuracy of ELISA were statistically calculated as 100%, 41.6% and 92.4%, respectively.

It was concluded that iELISA is highly sensitive, less specific and highly accurate for the serodiagnosis of Ovine Monieziosis.

Keywords:

Moniezia expansa, iELISA, sheep, parasitological examination.

INTRODUCTION

Monieziosis is one of the most important parasites of sheep which cause an important problem in sheep breeding. Monieziosis has been noticed to be the most abundant helminth parasites infesting sheep through postmortem examinations **(Becker *et al.*, 1981; Polec, 1990; Maziad and El-Nemr, 2002).**

Monieziosis is one of the most common tapeworm infections in sheep and goats caused by *Moniezia* sp. known to occur in the small intestine. The genus *Moniezia* is an important

tapeworm and has many final hosts such as cattle, sheep, goats and other wild ruminants. The most important species is *Moniezia expansa, which more frequently parasitizes sheep,* and goats compare to other ruminants **(Al-Qureishy, 2008; Jie *et al.*, 2013).**

Monieziosis constitutes a major problem in sheep raising countries as it can negatively affect the productivity **(Agyei, 2003;Haridy *et al.*, 2004)**. Lambs are more susceptible than yearling and adult to *M. expansa* infection and massive infection causes diarrhoea and reduced weight gain **(Elliott, 1986).**

The parasitological method of diagnosis of *Moniezia* infection is usually performed by faecal sample analysis in which the eggs can be detected or often observation of gravid proglottids in the faeces and around anus. Such diagnosis is unreliable because the parasite eggs are not found during prepatent period and it cannot provide complete information on percentage of animals that have infection **(Roepstorff, 1998).** Therefore, the serological assays provide the accurate means of pre-mortem diagnosis of *M. expansa* infection in sheep and goats.

Specific antigen and sensitive immunodiagnostic tests would be useful aid to control this infection **(Hassanain and Abdel-Rahman, 2000).**

The information on immunological diagnosis of cestode infection is restricted **(Lightowlers, 1990).** The crude antigen can detect anti-*M. expansa* antibodies in sheep **(Haralampidis, 1987; Polec, 1990).**

More precisely this study aims to evaluate iELISA as sero-diagnostic method for Ovine Monieziosis.

MATERIAL AND METHODS

2.1. Samples:

2.1. 1. Two hundred eighty-three faecal samples of sheep were collected to screen *M. expansa* infestation in sheep flocks at different localities of Giza governorate, Egypt, by parasitological examination according to **Soulsby (1986).**

2.1.2. Ninety two-serum samples were collected from the examined sheep to be tested by iELISA. A zero-day lamb serum was used as negative control in iELISA.

2.1.3. Adult worms of *M. expansa* were collected from slaughtered sheep at the Elmonieb abattoir and were used for the somatic antigen preparation.

2.2. Parasitological examination:

2.2.1. Concentration flotation technique:

This technique was adopted to diagnose the other eggs. According to **Soulsby (1986).**

2.2.2. Postmortem examination of the slaughtered sheep:

The slaughtered sheep were examined for the presence of *M. expansa* adult worms in the small intestine. The worms were collected for the somatic antigen preparation according to **Prasad *et al*. (2007).**

2.3. Preparation of Somatic Antigen:

The somatic antigen was prepared by following the procedure of **Prasad *et al.* (2007)** with slight modification by **Ananda *et al.* (2016)**. The adult worms of Moniezia recovered from the intestine of sheep were washed thoroughly in Hank's balanced salt solution. That, the worms were transferred to a screw-capped vial containing 0.15 M phosphate buffered saline (pH 7.2). The segments were triturated using a glass mortar and pestle. The contents were repeatedly frozen and thawed for four times and then disrupted by three times sonication using "Cole parmer ultrasonic Homogenizer "under 150 watt interrupted pulse output at 50% power cycle in ice bath for 20 seconds each time at 100 mAmp at 4°C at parasitological laboratory, Faculty of Veterinary Medicine. The suspension was centrifuged at 12000 rpm for 30 min in a refrigerated centrifuge (4°C). The supernatant was collected and used as the soluble antigen extract. The protease inhibitor phenyl methyl sulphonyl fluoride (PMSF) (Sigma, USA) was added at the concentration of 2 ml/ml of antigen. As the antigen was prepared in saline solution and stored at -20°C until further use.

2.4. Indirect Enzyme Linked Immuno Sorbent Assay (iELISA):

Indirect ELISA used to detect antibodies against *M. expansa* in the examined sheep sera; was applied as previously described by **Ananda *et al.* (2016)** as follows.

Flat bottom polystyrene 96 well ELISA plate was coated with 100 µl containing 2µg of somatic antigen in coating buffer in duplicates. The plate was incubated at 4°C overnight and washed thrice with washing buffer. The plates were incubated at 37°C for one hour after adding 100µl of blocking buffer (5% skimmed milk powder with PBS Tween-20) and washed thrice with PBS Tween-20. 100 µl serums (1:100 dilutions) with blocking buffer was added to all wells and incubated for one hour at 37°C. The plates were washed four times with washing buffer followed byadding 100 µl of1:1000 diluted anti-ovine conjugate (alkaline phosphatase) and incubated as above. The plates were washed five times with washing buffer. Then 100µl of substrate chromogen working solution was added. The reaction was stopped by adding 50µl of 2M H2S04. The absorbance values were read in ELISA reader at 450 nm.

The positive control and negative control were included in the assay in duplicate.

2.5. Determination of the Cut off Value:

Ten known negative serum samples were obtained from a zero-day lamb (serum).

This was used to determine the cut off value and was calculated by taking mean absorbance values of known negative sera plus three standard deviation. Any serum with OD values above the cut off value was considered as positive.

2.6. Evaluation of iELISA:

Sensitivity, specificity and accuracy of iELISA were conducted according to **Wayne *et al.*, (1987); Riegelman and Hirsch (1989); Dawson and Trapp (1990)** as follow**:**

$$\textbf{Sensitivity} = \frac{\textbf{True positive}}{\textbf{True positive} + \textbf{False negative}} \textbf{x 100}$$

$$\textbf{Specificity} = \frac{\textbf{True negative}}{\textbf{True negative} + \textbf{False positive}} \textbf{x 100}$$

$$\textbf{Accuracy} = \frac{\textbf{Truepositive} + \textbf{Truenegative}}{\textbf{Totalnumber } (n)} \textbf{x 100}$$

RESULTS

3.1. Faecal examination:

Prevalence of *Moniezia* expansa in the examined sheep was 28.3% (80/283) depending upon detection of Moniezia egg (photo-1 and 2) by concentration flotation technique of faecal samples.

Effect of sex, age and seasons on prevalence of *Moniezia* infested sheep are illustrated in (Table 1). The higher prevalence of *Moniezia* infested sheep were showed in female lambs and in winter season, while the lower prevalence of *Moniezia* infested sheep were recorded in aged ram (more than 3 years of age) in spring season.

Table (1): Effect of sex, age and seasons on prevalence of *Moniezia* infested sheep.

		No. of examined sheep	No. of *Moniezia* infested sheep	Prevalence% of *Moniezia* infested sheep
Sex	Male	68	24	8.5%
	Female	215	56	19.8%
	Total	283	80	28.3%
Age	3 m to ≤ 1 y	128	32	11.3%
	> 1 y to ≤ 2 y	74	18	6.4%
	> 2 y to ≤ 3 y	56	19	6.7%
	> 3 y	25	11	3.9%
	Total	283	80	28.3%
Season	Winter	100	33	11.7%
	Autumn	83	12	4.2%
	Summer	70	25	8.8%
	Spring	30	10	3.5%
	Total	283	80	28.3%

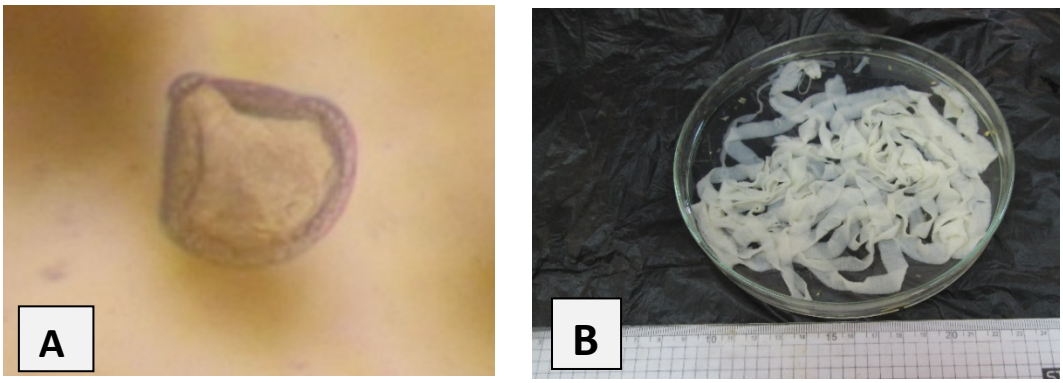

Photo (1): A: *Moniezia expansa* egg in the faecal sample of the infested sheep examined with concentration flotation technique; B: *Moniezia expansa* adult worm.

3.2. IELISA:

Eighty-seven serum samples out of 92 were positive by iELISA depending upon cutoff point of optical density0.103.Thisresultis illustratedin(Table 2).

Table (2): Indirect ELISA compared with gold standard parasitological examination.

		Parasitological examination (gold standard)		
		Positive (Diseased)	Negative (Non-diseased)	Total
IELISA	+ve	(T+) 80	(F+) 7	87
	-ve	(F-) 0	(T-) 5	5
Total		80	12	92

False positive (F+), False negative (F-), True positive (T+), True negative (T-).

3.3. Statistical evaluation of iELISA:

The sensitivity, specificity and accuracy of indirect ELISA test was calculated as 100%, 41.6 %, 92.4%, respectively when compared with gold standard test (parasitological examination) as shown in (Table 3). All the serum samples of sheep infested with *M. expansa* showed positive reaction in ELISA. However, 7 serum samples of sheep which did not harbor any *M. expansa* eggs showed positive reaction in ELISA.

Table (3): Statistical evaluation of iELISA.

Sensitivity	Specificity	Accuracy
100%	41.6%	92.4%

DISCUSSION

Moniezia expansa could be considered as the most important cestodes infesting sheep, causing gastrointestinal disorders and even death in sheep **(Yan *et al.,* 2013).**

The diagnosis of Monieziosis by using conventional methods is unreliable because, the parasite eggs are not found during pre-patent period and they do not provide any information on percent prevalence of infection **(Roepstorff, 1998).** Therefore, the immuno-diagnostic methods are more reliable and alternative methods in diagnosis of Monieziosis caused by all stages of tapeworm.

In the present study,the overall prevalence of (80) 28.3% was observed in sheep.

Anumol *et al.* (2011) reported 15.69 % prevalence of Monieziosis in goats. **Jena *et al.* (2018)** examined 1506 fecal samples of Chottanagpuri sheep in and around Ranchi and found that

329 (21.85%) infested with cestodes. **Etibar (2009)**; **Bashtar *et al.* (2011);** reported higher prevalence of *M. expansa* infection in sheep 74.0% in Egypt and 65.5% in Sherur Region of Nakhichevan Autonomous Republic, respectively.

Al-Qureishy (2008) conducted a slaughterhouse survey in Riyadh city, Saudi Arabia to record the prevalence of cestode parasites in sheep and found that 96.3% prevalence of *M.expansa*. **Ijaz *et al.*(2009),Bhat *et al.*(2012) and Poddar *et al.*(2017)** recorded infestation rate with *Monezia* 1.34%, 3.3% and 3.8%, respectively.

There was a seasonal variation in the rate of *Moniezia* infestation; the highest prevalence of Monieziosis was recorded in winter season (11.7%), followed by summer season (8.8%), then autumn (4.2%) and spring (3.5%). Nearly similar results were recorded by **Yeasmin *et al.* (2014); Jena *et al.* (2018).** The higher prevalence of infestation with monieziosis in winter seasons might be attributed to that, the level of infestation was somewhat higher during the rainy season than during the dry season. İn rainy season, favorable climatic condition and higher moisture and humidity that may encourage survival of cestodes and worms.

Prevalence of Monieziosis was higher in female sheep (ewe) (19.8%) than in male sheep (ram) (8.5%). The higher prevalence in females might be attributed to reproductive physiological stress during pregnancy and parturition, which lower their resistance and causes reduction of immune status, also, due to their enhanced grazing during lactation. These results disagree with **Bashir *et al.*(2012)** reported a significant difference ($P<0.05$) in the overall incidence of gastrointestinal parasites between male (75.6%) and female (44.8%) sheep data analysis based on sex. **Yeasmin *et al.*(2014)** detected higher prevalence of helminth in case of male sheep (81.58%) than female (72.73%).

The highest percentage of infestation with Monieziosis was recorded in lamb with 11.3% followed by 6.7% in group age (2-3 years), then 6.4% in age group (1-2 years) while the lowest percentage of infestation 3.9% in group age (over three years). These results were previously reported by **Bashir *et al.* (2012); Jena *et al.* (2018).**

The variation of age susceptibility might be attributed to acquired immunity that lacked in young lambs, as they were not exposed to previous infestation.

Young animals are more susceptible to worm infestation due their weaker immunological response. This can be linked to weaning stress, low live weight and fat reserves or inadequate nutrition. In contrast, **Yeasmin *et al.*(2014)** did not support these results**; Poddar *et al.* (2017)** who noticed that higher prevalence of infestation was observed in adult sheep than in young

sheep and lamb. The prevalence variation of *M. expansa* infection in sheep between the prevalence in different studies may be attributed to change in the environmental factors, the type of rearing, regional differences, seasons, age, sex, hygiene; number of samples screened and control measures, where sheep raised on high protein diet developed resistance to parasitic gastroenteritis **(Knox and steel, 1999)**. In addition, the changes in climatic condition may affect the degree of infection.

In the present study, a total 92 serum samples from sheep were used to detect the circulating antibodies against *Monezia expansa.* Eighty-seven serum samples of sheep were showed positive reactivity in ELISA with a sero-prevalence of 94.5%. However, only five serum samples of sheep showed negative reaction in ELISA. The sensitivity, specificity and accuracy of ELISA compared with gold standard parasitological examination was calculated as100 percentage, 41.6 %, and 92.4%, respectively**. Ananda *et al.* (2016)** studied the sero-prevalence of *Moniezia expansa* infection in sheep by Indirect-Enzyme Linked Immuno Sorbent Assay (iELISA) using somatic antigen. All the serum samples of sheep infested with *M. expansa* showed positive reaction in iELISA. However, 94 serum samples of sheep, which did not harbor any *M. expansa* worms, showed positive reaction in iELISA. The sensitivity and specificity of iELISA was calculated as 100% and 65.18%, respectively. In addition, 250 serum samples were also collected from migratory sheep in and around Shimoga for detection of circulating antibodies of *M. expansa* and found 94 serum samples positive by iELISA with a sero-prevalence of 37.6%. **Abdel-Megeed *et al.* (2014)** used ELISA for immuno-diagnosis of *M. expansa* infection in sheep, 53 out of 76 (69.7%) sheep serum samples were found positive for Monieziosis. This variation might be due to different number of samples, change in the preparation of the antigen, and the type of antigen used.

In indirect ELISA, the seven false positive samples might be due to the cross-reaction of *Moniezia expansa* with other helminths or the parasite eggs are not found during prepatent period **(Roepstorff, 1998)** or previous exposure or treated animals. This cross reactivity is one of the limiting factors for the development of serological tests against helminth infection **(Molina *et al.*, 1999).**

CONCLUSION

It was concluded that iELISA is highly sensitive, less specific and highly accurate for the serodiagnosis of Ovine Monieziosis. iELISA could be carried out for mass screening of Monieziosis among sheep population compared to routine parasitological methods, but it need another serodiagnostic test of high specificity to be performed together to overcome its low specificity.

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