CORRELATION BETWEEN *YERSINIA* SPP. FOUND IN FAECES AND B*RUCELLA SUIS* INFECTION IN WILD BOARS *

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***SUMMARY**: 379 wild boar faeces samples were collected from July 2005 to June 2006 and tested to detect* Yersinia *spp.. The same animals were submitted to bacteriological and serological tests to detect* Brucella *infection.*

62 Yersinia spp. strains were isolated. Out of them, 31 were identified as Y. enterocolitica*.*

The Chi^2 test has showed neither correlation between Yersinia *spp. and serological tests (X^2 = 0.009, p=0.97) nor between* Yersinia *spp. and* Brucella suis *isolation (X^2 = 0.03, p=0.87).*

Contrary to what occurs in cattle, seropositivity in naturally infected wild boars may reflect infection by Brucella suis *rather than by* Yersinia *spp.*

Keywords: *Yersinia spp.*, crossreactivity, *Brucella suis.*

***RESUME** : La recherche de* Yersinia *spp a été faite dans 379 fèces de sanglier récoltées entre Juillet 2005 et Juin 2006 ; chaque animal a été soumis à des tests de bactériologie et de sérologie pour confirmer l'infection par* Brucella*. Soixante deux souches de* Yersinia *spp. ont été isolées et 31 ont été identifiées comme* Y. enterocolitica*.*

Le Chi^2 test n'a démontré aucune corrélation entre l'isolement de Y.enterocolitica *et les tests sérologiques (X^2 = 0,009, p=0,97) ou entre l'isolement de* Yersinia *spp. et celui de* Brucella suis*.*

Au contraire de ce qu'on observe chez les bovins, on peut supposer que chez le sanglier la seropositivité brucellique est liée à l'infection par Brucella suis *et non pas à celle par* Yersinia *spp.*

Mots-clés: *Yersinia spp.*, réaction croisée, *Brucella suis.*

I - INTRODUCTION

Brucellosis and yersiniosis are diseases which affect animals as well as human beings.

Brucella infection 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 J., 2002].

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In Central Europe, including Italy, this infection is endemic; brown hare (*Lepus europaeus*) and wild boar (*Sus scrofa*) are the natural reservoirs of *B. suis* biovar 2.

Yersiniosis has been described in many species of domestic and free-living mammals and birds. Recently, several outbreaks of this emerging food-borne disease have been reported.

In wild boar infectious agents are *Yersinia pseudotuberculosis* and *Yersinia enterocolitica* and particularly, *Y. enterocolitica* O:9 shows similarity to the O-chain of *Brucella* spp., producing false seropositivity versus *Brucella* spp., as shown in particular in cattle [Garin-Bastuji B. *et al*., 1999; Godfroid J. *et al*., 2002].

The purpose of this work was to study the correlation between *Yersinia* spp. detection in faeces and serological response to *Brucella* spp. in naturally infected wild boar.

II - MATERIALS AND METHODS

1. SAMPLING

The complete test protocol includes sampling sera, organs (spleen and/or genital organs) and faeces from 379 wild boar hunted from July 1, 2005 to June 30, 2006 in areas where *Brucella suis* infection is endemic.

2. SEROLOGY

We used a technique described by Alton [Alton *et al.,* 1988] and optimised by the Italian National Institute of Health based on the Rose Bengal Test (RB) and the Complement Fixation Test (CFT).

3. *BRUCELLA SUIS* ISOLATION FROM ORGANS

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 into modified Thayer-Martin medium [Farrell, 1974; Martin *et al*., 1996] and *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 into 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 al*., 1994; Cloeckaert *et al*., 1995; Corbel *et al.,* 1983].

4. ISOLA-TION OF *Y. ENTEROCOLITICA* FROM FAECES

About 5 g of faeces were sent to the laboratory and tested. Phosphate buffered saline (PBS) was added to the samples in a ratio of 1:20 v/v.

The culture broths were incubated at 4°C for 21 days and weekly seeded on selective agar (CIN). In order to evaluate lactose fermentation and to proceed with the biochemical studies,If a suspected colony developed the colony was transplanted to MacConkey agar and blood agar and the plates were incubated at 30°C for 24 h. Urease study was performed on suspected colonies; in the presence of positive urease, identification was performed with a miniaturized API 20 E gallery. The strains confirmed as *Yersinia enterocolitica* were serotyped with monovalent antisera for somatic antigens.

III - RESULTS

A total of 62 faecal samples out of 379 gave positive results for *Yersinia* spp. 31 out of them were identified as *Y. enterocolitica* and then serotyped. The remaining samples were positive to *Y. kristensenii* (26), *Y. frederiksenii* (2), *Y. aldovae* (2) and *Y. intermedia* (1).

Serotyping has led to identify 12 strains of *Y. enterocolitica* (YE) O:9, 5 YE O: 5, 4 YE O:8 and 1 YE O:1,2; 9 strains didn't give any conclusive result.

The table below (table 1) shows the results of the serological and bacteriological tests. RB and CFT have been evaluated in parallel.

For purposes of statistical analysis we considered only cases in which *Yersinia* spp. were isolated from faecal samples (*Yersinia* positive/negative). Subsequently in order to evaluate the presence of *Yersinia enterocolitica* in wild boars, all strains were identified by means of API gallery [Stockes, 2000].

Table 1

Results and relative comparison of serological and bacteriological tests
(N: negative; P: positive)

***Brucella* spp.**		**Yersinia negative**	**YE +**	***Yersinia* + (other than YE)**	**Total**	**%**
Serological test	**Bacteriological test**					
N(299)	N(292)	244	25	23	48	16.4% (48/292)
	P(7)	6	1	0	1	14.2% (1/7)
P(80)	N(67)	56	4	7	11	16.4% (11/67)
	P(13)	11	1	1	2	15.3% (2/13)
Total	379	317	31	31	62	

IV - DISCUSSION

In wild boar groups, examined in different areas where *Brucella* infection is endemic, we should assume that seropositivity is most likely linked to the presence of *Brucella* spp. Table 2 shows *Yersinia* spp. detection has been indifferently obtained both from seropositive (16.2%) and seronegative animals (16.7%).

Chi-square test shows that *Yersinia* spp. detection in faeces and seropositivity to *Brucella* spp. are independent (χ^2 = 0.009, p=0.97).

Similarly, we have observed no correlation between *Yersinia* spp. and *Brucella suis* isolation (χ^2 = 0.03, p=0.87).

Thus, the agreement between *Yersinia* detection and serological/bacteriological test is poor, which strengthens the hypothesis that *Yersinia* presence is independent from the two considered tests, differently than observed in cattle.

Table 2

Results and relative comparison of serological test and *Yersinia* spp. isolation
(N: negative; P: positive)

***Brucella* spp. serological response**	***Yersinia* spp. isolation from / examined samples**
N(299)	16.7% (50/299)
P(80)	16.2% (13/80)

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