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BACTERIOLOGICAL AND IMMUNOLOGICAL PROFILES OF BRUCELLOSIS IN SHEEP AND GOATS (With 4 Tables)

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رؤية بكتريولوجية وامينولوجية عن مرض البروسيلات في الاغنام و الماعز

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تشخيص مرض البروسيلات في الاغنام و الماعز يحتاج الى أكثر من اختبار سيروولوجى ، ويفحص ١٠٥٦ غنمه و ٢٦٤ ماعز باستخدام اختبار الروزبنجال وجد أن ٢١ عينة بنسبة ١.٩٩% و ٧ عينة بنسبة ٢.٦٥% ايجابية لمرض البروسيلات على التوالي ، وكان معدل التوافق بين الحيوانات الايجابية لاختبار الروزبنجال و اختبار التجمع البطى في الاغنام (٨٦ و ٤٢%) وفى الماعز (٥٧ و ٤%). وكان معدل التوافق عالى وذلك باستخدام اختبار التجمع البطى المعدل بالاديتا وكذلك اختبار الريفانول (٨٠ و ٩٥%) فى الاغنام و (١٠٠%) فى الماعز . ولقد تم عزل أربع عترات ميكروب البروسيلات ميلتينسيس النوع الثالث من الغدد الليمفاوية من هذه الحيوانات.

SUMMARY

The diagnosis of Brucellosis in small ruminants requires the use of more than one serological test. Examination of 1056 sheep and 264 goats by RBPT revealed that 21 (1.99%) and 7 (2.65%) were positive reactors for brucellosis. The rates of the agreement between the positive RBPT and SAT reactors were (42.86%) and (57.14%) in sheep and goats respectively. A higher rates were obtained on using both EDTA-modified SAT and RT, (80.95%) in sheep and (100.00%) in goats. Four isolates of *Br. melitensis* biovar 3 were recovered from the lymph nodes from sheep and goats.

Key words: *Brucellosis - Sheep & Goats - Bacteriology - Immunology*

INTRODUCTION

Sheep and goat husbandry has been practised in Egypt in small farms and grassing animals. Such livestock and their products play an important

part in the economy of the area and in the nutrition of the populace, so that any disease which can be transferred from animal to animal and more seriously from animals to man requires more than passing recognition. Brucellosis, being a contagious disease of animals causing abortion and infertility, while its zoonotic nature gives rise to serious recurrent sickness in man, cases of which have been reported from time to time by the medical authorities.

A battery of tests is usually used in the serological diagnosis of brucellosis (Falade, 1978; Waghela et al., 1980 and Alton *et al.*, 1988), but the practice is time consuming and uneconomical when a large number of animals are involved. It was therefore decided to investigate whether the use of Rose Bengal plate test (RBPT) normally employed as a screening test (Morgan *et al.*, 1978) was by itself sufficient in the detection of ovine and caprine brucellosis. Thus, it was compared with standard agglutination test (SAT) and more sensitive tests as EDTA modified SAT (MacMillan, 1990) and rivanol test (RT) (Anczyhowski and Murat-Skwersk, 1979).

MATERIAL and METHODS

A total of 1320 blood samples were collected from 1056 sheep and 264 goats from Cairo, Giza and Assiut governorates.

All the blood sera were subjected to RBPT, and those gave positive reactions were furtherly subjected to SAT and RT according to Alton *et al.* (1988) and EDTA-modified SAT according to MacMillan (1990)..

On the other hand, a homogenised portions of lymph nodes of RBPT positive reactor sheep and goats were bacteriologically examined for brucella organisms using both direct culture and Guinea pig inoculation followed by culture on selective media. Suspected colonies were identified and typed into brucella biovar using the serological and biochemical methods according to Alton *et al.*(1988).

RESULTS

Out of the 1056 and 264 serum samples collected from sheep and goats, 21 (1.99%) & 7 (2.65%) were positive to RBPT respectively (Table 1).

Examination of the positive RBPT reactors of sheep and goats revealed that 9(42.86%) and 4(57.14%) were positive to SAT at end titre varied from 1/40 to 1/160 respectively. On the other hand, the results of the

confirmatory tests revealed that 17(80.95%) in sheep and 7(100.0%) in goats showed agglutination to both EDTA modified SAT and RT with end titre varied from 1/10 to 1/160 and 1/25 to 1/200 respectively (Tables 2 and 3).

Bacteriologically, 4 brucella isolates were obtained from the lymph nodes of sheep and goats and were identified and typed as *Br. melitensis* biovar 3. The four isolates were obtained from 3 sheep and one goat giving positive serological reaction to all the previously mentioned tests except one isolate was recovered from sheep giving suspicious reactin to SAT (Table 4).

DISCUSSION

Brucellosis, an acute or chronic contagious disease, is characterized by septicemia followed by localization of infection in lymph nodes and genital organs and is usually caused by *Br. melitensis*, but occasionally also by *Br. abortus*, species of bacterial pathogens that attack most mammals including man.

In this study, a total of 1320 serum samples obtained from 1056 sheep and 264 goats were examined firstly by RBPT as a screening test and positive ones were reexamined by SAT, EDTA modified SAT and RT to clear up the state of infection in such animals.

From the afore-mentioned results, it is clear that, the discrepancy between the higher number of reactors detected by RBPT than the other tests is due to the fact that RBPT is a highly sensitive test (Nicoletti, 1969) which can detect low titre as in cases of chronic brucellosis, that can not be considered positive by the quantitative tests. Moreover, samples positive to RBPT and negative to SAT could mean that these animals were suffering from chronic brucellosis.

On the other hand, EDTA modified SAT and RT identify more positive samples in both sheep and goats (Table, 2 and 3) which were initially suspicious by SAT, a result that agree with Falade(1978) and Weghela *et al.* (1980) who detected positive reactors of sheep and goats showed low titres by SAT. This difference in number of positives among SAT and the confirmatory test could be explained on the assumption that SAT is 10 times more sensitive to IgM than IgG1 and IgG2 antibodies (Allan *et al.*, 1976).

Moreover, in the confirmatory test, EDTA modified SAT and RT, brucella antigens react with IgG antibodies besides, the Fab portion of IgM antibodies in EDTA modified SAT. Therefore, these tests are regarded as the tests nearest to reflecting the true status of brucellosis in animal's, where reduction, persistence or increase in titre can occur (Nicoletti, 1969; Garin *et*

al., 1985 and MacMillan, 1990). Meanwhile negative confirmatory tests in low titre SAT in sheep indicated that EDTA modified SAT and RT eliminate such non specific reactors to SAT (Nielsen and Duncan, 1982 and Falade, 1983).

The detailed bacteriological examination of the 28 lymph nodes revealed that the best chance for isolation could be obtained from animals with high blood titre (Shini and Tabatabazy, 1981 and El-Gibaly, 1993). One isolate could be obtained from suspicious sheep to SAT, a finding which supports the confirmatory test result (Ibrahim et al., 1993). The obtained four isolates were typed as *Br. melitensis* biovar 3 (Table, 4).

Finally, it seems valuable for reference laboratories to use EDTA modified SAT and/or RT besides, the conventional tests (RBPT and SAT) used routinely for diagnosis of brucellosis in ovine and caprine hand to hand with bacteriological examination to assure the actual status of the animals and supports the serological findings specially in animals giving inconclusive serological results.

REFERENCES

- Allan, G.S.; Charppel, R.J.; Williamson, P. and McNaught, D.J. (1976): A quantitative comparison of the sensitivity of serological tests for bovine brucellosis to different antibody classes. *Journal of Hygiene*, 76: 287-295.
- Alton, G.G.; Jones, L.M.; Angus, R.D. and Veger, J.M. (1988): Techniques for brucellosis laboratories. *Inst. Nat. De la Rec. Agronomique, INRA, Paris*.
- Anczyhowski, F. and Murat-Skwarak, P. (1972): On the value of the rivanol test in routine diagnosis of bovine brucellosis. *Pol. Arch. Water.*, 15: 205-208.
- El-Gibaly, S.M. (1993): Correlation between sero tests and isolation of *Brucella melitensis* in an infected sheep farm. *2nd Sci. Cong., Egyptian Society for Cattle Diseases, 5-7 Dec. 1993, Assuit, Egypt*.
- Falade, S. (1978) A comparison of three serological tests in the diagnosis of caprine brucellosis. *Res. Vet. Sci.*, 24:376-379.
- Falade, S. (1983): Some observations on the use of the Rose Bengal Plate, tube agglutination, heat inactivated and rivanol tests in caprine brucellosis. *Top. Vet.*, 1: 49-52.

- Garin, B.; Trap, D. and Gaumont, R. (1985):* Assessment of the EDTA seroagglutination test for the diagnosis of bovine brucellosis. *Vet.Rec.*, 117: 444-445.
- Ibrahim, I.G.A.; Bassiony, M.M.; Farag, Y.A.; Shalaby, M.N.H.; Kholeaf, Z.M. and Farid, A. (1993):* Evaluation of brucellosis status in low titred bovine reactors. *Egypt. Soc. Anim. Reprod. Fert.*, 5th Annual Cong., Cairo Jan. 26-28, 1993.
- Mac Millan, A.P. (1990):* Conventional serological tests. Edited by Nielsen, K.H. and Duncan, J.R., *Animal brucellosis. Int. Stand. Book. No. 0-8493-58787. Library of Congress, Card No.: 89-25248. USA.*
- Morgan, B.W.J.; Mackennon, D.J. and Gill, K.P.W. (1978):* Standard laboratory techniques for the diagnosis of brucellosis. *Central Vet. Lab. New Haw. Weybridge.*
- Nicoletti, P. (1969):* Further evaluation of serological procedures used to diagnose brucellosis. *Am.J. Vet. Res.*, 30: 1811-1815.
- Nielsen, K.H. and Duncan, J.R. (1982):* Demonstration that non-specific bovine *Brucella abortus* agglutinine is EDTA-Labile and not calcium dependent. *J. Immunol.*, 129: 366-369.
- Shini, A. and Tabatabazi, A.H. (1981):* Pathological, bacteriological and serological responses of ewes experimentally infected with *Br. melitensis*. *Bull. off. Int. Epizoot.*, 93: 1411-1414.
- Weghela, S.; Wandera, J.G. and Wagner, G.G. (1980):* Comparison of four serological tests in the diagnosis of caprine brucellosis. *Res. Vet. Sci.*, 28: 168-172.

Table 1: The results of RBPT on sera of sheep and goats.

Animal species	No. of examined sera	Results of RBPT			
		Positive		Negative	
		No.	%	No.	%
Sheep	1056	21	1.99	1035	98.01
Goats	264	7	2.65	257	97.35

*Percentage is computed according to the number of examined sera.

Table 2: Correlation between SAT titres and confirmatory tests results in sheep

confirmatory tests end titres	SAT end titres							Total No.	%
	1/10 2(9.5%)	1/20 10(47.62%)	1/40 2(9.5%)	1/80 5(23.81%)	1/160 2(9.5%)	1/320 2(9.5%)	1/640 1(4.76%)		
EDTA mod. SAT	-ve	2	2	-	-	-	-	4	19.05
	1/10	-	-	-	-	-	-	-	-
	1/20	-	8	1	-	-	-	9	42.86
	1/40	-	-	1	2	-	-	3	14.29
	1/80	-	-	-	3	-	-	3	14.29
	1/160	-	-	-	-	2	-	2	9.5
RT	-ve	2	2	-	-	-	-	4	19.05
	1/25	-	8	2	-	-	-	10	47.62
	1/50	-	-	-	3	-	-	3	14.29
	1/100	-	-	-	2	-	1	3	14.29
	1/200	-	-	-	-	-	1	1	4.76

* Percentage is computed according to the number of positive RBPT sera in sheep.

Table 3: Correlation between SAT titres and confirmatory tests results in goats

confirmatory tests end titres	SAT end titres							Total No.	%
	1/10	1/20	1/40	1/80	1/160	1/320	1/640		
EDTA mod. SAT	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-
1/10	-	1	-	-	-	-	-	1	14.29
1/20	-	2	-	-	-	-	-	2	28.57
1/40	-	-	1	-	-	-	-	1	14.29
1/80	-	-	1	-	-	-	-	1	14.29
1/160	-	-	-	-	2	-	-	2	28.57
RT	-	-	-	-	-	-	-	-	-
-ve	-	-	-	-	-	-	-	-	-
1/25	-	3	-	-	-	-	-	3	42.86
1/50	-	-	1	-	-	-	-	1	14.29
1/100	-	-	1	-	-	-	-	1	14.29
1/200	-	-	-	-	2	-	-	2	28.57

* Percentage is computed according to the number of positive RBPT sera in goats.

Table 4: Correlation between positive Brucella isolates and serological tests

Animal species	No of isolate	SAT end titres							EDTA mod. SAT end titres					RT end titres				
		1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480	1/40960	1/81920	1/163840	1/327680		
Sheep	1																	
	2		1															
	3																	
Goats	1																	
	1																	

