

BRUCELLOSIS IN WILD BOARS IN PIEDMONT REGION*

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SUMMARY : After four years of Wildlife Surveillance Plan application, our activity has been improved and spread in all Piedmont region territory. Blood samples and tissue specimens had been taken from hunted or dead found Wild Boars (*Sus scrofa*) from all Piedmont territory.

These samples had been tested by RBT and CFT according to bibliography reported methods. Animals tissues had been also tested by bacteriological isolation.

In our 2000-2003 activity we tested: 3406 serum specimens by CFT and we found 234 (6,87%) positive samples and 3172 negative samples; 2933 serum specimens by RBT and we found 192 (6,55%) positive samples and 2741 negative samples; 940 tissue specimens for bacteriological isolation and we found 79 (8,40%) positive samples [62 *B. suis* bv1; 1 *B. suis* bv2, 16 *B. melitensis* bv3] and 861 negative samples.

In Piedmont, cultural tests confirm that wild boar brucellosis seropositivity is specific. At present, on the ground of these data is possible to make an estimate of infection prevalence only in one area, where the disease has been particularly monitored.

Keywords : Brucellosis, wild boar, Piedmont.

RÉSUMÉ : Après quatre ans d'application du plan de surveillance de la faune sauvage, notre activité a augmenté et s'est étendue à toute la région du Piedmont.

Des prélèvements de sang et d'organes ont été effectués sur des sangliers morts ou tués à la chasse dans tout le Piedmont. Les sérums ont été étudiés par fixation du complément ou par le test au rose bengale et les tissus soumis à l'isolement bactériologique.

De 2000 à 2003, 3 406 sérums ont été étudiés en fixation du complément (234 positifs, soit 6,87%) et 2 933 en rose bengale (192 positifs, soit 6,55%). A partir de 940 échantillons d'organes, 79 ont permis l'isolement d'une souche, soit 8,4%: 62 *B. suis* bv1; 1 *B. suis* bv2; 16 *B. melitensis* bv3.

Au Piedmont, la mise en culture confirme la valeur du dépistage sérologique de la brucellose du sanglier.

Mots-clés : Brucellose, sanglier, Piedmont.



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I – MATERIALS AND METHODS

These samples had been tested by rose bengal test (RBT) and complement fixation test (CFT) according to bibliography reported methods. Instead of using serum sample, when haemolytic, insufficient or polluted, we used lung-tissue extract considering that serology from this material has good sensitivity (96-100%) and specificity (68-100%). According to Waller and Mörner technique adjusted by Ferroglio *et al.* (2000), we had extracted serum starting from 10gr of lung-tissue obtained from the dorsal margin of the diaphragmatic lobe, cutting 2,5gr of tissue and leave at room temperature for 20 minutes in tubes containing 2 ml PBS at pH 7,2, then vortexing and centrifugating at 800 g for 10-15 minutes and collecting finally the supernatant.

This material had been treated as serum. Animals tissues had been also tested by

bacteriological isolation: we tested udder, lymphonodes, uterus, spleen and testicles. Total number of sampled specimens are summarized below in table I.

Sampled tissues' specimens had been homogenised, suspended with sterile physiological solution and inoculated on selective *Brucella* medium enriched with equine serum (10%), Vancomycin, Cycloheximide, Nystatin, Nalidixic acid, Bacitracin and Polymyxin B sulfate. Suspected cultures had been incubated in CO₂ (5%) modified atmosphere at 37° C. Colonies growing control had been effectuated till 10 days after inoculation. Isolated strains had been subcultured, coloured and biochemical tested.

Table I

Results of bacteriological and serological tests

Samples	2000-20043		
	CFT	RBT	Isolation
Positive	234	192	79
Negative	3 172	2 741	861
Total	3 406	2 933	940

II - RESULTS

Results of our researches are summarized in table II.

We found 79 strains of *Brucella* spp. identified as follow:

- 62 *B. suis* bv 1;
- 1 *B. suis* bv 2;
- 16 *B. melitensis* bv 3.

Comparing our results between bacteriological isolation and serology and between Complement Fixation Test and Rose Bengal Test we found these agreement percentage (tables III and IV).

Table II

Results of bacteriological and serological tests, year by year (percentages)

Wild Boars Results	2000			2001			2002			2003		
	CFT	RBT	Isol.	CFT	RBT	Isol.	CFT	RBT	Isol.	CFT	RBT	Isol.
Positive	5,7	2,2	0,0	2,3	3,4	0,0	7,7	10,5	7,8	10,1	8,0	9,7
Negative	94,3	97,8	100,0	97,7	96,6	100,0	92,3	89,5	92,2	89,9	92,0	90,3
Total	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0

Table III

Agreement between serology and isolation :
32%

Results comparing				
2002-2003		Serology		
		Pos.	Neg.	Total
Isol.	Pos.	47	17	64
	Neg.	104	365	469
	Total	151	382	533

Table IV

Agreement between CFT and RBT :
53,5%

Results comparing				
2002-2003		CFT		
		Pos.	Neg.	Total
RBT	Pos.	102	52	154
	Neg.	92	1 421	1 513
	Total	194	1 473	1 667

III - CONCLUSIONS

In Piedmont, cultural tests confirm that wild boar brucellosis seropositivity is specific. Isolation from lesionless genital organs is according to bibliographic data about short infection consequences on fertility. At present, on the ground of these data is possible to make an estimate of infection prevalence only in one area, where the disease has been particularly monitored. About our future goals, we want technically to found and validate alternative diagnosis methods. Moreover, we want to clear up epidemiological situation on

the territory concerning to all different *Brucella* species and biovar, to keep a good passive surveillance system in order to analyse each macroscopically lesion showed by hunted heads and to sequence isolated strains.

In our region, is important to improve sanitary monitoring of hunted and dead found wild boars choosing control areas and sampling on the basis of occupied geographic area and estimate population density.

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