Journal of Animal and Veterinary Advances 9 (13): 1880-1884, 2010
ISSN: 1680-5593
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Diagnosis of *Psoroptic mange* in Sheep by Modified Modified ELISA Test

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Abstract: The present study used modified ELISA to detected the specific antibodies to *Psoroptes ovis* by using whole mite extract from naturally infested sheep. The total sera was 96 which include 46, 29, 21 from Lambs, Ewes, Rams, respectively and 24 sera from experimentally infested Lambs with *P. ovis* which include 12 sera consider as negative control and 12 sera which consider as positive control. The cut-off level of negative control sera was 0.532, specificity and sensitivity according to the cut-off were 81.1 and 81.4%, respectively to all groups and when determined to each group the specificity was 91.7% in Ewes, 80% in Lambs and 75% in Rams. The sensitivity was 100% in Lambs and Rams and 52% in Ewes. In comparison with sensitivity to microscopical examination of skin scraping which was 36% in Lambs, 41.6% in Ewes and 20% in Rams. These results were indicated to that modified ELISA is a simple, accurate test and could be used for monitoring of infestations of *P. ovis* in naturally infested sheep. The prevalence of Psoroptic mange in sheep is depending on results of ELISA was 44.7% while in Lambs, Ewes and Rams the prevalence of mange was 45.6, 58.6 and 23.8%, respectively. High prevalence of Psoroptic mange in Hamdani breed was 71.4% in comparison with Awassi breed it was 37.3% .

Key words: *Psoroptes ovis*, ELISA, *Psoroptic mange*, lambs, comparison, Iraq

INTRODUCTION

The sheep scab mite *Psoroptes ovis* is an obligate ectoparasite of sheep causing a highly contagious condition which if left untreated can result in emaciation and even death (Tarryn, 1974). Infestation by this parasite provokes sever exudative skin lesion associated with intense pruritis which lead to rubbing, scratching, wool loss, wounding and fitting (Bates, 1997; Van den Broek and Huntley, 2003). Psoroptic sheep scab is of economic important and a notifiable in many countries where the incidence of this disease has increased over the last years dispite compulsory control measures (Clark *et al*., 1996). The traditional diagnosis of *P. ovis* infestation is based on clinical observation and microscopical detection of mite in skin scrapings, since clinical symptoms are not pathognomic and examination of skin scrapings using microscopic techniques exhibit only low to medium sensitivity (Meleney and Christy, 1978). The diagnosis of *P. ovis* infestation in sheep based on these traditional methods is unsatisfactory (Ochs *et al*., 2001). Clinical outbreak of mange usually are notified during winter whereas *P. ovis* infestation remain in a latent form during the grazing season. Cryptic anatomical sites of sheep such as infra-orbital and inter digital fossa or external auditory canal are discussed as potential location where mite may survive during latent phase of the infestation (Kirkwood, 1985). Since Fisher (Fisher, 1972) demonstration specific antibodies against psoroptes crude extracts in *P. ovis* infested sheep several assays have been developed for antibody detection in sheep infested with psoroptic mite (Fisher, 1983a, b; Wassall *et al*., 1987; Boyce *et al*., 1991; Lonneux *et al*., 1996). In this study we evalution of modified ELISA for detection serum antibodies against *P. ovis* in sheep.

MATERIALS AND METHODS

Animals: The total animal was 326 which include 230 from Awassi breed and 96 from Hamdani breed which is naturally infested with *P. ovis* in the university of mosul/collage of veterinary medicine and collage of Agriculture divided into three groups Lambs (1-4 month) 86, Ewes (2-5 years) 171 and Rams (2-7 years) 69 (Table 1).

Experimentally infested animals: We used 30 Lambs aged 1 month which born in the farm of agriculture collage which not exposure to infestation with *P. ovis*, Isolated these animal with his dam for about 3 month housed under helminth and arthropod free condition.

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Table 1: Sources and description of samples that used in ELISA test

Groups	No.	Clinical signs	No. of samples Skin scraping	Serum	Types of Infestation	Source
Lambs	86	Pruritus	11	46	*Psoroptes ovis*	Collage of veterinary medicine and collage of agriculture
Ewes	171	Pruritus with wool loss	12	29	*Psoroptes ovis*	Collage of veterinary medicine and collage of agriculture
Rams	69	Sever skin lesion with pruritus	5	21	*Psoroptes ovis*	Collage of veterinary medicine and collage of agriculture
Total	326		28	96		

Samples

Skin scrapings: Skin scrapings was collected from 28 sheep which include 11 from Lambs, 12 from Ewes and 5 from Rams (Table 1), Skin scrapings were digested in 10% (w/v) potassium hydroxyel (KOH) for at least 4 h at room temperature and examined in a Petri dishes using stereomicroscope with transillumination (50X), detected mite were transferred to microscopic slides for morphological differentiation (100x) (Sanders *et al*., 2000).

Collection of *P. ovis* by using modified mite collector equipment: This equipment made according to Thind and Ford (2003) with some modification and it is consider a portable equipment is easy to use under field condition this method for mite collection consider a simple, robust, reliable method that could remove sheep scab mite in large numbers without damaging them and with minimal discomfort to the sheep (Thind and Ford, 2003). The equipment consist of four main part A-sampling arena, B-filtration unite, C-peristaltic pump, D-a reservoir contain saline (Fig. 1).

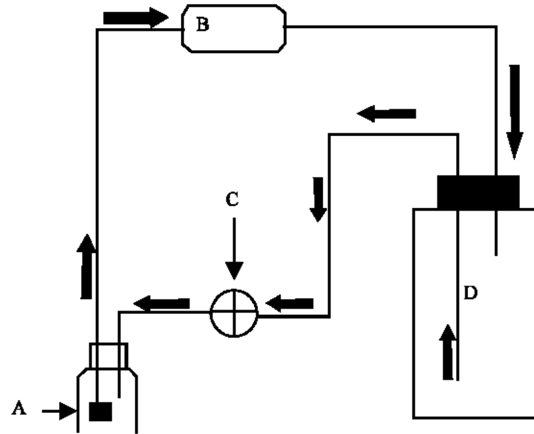

Fig. 1: Diagram of the mite collector A-sampling arena, B-filtration unite, C-peristaltic pump, D-a reservoir contain saline

Blood samples: Blood sample was collected from jugular vein from 96 sheep including 75 Awassi breed and 21 Hamdani breed to abtain serum which is divided into 3 groups, 46 from Lambs, 29 from ewes and 21 from Rams (Table 1). The serum samples stored -20°C until used (Ochs *et al*., 2001).

Experimental infestation with *P. ovis*: Thirty Lambs were used in this experiment were divided in two groups (15 Lambs group^{-1}). The first group was experimentally infestated with *P. ovis* approximately 50 female adult mite by deposition of mite on the back, All animals developed typical clinical singe except three, blood samples were taken weekly from jugular vein after clinical sings appear and consider as positive control while the scond group left under helminth-arthropod free condition, blood samples were collected from 8 weeks from began the experiment and consider as a negative control, negative and positive control were used in ELISA to determined cut-off value (Ochs *et al*., 2001).

Soroptes ovis Whole Mite Extract (WME): *P. ovis* Whole Mite Extract (WME) was prepared from 5000 living mite in all stages (adult, nymph and larvae) from donor infested sheep. The living mite washed by vortex for about 5 min in ice-cold Phosphate Buffered Saline (PBS PH 7.2) followed by 1% Sodium Dodecylsulphate (SDS) at room temperature in an attempt to remove any host immunoglobulin which might have been adhering to the external surface, the mite were then washed 10 more times in ice-cold PBS to remove the SDS residual, mites were then homogenized and sonicated in ice-cold PBS homogenized repeated three time in about 5 min in 500 cycle min^{-1}, the final homogenate was centrifuged at 3000 cycle min^{-1} for 20 min (at 4°C), the supernatant (WME) was filtrated by using (Nitrocellulose filter paper 0.45 μm) (Smith and Pettit, 2004) and then protein content of the supernatant was determined in accordance to modified folin lowery method (Schacterle and Pollack, 1973) and finally WME stored in -20°C.

Titration of antigen for ELISA test: Different concentration were prepared (2.3, 4.6, 9.3, 18.7, 37.5, 75, 150, 300, 600) mg Protein mL^{-1} PBS to determined the best titer which is the minor titer give positive result in ELISA test (Van den Broek, 2003).

Modified enzyme-linked immunosorbent assay: Serum samples were assayed in duplicates, microtiter plate wells filled with 100 μL of mite extract (10 μg/1 mL PBS) and

incubated overnight at 37°C after that incubated with 10% w/v skimmed milk powder overnight at 37°C. The wells were filled (100 μ) with serum of sheep and incubated for 2 h at 37°C. The plate were emptied given a standard wash filled and emptied three time with wash buffer, 100 μL $well^{-1}$ of Rabbit anti-sheep IgG conjugated to alkaline phosphatase diluted 1:50 with PBS was added to each well after the incubation of 2 h at 37°C. The plate were washed three time and 100 μL $well^{-1}$ of substrate solution (Tetraethyl benzidine) was added to each well and after 1 h in dark the color of reaction was stopped by the addition of 100 μL Hcl. OD was measured at 405 nm with Automated microplate reader. The results are expressed as antibody unites which were calculated as percentage of the OD of positive reference serum minus the cut-off value OD percentage = OD(X)-OD(-)/OD(+)-OD(-) X 100, Cut-off = $\bar{X}$ -3 S.D of negative control, OD(X) = OD of suspected serum, OD(-), OD (+) = OD of negative and positive serum (Jacober *et al*., 2006).

Comparison of microscopical examination of skin scrapings with ELISA test in all group as well as determine the prevalence of psoroptic mange in different breed (Awassi and Hamdani), determination the percentage of specificity and sensitivity in the following formula:

$$\text{Percentage of specificity} = (A-B)/A \times 100$$

Where:

A = Total number of non-infested animal which having negative result in ELISA

B = Number of non-infested animal which have OD above cut-off value

$$\text{Percentage of sensitivity} = (C-D)/C \times 100$$

Where:

C = Number of infested animal

D = Number of infesed animal which have OD under cut-off value

Statistical correlation were estimated using the student's t-test by using sigma stat for windows version 3.10 copyright© 2004 systat software inc.

RESULTS AND DISCUSSION

About 12 of experimentally infested lambs were showed typical clinical signs: Rubbing, yellow scabs during 9-21 days after exposure to adult female *P. ovis* the mean OD value (2.15, SD = 0.075) of the positive control was about five time higher than the mean OD value (0.449, SD = 0.027) of negative control. The intra-and inter-plate variation was narrowing by using duplicated plates. The prevalence of Psoroptic mange was 44.7% according to ELISA, 43 sera were positive from 96 and the prevalence to every groups were in Lambs 45.7, ewes 58.6 and Rams 23.8 (Table 2). While the prevalence of Psoroptic mange in Awassi breed was less than Hamdani breed which was 37.3% in Awassi compared with 71.4% in Hamdani, 28 sera was positive from 75 sera in Awassi breed while in Hamdani 15 sera was positive from 21 sera (Table 3).

When compared the results of ELISA test with skin scrapings we found 22 sera positive to ELISA from 28 sera (78.6%) compared with skin scrapings 10 animal positive to skin scraping from 28 (35.7%) and to every group: Lambs 100% positive to ELISA test compared with 36.4% positive to skin scraping, Ewes 50% positive to ELISA test compared with 41.6% positive to skin scraping and Rams 100% positive to ELISA test compared with 20% positive to skin scraping (Table 4).

The ELISA test show high efficiency to detect *Psoroptic mange* in the form (clinical and subclinical) this depend on specificity and sensitivity. The sensitivity in Ewes was 52.9% while 100% in both Rams and Lambs, the specificity was 80% in Lambs, 91.7% in Ewes and 75.0% in Rams (Table 5).

The ELISA described in the present study was more specific to detected psoroptic mange in subclinical form than microscopical examination of skin scrapings. The choice of cut-off values is a critical element in

Table 2: The prevalence of psoroptic mange according to ELISA

Groups	Serum number	Negative sera		Positive sera	
		Number	Percentage	Number	Percentage
Lambs	46	25	54.3	21	45.7
Ewes	29	12	41.3	17	58.6
Rams	21	16	76.1	5	23.8
Total numbers	96	53	55.2	43	44.8

Table 3: The prevalence of psoroptic mange in awassi breed and hamdani breed according to ELISA

Breed	Sample number	Positive	
		Number	Percentage
Awassi	75	28	37.3
Hamdani	21	15	71.4
Total	96	43	44.8

Table 4: Comparison the results of ELISA test with skin scraping

Groups	Samples	Positive serum		Positive to microscopica examination	
		Number	Percentage	Number	Percentage
Lambs	11	11	100.0	4	36.4
Ewes	12	6	50.0	5	41.7
Rams	5	5	100.0	1	20.0
Total	28	22	78.6	10	35.7

Table 5: The efficiency of ELISA test (specificity and sensitivity) to detect Psoroptic mange

Groups	Specificity (%)	Sensitivity (%)
Lambs	80.0	100.0
Ewes	91.7	52.9
Rams	75.0	100.0

immunoassay design, An advantage of using the mean plus 3DS is that it minimize the risk of false positive, thereby increasing the predictive value of positive test result (Hollanders *et al*., 1997). The observed low sensitivity of the with ELISA 81.4% may be due to false negative samples, the sensitivity was much lower for Ewes 52.2% than Rams and Lambs 100% this difference is due to 8 Ewes clinically infested with *Psoroptes ovis* but have low titer of antibodies below the cut-off level (false negative) and this may be these Ewes in the first stage of disease and not reach to seroconversion or may be these animal treated recently with endectocides (ivermectine) (Arlian and Morgan, 2000).

While the specificity was 81.1% is due to 10 samples show false positive results, these animal don't show any clinical signs of mange this is due to latent form of psoroptic mange, the presence of false positive is due to cross reaction with other ectoparisites like Dermatophgoides pteronyssinus (Stewart and Fisher, 1986), *Chorioptes bovis*, *Sarcoptes scabiei*, *Notoderes cati* (Matthes *et al*., 1996) and ticks (Wozniak *et al*., 1996) this reason is impossible because all skin scraping examined and don't show any any of these ectoparisites. In this study we discussed the releshinship between microscopical examination of skin scraping and ELISA test as (Ochs *et al*., 2001) described which these animal have antibodies without any clinical sings this is due to latent phase of disease, this really occur in Rams which have antibodies with negative result in microscopical examination.

According to ELISA result Ewes and Lambs show highly prevalence comparison with Rams this may be because Lambs (1-2 month) persist presence with her mother during lactation period as well as maybe because presence of maternal antibodies in colostrum which cause high antibodies titer in Lambs in omparison with Ewes especially in Lambs under 1 month, as Butler (1999) said the level of humeral immunity against parasite in colostrum in Lambs more than in Ewes and the IgG is the common type in colostrum and small amount of IgM, IgA and the amount of IgG1 more than IgG2, the way that IgG pass to the endothelium of udder to colostrum in ruminant through (FCRN: neonatal Fc receptors) while IgA and IgM pass through polymeric Immunoglobulin receptor (Mayer *et al*., 2002) after birth in few hour absorption occur to these antibodies in neonatal intestine in way pinocytotic absorption and then decrease the level of these antibodies in milk (Butler, 1999; Mayer *et al*., 2002) and some of these Immunoglobuline specially IgG1 and IgA present in milk for long time but (Van den Broek *et al*., 2000) said that IgA is not important in immunity against *P. ovis* in sheep.

CONCLUSION

In this study we discovered significant differences in severity of infestation with *P. ovis* and prevalence between breeds of sheep, the severity of skin lesions and the number of mites in Hamdani breed more than in Awassi breed that precence in the same location and have same strain of *P. ovis* (Fourie *et al*., 1997).

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