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SEROLOGICAL STUDIES OF ANIMAL BRUCELLOSIS IN ALGERIA
(With 3 Tables)

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دراسة سيرولوجية عن مرض البروسيلا في الحيوان
بولاية تيارت (الجزائر)

حبيب عقاد

تم جمع ٥٤٤ عينة دم (٢٣٠ من الماعز ، ١٧٢ من الأبقار و١٤٢ من الأغنام) لمعرفة نسبة الإصابة بمرض البروسيلا في مجترات منطقة ولاية تيارت (غرب الجزائر) وذلك خلال عام ٢٠٠٢ ، وكذلك لمقارنة مختلف الإختبارات المستعملة وهي ٦: اختبار الروزبنجال ، الطبقي المحمض ، المتوازن التشريحي ، الريفانول ، التلبد الأنبوبي ، المركبثواناتول وإختبار إرتباط المضاف. أوضحت الدراسة أن نسبة الإصابة ٢,٢٢% في الماعز ، ٢,٣٥% بين الأبقار و ١,٤٢ في الأغنام. أما نسبة الإصابة بين الماعز ، الأبقار والأغنام بواسطة الاختبارات ٦: اختبار الروزبنجال ، ختبار الطبقي المحمض ، اختبار المتوازن التشريحي ، الريفانول ، التلبد الأنبوبي ، المركبثواناتول وإختبار إرتباط المضاف فهي (٢,٦% ، ٣,٠٥% ، ٢,٦% ، ٢,٢٢% ، ٢,٢٢% و ٢,٩%)،(٢,٩% ، ٣,٥% ، ٢,٩% ، ٢,٩% ، ٢,٩% و ٢,٣٥%) ، (١,٤٢% ، ١,٤٢%، ١,٤٢% ، ١,٤٢% ، ١,٤٢% و ١,٤٢%) على التوالي. لقد وجد أن كل من اختبار الروزينجال والمحمض المتوازن التشريحي أكثر كفاءة في إجراء عمليات المسح السيرولوجي للحيوانات المصابة بالبروسيلا لأنهم أكثر حساسية. أما اختبار التلبد الأنبوبي واختبار المركبنواتانول فإنهم أقل حساسية ودقة. اختبار الريفانول أقل حساسية وأكثر دقة بالنسبة للإختبارين الآخرين ، بينما اختبار إرتباط المضاف فهو الأقل حساسية والأكثر دقة بمقارنته بالاختبارات الأخرى.

SUMMARY

A serological study was carried out on 230, 172 and 142 sera of goats, cattle and sheep to estimate the incidence of brucella infection among them) in Tiaret wilaya (department) during the year 2002. All samples were serologically examined by Rose Bengal plate test (RBPT), Buffer acidified plate test (BAPAT), Rivanol test (Riv. T), Tube agglutination test (TAT), Mercaptoethanol test (MET) and Complement fixation test

(CFT). The obtained results indicate that the incidence of brucellosis among goats, cattle and sheep were (2.22 %, 2.35 % and 1.42 %) respectively. The brucella seroprevalence proportion among goats, cattle and sheep by different six serological tests (RBPT, BAPAT, Riv.T, TAT, MET and CFT) were (2.6 %, 3.05 %, 2.6 %, 2.22%, 2.22 % and 2.22 %), (2.9%, 3.5 %, 2.9%, 2.9%, 2.9%, 2.35 %) and (1.42 %, 1.42 %, 1.42 %, 1.42%, 1.42 %, 1.42 %). Also it was found that RBPT, BAPAT, are more efficient and sensitive in screening work and detection of brucella infection, on other hand TAT and MET test are poor sensitive and specific, Rivanol test is more specific and les sensitive than the previous, while CFT is the most specific in comparison with the previous tests.

Key words: Brucella- serology, goats, cattle, sheep,Tiaret.

INTRODUCTION

In Algeria brucellosis is still remaining one of the most important serious recognized disease problems. Intensive governmental efforts were done to minimize widespread of brucella infection among our livestock to an acceptable level.

Unfortunately, investigations carried in Algeria to determine the prevalence are insufficient.

The data purchased by the direction of veterinary services regarding animal concern mainly the bovine and caprine species, but are incomplete because they touch only a few percent of our livestock, while the sheep are not tested regularly.

The incidence of brucella among the different species differs from a locality to another. This variation explains largely the apparition of some human micro-epizooties like those of Ghardaia (south Algeria) in 1985 with 600 cases, attributed to consumption of goats milk (Boudilmi *et al.*, 1991).

For this reason and for economic significance of the disease in different animal species, this investigation was carried out to throw light upon the followings:

- Study the prevalence of the disease among some different species (goats, cattle and sheep).
- Evaluate the sensitivity of some serological tests including (Rose Bengal Test, Buffer Acidified Plate Test, Tube Agglutination Test, Mercaptoethanol Test, Rivanol Test and complement Fixation Test).

MATERIALS and METHODS

Materials:

Samples: A total of 544 blood samples were collected from goats (230), cattle (172) and sheep (142) from different localities at Tiaret Wilaya during 2002.

Antigens:

Antigens for RBPT, BAPAT, SAT, Rivanol test and CFT were obtained from the veterinary serum and vaccine research institute, Abbassia, Cairo, Egypt.

Reagent and solution:

Haemolysin for sensitization of sheep erythrocytes in the presence of complement was obtained from Sigma laboratory, USA.

Guinea pig complement and Mercaptoethanol was manufactured by brucella department, animal health research institute (AHRI), Dokki, Cairo, Egypt.

Rivanol solution was obtained from the veterinary serum and vaccine research institute, Abbassia, Cairo, Egypt.

Alsever's solution for preservation of sheep erythrocytes was purchased as described by Alton *et al.* (1988). The sera were stored at -20°C till the serological tests were performed.

The sera were subjected to 6 serological tests, by Rose Bengal plate test (RBPT), Buffer acidified plate test (BAPAT), Tube agglutination test (TAT), Mercaptoethanol test (MET), Rivanol test (Riv. T) and Complement fixation test (CFT).

1- History of investigated animal: There were a close contact between cattle, sheep and goats.

2- Animals: This study was performed on 544 animal belonged to different areas in Tiaret department.

Methods:

1- The RBPT was carried out according to Morgan *et al.* (1969). The BAPAT was done according to the procedure of the National Vet. Serv. Lab. (NVSL). Ames, Iowa, USA (Anon, 1984). TAT was done but with a modification to start with a dilution of 1/10, using the European assay, according to the method described by Alton *et al.* (1988)

2- Rivanol test (Riv. T) was done according to Alton *et al.* (1988).

3- MET was done according to Alton *et al.* (1975).

4- The CFT was done using the cold technique in micro-titers plates depending on 50 % haemolysin of sheep erythrocytes as described by (Alton *et al.*, 1988), the complement antigen and haemolysin were standardized and titrated before conducting the test. Each serum sample was diluted to 1:4 barbital buffered inactive at 56° C for 36 mn.

The sensitized sheep erythrocytes were prepared by mixing equal volumes of diluted haemolysin in 3% of standardized erythrocytes solution suspension and left for 15 mn at room temperature for sensitization. A titer of 1:4 or higher was considered positive.

RESULTS and DISCUSSION

Brucellosis remains a serious problem for both the veterinary and medical professions.

Despite the relative insufficient population studied, the results showed that brucellosis is widespread at the area (Tiaret Department), but there is a need to other investigations in different areas to have an exact idea upon the incidence of this zoonose at Algeria.

From these results, in this study, we remark that brucellosis is widespread among cattle followed by goats and sheep.

The percent of screening using RBPT in goats, cattle and sheep was 2.6%, 2.9% and 1.42% respectively with total percent of 2.39% in the studied population while that recorded by BAPAT was 3.05%, 3.50% and 1.42% with total percent of 2.76 % (Table 1).

Table 1: Results of screening tests.

Specie	Total	Screening tests					
		RBPT			*BAPAT*		
		Positive	Negative	%	Positive	Negative	%
Goats	230	6	224	2.6	7	223	3.05
Cattle	172	5	167	2.90	6	166	3.50
Sheep	142	2	140	1.42	2	140	1.42
Total	544	13	531	2.39	15	529	2.76

RBPT: Rose Bengal Plate test.
BAPAT: Buffered acidified plate antigen test

These results showed sensitivity and superiority of BAPAT as to that recorded by Montasser *et al.* (2002) and from this study we

recommend BAPAT to be introduced in the program of brucellosis control, because is more sensitive as screening test and can complete RBPT.

The BAPAT detects a higher number of positives compared with other serological test. This is attributed to the final pH of a serum-antigen mixture of 4.02 +/- 0,004 (Alton *et al.*, 1988).
This pH enables some of the Igm beside IgG, IgG1, IgG2 and IgA to share in the reaction (Wright and Nielson, 1990).

On the other hand, the relatively acidic pH of the RBPT (3.65) allows lesser amounts of IgM to share in the reaction, since this class of immunoglobulin is known to be acid labile (Allan *et al.*, 1976).

Buffered antigen tests are suitable for screening herd and individual animals (OIE, 1992).

The results of confirmatory tests (table 2 and 3) showed more positive reactors by TAT and MET (2.20 %) followed by Riv. T and CFT (2.02 %).

For the Rivanol test, the results were 2.60, 2.90 and 1.42 in goats, cattle and sheep respectively with total percent of 2.02% (Table 2). This revealed its lowest incidence compared with the 2 previous tests depending upon the fact that this test precipitates immunoglobulin IgM (Montasser *et al.*, 2001).

Table 2: Results of serological confirmatory tests.

Specie	Total	Confirmatory tests					
		Rivanol test			TAT		
		Positive[a]	Negative	%	Positive[b]	Negative	%
Goats	230	6	224	2.60	5	225	2.22
Cattle	172	5	167	2.90	5	167	2.90
Sheep	142	2	140	1.42	2	140	1.42
Total	544	11	531	2.02	12	532	2.20

TAT: Tube agglutination Test
a: positive results to Riv. T was considered with titers of 1/25 or higher
b: positive results for TAT was considered with titers of 1/10 or higher

The class of immunoglobulin which appeared after the beginning of infection is the IgM witch was not detected by Rivanol test, since the test detects only IgG of infected animals. Than, the Rivanol test can not detects animals in the earlier stage of infection and can't replace CFT.

Concerning the CFT, its revealed 2.22 % in goats, 2.35 % in cattle and 1.42% in sheep, with total prevalence of 2.02% (Table 3). Compared with SAT and MET, whose showed a same prevalence of 2.22, 2.90 and 1.42 in goats, cattle and sheep respectively with total prevalence of 2.20, it was indicated that SAT and MET were less more sensitize, knowing that SAT and MET are IgM detector (Alton *et al.*, 1975) and CFT IgG$_1$ detector mainly (Larsen *et al.*, 1988).

Table 3: Results of confirmatory tests.

Specie	Total	Confirmatory tests					
		MET			*CFT*		
		Positive[b]	Negative	%	Positive[c]	Negative	%
Goats	230	5	225	2.22	5	225	2.22
Cattle	172	5	167	2.90	4	168	2.35
Sheep	142	2	140	1.42	2	140	1.42
Total	544	12	532	2.20	11	533	2.02

MET: Mercaptoethanol test
CFT: Complement fixation test
a: positive results of MET was considered with titers superior or equal to 1/10.
b: positive results for CFT was considered with titers superior or equal to 1/4.

The complement fixation test is considered as the best confirmatory test for the diagnsis of brucellosis. When the disease becomes chronic the titers detected by SAT tend to be negative whereas titers detected by CFT stay in perceptible level (Mathias and Pinto, 1983).

The prevalence of brucellosis in sheep is the same in all the 6 tests (1.42%), this is probably due to equal titers of different class of immunoglobulin in the studied animals.

However, the ideal standard test that's confirms the disease is the isolation of the bacterial agent (Radostits *et al.*, 2000). From our knowledge, isolation of brucella has not been done and reported in Algeria, so we continue to investigate with serological methods in both human and animal individuals, but sometimes, animals with low serological titers, often missed during the control, can play a great act as they may transmit the infection.

The total incidence we found (2.02%) doesn't reflect the real prevalence of brucellosis in Algeria, since our sample was not big but this always confirms that this disease remains as a major both health public and economic problem.

However, every year, we record about 3500 cases of animal brucellosis for all species (INSP, 2000).

The results for cattle (2.35%) are higher to those declared by the direction of veterinary services (DSV, 2001-2002); for example, this authority signaled a percent of positive cases variable between 0.60% to 1.50% from 2001 to 2002, knowing that every month, only 6000 to 8000 cattle are tested witch represent only about 11.5 % on the total of the national dairy bovine strength.

It is evident that several investigations were to be done in different departments of Algeria in order to have more precise informations about epidemiology of brucellosis among animals and human.

However, the massive importation of cattle may increase the incidence of brucella in Algeria and require that only brucellosis free animals be accepted in order to exclude the possible importation of a new Brucella species to our country.

In Algeria the serological tests regarding the bovine under control are RBT and CFT, which appeared to be acceptable. Unfortunately, in infected farms, sometimes, a few months after the slaughtering of the declared animals, the brucellosis came for the second time with its catastrophic consequences.

The reason was that the control was made every 6 months without regarding the medical statute of the herd (declared infected or no); it was better that in infected herd, systematic control has to be done every 21 days in order to detect a new case, especially for animals with suspicious low titers.

The results for (2.22% and 1.42%) are very low in comparison with those obtained in 1986-1989 by Boudilmi and Benhabyles (1991) in western Algeria with 12.01% in goats and 2.18% in sheep; the percentages of infected flocks were 43.5% and 42% in sheep and goats flocks respectively.

Also with those recolted by (Ould-Ali, 2000); 4% in goats and 2.53% in sheep in 1999 for Tiaret department.

Every month, less than 140 cases of caprine brucellosis are declared and the total tested represents only about 8% of the caprine strength (DSV, 2001-2002).

This difference can be explained by the official test used, witch is only RBPT for goats and sheep. In our opinion, it must to be completed by a confirmatory test like CFT.

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Since the cited animals lived together, more attention is to be done for the epidemiological role of goats in the incidence and prevalence of brucellosis. But till now, little is done to test and prevent brucellosis in goats and sheep.

It is necessary to inform about danger of consumption of raw milk and milk products. The farmers need to be informed about the cross infection of goats brucellosis and sheep brucellosis to bovine brucellosis and final conduction to human brucellosis.

Some habitual traditions as the consumption of raw milk, the free circulation and the non-identification of animals explain that that the national policy against brucellosis (test and slaughter) can not be successfully, knowing that the vaccination is not done in Algeria (Aggad, 2002). More than 80 % of human infection is due to the consumption of raw milk and milk products. This explains the apparition of micro-epizooties as that of Ghardaia in 1985 (Boudilmi and Benhabyles, 1991).

A screening investigation revealed the presence of antibodies in over than 30 % of a population in western Algeria (Aggad, 2003). Finally, in place of test and slaughter policy used till now in Algeria, his complementarity by vaccination of ruminants (MZCP Report, 1998; Benkirane, 2001).

Of course, the choice of vaccine to be used has to be studied before starting to avoid bad consequences as post-vaccinale abortions.

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