TREND OF *BRUCELLA SUIS* INFECTION IN WILD BOAR IN PIEDMONT REGION (2002-2005) *

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***SUMMARY** : Brucellosis caused by* Brucella suis *is a zoonosis common to different animal species, including the wild boar. Due to the numerous wild boar population in Piedmont, since 2000 a monitoring programme was implemented to check their health status, wherein serological monitoring for Brucellosis is one of the programme targets.*

After serological positivity to the infection was found in some herds in 2001, typing of the infectious agent was performed by bacteriological examination (BE). Serum samples from 2688 wild boars were examined by the Rose Bengal Test (RBT) and 3535 by the Complement Fixation Test (CFT); samples from 1864 animals were analyzed by bacteriology.

In 160 animals Brucella spp*. was isolated by bacteriological determination.*

Brucella infection may be considered endemic in some areas in Piedmont Region.

Keywords: *Brucella suis*, serology, bacteriology.

***RESUME** : La brucellose due à* Brucella suis *est une zoonose commune à différentes espèces animales, y compris le sanglier. En raison d'une population importante de sangliers au Piémont, depuis 2000 un programme de surveillance a été établi, comprenant en particulier une surveillance sérologique de la brucellose.*

L'obtention de résultats sérologiques positifs en 2001 a conduit à des vérifications bactériologiques. Ont été analysés : les sérums de 2 688 sangliers par le test au rose bengale, de 3 535 sangliers par fixation du complément et des prélèvements de 1 864 sangliers par bactériologie.

Brucella spp *a été isolée chez 160 animaux.*

L'infection brucellique peut être considérée comme enzootique dans certaines zones du Piémont.

Mots-clés : *Brucella suis*, sérologie, bactériologie.

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I - INTRODUCTION

Brucellosis is a systemic infectious disease caused by Gram negative bacilli, belong to genus *Brucella*.

Brucella infections have also been reported in a wide variety of animals, including wildlife.

Brucellosis caused by *Brucella suis* is a zoonosis common to different animal species and although *B. suis* (biovar 1, 2 and 3) is still widely distributed in the world, prevalence in domestic pigs is low, with the exception of South-East Asia and South America [Godfroid, 2002, Davis, 1990]. In Europe, brown hare (*Lepus europaeus*) and wild boar (*Sus scrofa*) are the natural reservoir of *B. suis* biovar 2.

After serological positivity to the infection was found in some herds, typing of the infectious agent was performed by bacteriological examination (BE).

Reported and controlled in Northern Europe, the *Brucella suis* bv 2 range has recently increased; the infection has also been reported in Belgium [Godfroid *et al.*, 1994], France [Hars *et al.*, 2000] and Italy [Dondo *et al.*, 2003 ; Quaranta *et al.*, 1995] in the last decade.

Since its implementation five years ago, the Wildlife Brucellosis Surveillance Plan has been improved and extended across the entire region of Piedmont. Blood samples are taken from hunted or dead found wild boars (*Sus scrofa*). The samples are tested by RBT and CFT according to the methods described in Alton [Alton *et al.*, 1988]. Instead of using serum samples when hemolytic, insufficient or polluted, we use lung extracts according to the Waller and Mörner technique [Morner *et al.*, 1988 ; Waller *et al.*, 1980] adjusted by Ferroglio [Ferroglio *et al.*, 2000]). Some animal tissues are also tested by bacteriological isolation [Alton *et al.*, 1988]. All samples are tested by the laboratories at the Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Val d'Aosta in Turin and typed by the National Reference Centre for Brucellosis (Istituto Zooprofilattico Sperimentale di Abruzzo e Molise) in Teramo.

II - MATERIALS AND METHODS

Serology – We used a technique described by Alton [Alton *et al.*, 1988] and optimised by the Italian National Institute of Health.

Bacteriology – Bacteriological isolation was also performed on animal tissues (uterus, spleen and testicles). 20 g of tissue were macerated in 25 ml of sterile saline in a Stomacher® (International Pbi). The whole material was inoculated onto *Brucella* medium developed by Farrell [AA VV, 2004], enriched with 10% horse serum and selective Supplement and incubated at 37°C in an atmosphere containing 5% CO_2 for at least 10 days. All colonies resembling *Brucella* were seeded onto blood agar medium and incubated for a further 2 days before re-examination. If *Brucella* was suspected with Stamp's staining [Alton *et al.*, 1988], the colonies were then identified to species by classical techniques [Alton *et al.*, 1967].

Isolated strains were seeded and typed by biochemical [Alton *et al.*, 1988] and molecular tests [Bricker et Halling, 1994 ; Cloeckaert *et al.*, 1995 ; Corbel et Thomas, 1983].

III - RESULTS

The sampling, that has been made in the period 2002-2005 has turned out not homogeneous in the various considered zones of the Piedmont Region.

Results of serology and bacteriology are summarised in table 1.

We found 160 strains of *Brucella* spp.

Brucella strains came from several different areas of the region.

Table 1

Results of serological and bacteriological investigation during the years 2002-2005

	Rose Bengal test		Complement fixation test		Bacteriology	
	POS	TOT	POS	TOT	POS	TOT
2002	83 (10%)	792	79 (8%)	1026	33 (8%)	422
2003	70 (8%)	877	114 (10%)	1133	47 (7%)	637
2004	80 (11%)	686	113 (9%)	1292	54 (10%)	556
2005	126 (12%)	1042	215 (12%)	1863	53 (6%)	902

IV - DISCUSSION

Cultural tests have confirmed that wild boar brucellosis seropositivity is specific in Piedmont. According to data from the literature [Garin-Bastuji, 1997], isolates from lesion less genital organs (uterus and testicles) have revealed short infection with consequences on fertility.

From current data, mapping of serological and bacteriological positivity points to a wide distribution of the infection throughout the region.

In some areas (La Mandria Regional Park, ATCCN4, ATCCN5), characterized by constant percentage of seropositivity since 2002 and by high percentage of bacteriological isolation, Brucella infection has to be considered endemic.

In other areas (Collina di Superga Park, ATCVC2), seropositivity has remained constant since 2002-2003 and it has been confirmed by bacteriological exam since 2004.

In the past 5 years, single outbreaks have occurred in territories retiring with endemic areas (the Province of Asti, CAVCO1, CATO3, ATCCN3, ATCCN2).

Considering the difficulties of conducting a wild boar census and the fragmentary nature of the available data, no exact figures can be given about the prevalence of infection.

In Piedmont improvements to the surveillance of hunted and dead found wild boars can be made by selecting control areas and by sampling based on the geographic area occupied and the estimated population density.

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