Brucella suis infection in domestic pigs and wild boar in Croatia

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Summary
Between the years 2000 and 2004, 93,107 sera from 1,997 pig herds in 11 regions of Croatia were tested for the presence of antibodies against brucellosis. Positive results were observed in 67 herds from seven regions (mean individual prevalence: approximately 1%; herd prevalence: 3.4%). The herds from all but two of the infected farms were reared outdoors and thus almost certainly came into contact with wildlife. From 2003 to 2004, 424 sera, which were randomly collected from hunted wild boar (Sus scrofa), were also tested and shown to have a mean seroprevalence of 27.6%. Brucella was isolated from 88 out of 151 serologically positive pigs (58.3%) and 7 of the 93 (7.5%) wild boar which were randomly submitted for bacteriological study. All but three isolates were Brucella suis biovar 2; the others being biovar 3.

These results suggest that brucellosis is enzootic in Croatian populations of wild boar. These populations represent a potential disease reservoir for free-range pig farms, as they do in other countries of Central and Western Europe. This is the first report of B. suis biovar 3 in swine and wild boar in Europe, which is an issue of serious concern for public health.

Keywords

Introduction
Brucellosis is a zoonosis of worldwide importance, caused by several species of the bacterial genus Brucella. Biovars 1, 2 and 3 of Brucella suis infect primarily domestic and feral pigs, as well as wild boar (Sus scrofa). The most common manifestations of the disease are:
– abortion at any stage of gestation
– stillbirth
– weak piglets in sows
– orchitis in boars.

Arthritis with lameness and occasional posterior paralysis may also be seen in both sexes.

Brucella suis biovar 1 is common in South America (25, 28, 31) and Asia (34), while B. suis biovar 1 and 3 have both been reported in the United States of America (USA), Australia and the People’s Democratic Republic of China (3, 5, 9, 30). The most commonly isolated strain in Central, Eastern and Western Europe is B. suis biovar 2, whose natural reservoir is the wild boar and/or the European hare (Lepus europaeus) (6, 15, 17, 18, 20, 22, 33).

Porcine brucellosis was eradicated in most European countries in the 1970s, through the industrialisation of pig farming. For this reason, US regulations do not require surveillance of brucellosis in the European pig industry and nor does the European Union (EU), except for intra-
Community trade in living animals, semen and embryos (11, 12). According to Godfroid and Kasbohrer (17), brucellosis has not been recorded in domestic pigs in Belgium since 1969 nor in the Netherlands since 1973. Moreover, it has never been recorded in Finland, Sweden, the United Kingdom (UK) or Norway (17). However, the disease re-emerged in the 1990s on pig farms that rear their animals outdoors, as a result of spill-over infection from hares or wild boar in Denmark, France and Germany (1, 15, 21, 22). *Brucella suis* biovar 2 infection in pigs has also been reported in Austria, Hungary, Portugal, Spain and Switzerland (10, 17).

*Brucella suis* biovar 2 has been described in hares and wild boar in other Central European countries, including:
- the Czech Republic (20)
- Croatia (8)
- Hungary (23)
- Poland (33)
- Slovakia (20, 26)
- Slovenia (4).

Godfroid *et al.* (18) and Garin-Bastuji *et al.* (14, 15) have reported the occurrence of brucellosis in wild boar, due to *B. suis* biovar 2, in Belgium and France, respectively. Positive serological reactions to brucellosis in wild boar ranged from 20% to 60% in France and reached 39% in the Belgian study. In both countries, *B. suis* biovar 2 was isolated from approximately 10% of hunted animals. Similar observations were reported in Switzerland (24), and Kautzsch *et al.* (22) described hares and wild boar as natural reservoirs of *B. suis* biovar 2 in Germany.

Transmission of *Brucella* from wild boar to domestic pigs has never been conclusively proven. However, as direct (genital) contact does occur between these two species, as demonstrated by the observation of hybrid piglets (19), this is a possible means of spreading the bacteria from wild to domestic swine.

The aim of this study was to describe the situation of brucellosis in domestic pigs and wild boar in Croatia, using serological and bacteriological methods. The tested population consisted of pigs that had either been kept traditionally, on pasture, or which came from large commercial farms. Randomly selected, hunted wild boar were also included in the study.

**Materials and methods**

**Study population**

The domestic pig population that was tested included 93,107 animals from 1,997 flocks, in 11 different provinces of Croatia:

- Bjelovarsko-bilogorska
- Brodsko-posavska
- Koprivničko-križevačka
- Međimurska
- Osječko-baranjska
- Požeško-slavonska
- Sisačko-moslavačka
- Varaždinska
- Virovitičko-podravska
- Vukovarsko-srijemska
- Zagrebačka.

All these areas are on the mainland of Croatia, where intensive, semi-intensive and free-range pig holdings are concentrated. The domestic pig population consisted of:

- gilts aged over seven months
- breeding sows
- boars bred either for natural mating or for producing semen for artificial insemination.

The wild boar population employed during the tests included 424 wild boar, hunted between 2003 and 2004, from six different provinces, containing the most important hunting grounds in Croatia:

- Brodsko-posavska
- Koprivničko-križevačka
- Osječko-baranjska
- Požeško-slavonska
- Sisačko-moslavačka
- Vukovarsko-srijemska

These wild boar included 235 females and 189 males, aged from six months to five years, and weighing from 21 to 135 kg.

**Serological examination**

**Collection of blood samples**

Domestic pigs were bled by venipuncture from the vena cava cranialis, vena jugularis or the ear vein. In wild boar, blood was collected from the thoracic cavity, the heart or the pericardium, immediately after shooting.

**Serological tests**

Sera were screened by the rose bengal test (RBT) and confirmed by the complement fixation test (CFT) and the indirect enzyme-linked immunosorbent assay (ELISA). The RBT and CFT were performed according to World Organisation for Animal Health (OIE) standard procedures (33), using antigens (Institut Pourquier, Montpellier,
France) fitting the OIE requirements (35). The indirect ELISA (i-ELISA) was also performed according to the standard OIE procedure (35), except that the antigen, conjugate and substrate were a CFT antigen (Croatian Veterinary Institute, Zagreb), protein-G (Sigma, Germany) and 3', 3', 5', 5'-tetramethylbenzidine (Sigma), respectively. Sera that gave an optical density (OD) that was higher than the mean OD of the negative sera (the negative threshold) plus two standard deviations were considered positive (2). Sera with OD values lying between the negative and positive thresholds were considered doubtful.

Individual results were considered positive when a positive RBT was confirmed either by a positive iELISA or CFT (> 20 IU/ml) result. A positive result in the RBT, associated with doubtful reactions in either the iELISA or the CFT (> 8.3 but < 20 IU/ml), was considered ‘suspect’.

**Bacteriological examination**

**Collection of tissue samples**

Tissue samples from pigs and wild boar were collected during slaughtering or while dressing the carcass, respectively. The material included lymph nodes (parotid, sub-mandibular, retro-pharyngeal, portal, sub-iliac, mesothelial and supra-mammary) and reproductive organs (testes and uterus).

Samples were collected from 151 serologically positive pigs from five regions, comprising:

- 85 samples of uterus
- testes from 54 boar
- lymph nodes from 66 pigs and 12 aborted piglets.

Immediately after the wild boar in the study had been hunted and shot, tissue samples were taken from 93 animals from four regions, comprising:

- uterus samples from 45 animals
- testes samples from 48 animals
- lymph node samples from 42 animals.

**Bacteriological analysis**

Cultures were performed according to Alton et al. (2). Briefly, tissue samples homogenised in phosphate-buffered saline in a stomacher were directly cultured on non-selective nutritive blood agar (Merck), with 5% defibrinated sheep blood and the selective Farrell’s medium (2). One plate of each medium was incubated at 37°C in normal atmosphere and one in the presence of 5% to 10% CO₂. The growth and morphology of the colonies were monitored daily, up until ten days of incubation.

The suspect isolates were confirmed by a *Brucella* genus-specific polymerase chain reaction (PCR), targeting the gene encoding for the BSCP31 outer membrane protein and amplifying a fragment of 443 base pairs (bp) (32).

*Brucella* strains were finally biotyped according to Alton et al. (2). In brief, the strains were tested for lysis by phages Tbilisi at routine test dilution (RTD) and 10^4 × RTD, Weybridge and Izatnagar1 at RTD, and for oxidase, urease, CO₂ requirement, H₂S production, growth on thionin and basic fuchsin at 20 μg/ml and agglutination with anti-A and anti-M monospecific sera.

**Results**

**Serological findings**

**Domestic pigs**

Positive reactions were found in 766 animals (0.8%) and suspect reactions in 223 pigs (0.2%), originating from 67 flocks in seven of the 11 sampling regions (Tables I and II).

**Wild boar**

Positive reactions were found in 98 animals (23.1%) and suspect reactions in 19 animals (4.5%), from five of the six sampling regions (Table III).

**Bacteriological findings**

**Pigs**

*Brucella* spp. identified by PCR were isolated from 88 out of the 151 serologically positive pigs examined (58.3%). According to conventional biotyping results, the most commonly isolated strain was *B. suis* biovar 2. However, *B. suis* biovar 3 was isolated on two occasions from the stomach contents of aborted piglets from the Osječko-baranjska (Djakovo) region (Table IV).

**Wild boar**

Seven out of the 93 (7.5%) wild boar yielded positive cultures, with isolation of *B. suis* biovar 2 on six occasions and biovar 3 in one case (Table V).

The geographical distribution of pig and wild boar isolates is presented in Figure 1.

**Gross pathology findings**

The macroscopic examination of the seropositive pigs revealed several cases of orchitis and/or epididymitis, with focal purulent necroses of various sizes in males (Fig. 2)
Table I
Seroprevalence of brucellosis in domestic pigs and pig herds in Croatia from 2000 to 2004

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of herds tested</th>
<th>Seropositive herds</th>
<th></th>
<th>No. animals tested</th>
<th>Seropositive animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% (95% CI)</td>
<td>No.</td>
<td>% (95% CI)</td>
<td>No.</td>
</tr>
<tr>
<td>2000</td>
<td>325</td>
<td>3.08 (2.12-4.03)</td>
<td>17,783</td>
<td>0.79 (0.73-0.86)</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>420</td>
<td>5.95 (4.80-7.11)</td>
<td>14,670</td>
<td>0.86 (0.78-0.94)</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>398</td>
<td>1.51 (0.9-2.12)</td>
<td>23,948</td>
<td>0.89 (0.82-0.95)</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>505</td>
<td>3.96 (3.09-4.83)</td>
<td>20,951</td>
<td>0.71 (0.65-0.77)</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>349</td>
<td>1.72 (1.02-2.41)</td>
<td>15,755</td>
<td>0.88 (0.80-0.95)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1,997</td>
<td>3.36 (2.95-3.76)</td>
<td>93,107</td>
<td>0.82 (0.79-0.85)</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval

Table II
Regional distribution of the seroprevalence of brucellosis in pigs in Croatia from 2000 to 2004

<table>
<thead>
<tr>
<th>Region</th>
<th>Analysed sera (No.)</th>
<th>Positive reactions</th>
<th></th>
<th>Suspect reactions</th>
<th>All reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% (95% CI)</td>
<td>No.</td>
<td>% (95% CI)</td>
<td>No.</td>
</tr>
<tr>
<td>VS</td>
<td>2,535</td>
<td>13.33 (12.66-14.01)</td>
<td>59</td>
<td>2.33 (2.03-2.63)</td>
<td>397</td>
</tr>
<tr>
<td>GB</td>
<td>54,391</td>
<td>0.29 (0.27-0.32)</td>
<td>110</td>
<td>0.20 (0.18-0.22)</td>
<td>269</td>
</tr>
<tr>
<td>BP</td>
<td>5,656</td>
<td>1.56 (1.39-1.72)</td>
<td>5</td>
<td>0.09 (0.05-0.13)</td>
<td>93</td>
</tr>
<tr>
<td>PS</td>
<td>1,824</td>
<td>1.43 (1.15-1.70)</td>
<td>7</td>
<td>0.38 (0.24-0.53)</td>
<td>33</td>
</tr>
<tr>
<td>VP</td>
<td>12,887</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BB</td>
<td>790</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M</td>
<td>5,186</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>V</td>
<td>2,273</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>KK</td>
<td>1,322</td>
<td>0.08 (0.00-0.15)</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Z</td>
<td>4,802</td>
<td>0.04 (0.01-0.07)</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>SM</td>
<td>1,441</td>
<td>10.55 (9.74-11.36)</td>
<td>42</td>
<td>2.91 (2.47-3.35)</td>
<td>194</td>
</tr>
<tr>
<td>Total</td>
<td>93,107</td>
<td>0.82 (0.79-0.85)</td>
<td>223</td>
<td>0.24 (0.22-0.26)</td>
<td>989</td>
</tr>
</tbody>
</table>

Table III
Seroprevalence of brucellosis in wild boar in Croatia from 2003 to 2004

<table>
<thead>
<tr>
<th>Region</th>
<th>Analysed sera (No.)</th>
<th>Positive reactions</th>
<th></th>
<th>Suspect reactions</th>
<th>All reactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>% (95% CI)</td>
<td>No.</td>
<td>% (95% CI)</td>
<td>No.</td>
</tr>
<tr>
<td>VS</td>
<td>106</td>
<td>25.47 (21.24-29.70)</td>
<td>10</td>
<td>9.43 (6.59-12.27)</td>
<td>37</td>
</tr>
<tr>
<td>OB</td>
<td>104</td>
<td>38.46 (33.69-43.23)</td>
<td>4</td>
<td>3.85 (1.96-5.73)</td>
<td>44</td>
</tr>
<tr>
<td>BP</td>
<td>95</td>
<td>4.21 (2.15-6.27)</td>
<td>0</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>PS</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>KK</td>
<td>63</td>
<td>17.46 (12.68-22.24)</td>
<td>1</td>
<td>1.59 (0.01-3.16)</td>
<td>12</td>
</tr>
<tr>
<td>SM</td>
<td>55</td>
<td>29.09 (22.97-35.22)</td>
<td>4</td>
<td>7.27 (3.77-10.77)</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>424</td>
<td>23.11 (21.07-25.16)</td>
<td>19</td>
<td>4.48 (3.48-5.49)</td>
<td>117</td>
</tr>
</tbody>
</table>

Legislation on porcine brucellosis in Croatia includes compulsory serological examination of all boars kept for natural covering or artificial insemination and 20% of breeding animals on large farms. Furthermore, every abortion in a sow must be notified and investigated for brucellosis.

During this five-year investigation, 93,107 swine sera were examined, from approximately 2,000 herds in 11 regions. The mean seroprevalence was low (1% in individual animals and 3.36% in herds). Far higher individual rates were found in two particular regions: Vukovarsko-srijemska (15.7%) and Sisačko-moslavačka (13.5%).

Active disease was specifically identified on two commercial farms (both with about 1,000 breeding sows), in the eastern part of the country (Vukovarsko-srijemska and Osječko-baranjska). Brucellosis was eradicated on one of these farms (the Đakovo farm in Osječko-baranjska) by...

### Table IV
Geographical origin and biovars of *Brucella suis* strains isolated from pigs in Croatia from 2000 to 2004

<table>
<thead>
<tr>
<th>Region</th>
<th>Testes</th>
<th>Uterus</th>
<th>Aborted foetuses</th>
<th>Lymph nodes</th>
<th>Examined</th>
<th>Positive (%)</th>
<th>Biovars identified (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS</td>
<td>18</td>
<td>46</td>
<td>2</td>
<td>24</td>
<td>66</td>
<td>40 (60.6)</td>
<td>biovar 2 (40)</td>
</tr>
<tr>
<td>OB</td>
<td>16</td>
<td>32</td>
<td>2</td>
<td>20</td>
<td>50</td>
<td>27 (54.0)</td>
<td>biovar 2 (25) biovar 3 (2)</td>
</tr>
<tr>
<td>BP</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2 (100)</td>
<td>biovar 2 (2)</td>
</tr>
<tr>
<td>PS</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>5</td>
<td>11</td>
<td>4 (36.4)</td>
<td>biovar 2 (4)</td>
</tr>
<tr>
<td>SM</td>
<td>14</td>
<td>5</td>
<td>8</td>
<td>15</td>
<td>27</td>
<td>15 (55.6)</td>
<td>biovar 2 (15)</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>85</td>
<td>12</td>
<td>66</td>
<td>156</td>
<td>88 (56.4)</td>
<td>biovar 2 (86) biovar 3 (2)</td>
</tr>
</tbody>
</table>


### Table V
Geographical origin and biovars of *Brucella suis* strains isolated from wild boar in Croatia from 2003 to 2004

<table>
<thead>
<tr>
<th>Region</th>
<th>Testes</th>
<th>Uterus</th>
<th>Lymph nodes</th>
<th>Examined</th>
<th>Positive (%)</th>
<th>Biovars identified (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS</td>
<td>16</td>
<td>10</td>
<td>9</td>
<td>26</td>
<td>2 (7.7)</td>
<td>biovar 2 (2)</td>
</tr>
<tr>
<td>OB</td>
<td>10</td>
<td>18</td>
<td>12</td>
<td>28</td>
<td>2 (7.1)</td>
<td>biovar 2 (2)</td>
</tr>
<tr>
<td>KK</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td>22</td>
<td>0 –</td>
<td>–</td>
</tr>
<tr>
<td>SM</td>
<td>10</td>
<td>7</td>
<td>15</td>
<td>12</td>
<td>3 (17.6)</td>
<td>biovar 2 (2) biovar 3 (1)</td>
</tr>
<tr>
<td>Total</td>
<td>48</td>
<td>45</td>
<td>41</td>
<td>93</td>
<td>7 (7.5)</td>
<td>biovar 2 (6) biovar 3 (1)</td>
</tr>
</tbody>
</table>

VS: Vukovarsko-srijemska, KK: Križeva-koprivnička, OB: Osječko-Baranjska, SM: Sisačko-moslavačka

and metritis in females. Placentas were hyperaemic and oedematous, with yellowish purulent cheese-like miliary follicles (Fig. 3).

**Discussion**

Swine brucellosis is a disease characterised by infertility and abortion in sows, deaths of piglets and orchitis in boars. Genital discharge is the most important source of infection. In uninfected flocks, the infection occurs after the introduction of infected animals. On many occasions, abortion is the only sign but it may be overlooked if animals are kept in groups. In such circumstances, members of the group usually eat the aborted foetuses and placentas. Another potential method of transmission in swine kept on pasture is direct and indirect contact with infected wild boar.

Legislation on porcine brucellosis in Croatia includes compulsory serological examination of all boars kept for natural covering or artificial insemination and 20% of breeding animals on large farms. Furthermore, every abortion in a sow must be notified and investigated for brucellosis.

During this five-year investigation, 93,107 swine sera were examined, from approximately 2,000 herds in 11 regions. The mean seroprevalence was low (1% in individual animals and 3.36% in herds). Far higher individual rates were found in two particular regions: Vukovarsko-srijemska (15.7%) and Sisačko-moslavačka (13.5%).

Active disease was specifically identified on two commercial farms (both with about 1,000 breeding sows), in the eastern part of the country (Vukovarsko-srijemska and Osječko-Baranjska). Brucellosis was eradicated on one of these farms (the Đakovo farm in Osječko-baranjska) by...
stamping out. The other infected farm (Ovčara-Vukovar, in Vukovarsko-srijemska) acquired the infection during the last Serbo-Croatian war (which began in 1991). So far, this farm has been subjected to the ‘testing and slaughtering’ eradication scheme. Unfortunately, the infection still persists. On both farms, B. suis biovar 2 was confirmed on several occasions. This confirms the real difficulty in eradicating brucellosis from large farms without using stamping out.

In some of the other areas where brucellosis was found, pigs are often kept traditionally on pastures and pig breeding is mostly extensive and based on natural covering. This increases the possibility of contact among pigs from different owners as well as with wild boar (6). This fact is especially highlighted in Sisačko-moslavačka county, alongside the Sava river on the southern border of the province, most particularly in the micro-location called ‘Lonjsko polje Nature Park’. This park has a surface area of 506 km², between Sisak in the west and Nova Gradiška in the east, and is the largest area of protected wetlands in Central Europe. In this region, pigs cohabit with other species of domestic animals and mate naturally. The highest seroprevalence found during this study was...
recorded in this location. The seroprevalence rate of brucellosis observed in similarly kept animals from other regions was much lower. The positive results from eastern Croatia (Osječko-baranjska and Vukovarsko-srijemska) are related primarily to the large commercial farms described above.

In view of the low specificity of serological tests for porcine brucellosis (35), it seems probable that the positive serological results were due, at least in part, to *Yersinia enterocolitica* O:9 or other cross-reacting bacteria. To decrease the false positive reactions, individual results were considered positive only when a positive RBT was confirmed either by a positive iELISA or CFT. They were considered suspect when associated with doubtful reactions in the iELISA or CFT, with a probability of specific reactions being higher for positive animals than for suspect ones. Since cross-reactions could not be totally excluded, the seropositivity rate is assumed to give a rough evaluation only of the prevalence rate of *Brucella* infection.

In the region of Vukovarsko-srijemska, which had a high percentage of seropositive pigs, there is also a high percentage of seropositive wild boar. However, in the region of Osječko-baranjska, which demonstrated a low percentage of seropositive pigs, there is also a high percentage of seropositive wild boar. This apparent discrepancy may simply be related to the numbers of tested animals, hence, this could be a selection bias. The apparent selection bias could also be due to the potential for contact between pigs and infected wild boar. In Vukovarsko-srijemska, pigs are usually free range, with considerable potential for contacts between the domestic and wild populations throughout the year. Conversely, in Osječko-baranjska, most domestic pigs are intensively farmed and very few pigs are free range. Among the latter, only gravid sows are allowed to range freely during the summer months. It is probable that, in Osječko-baranjska, while the prevalence of the infection is high in wild boar, very few pig farms are exposed to the wild boar population.

Bacteriological examination of the samples submitted from five regions produced *Brucella* isolates from 88 out of 151 seropositive pigs. Most of the isolated strains (86) belonged to *B. suis* biovar 2. However, the two remaining strains, isolated from the stomach contents of aborted piglets from the farm in Djakovo, belonged to biovar 3 of *B. suis*.

From 2003 to 2004, this investigation was extended to wild boar from six counties, and 117 out of 424 serum samples examined were found to be positive or suspect (27.6%). *Brucella* strains were isolated from seven of the 93 (7.5%) tested animals from four counties. Six strains were identified as *B. suis* biovar 2, and one as *B. suis* biovar 3 (from Sisačko-moslavačka). This strain was isolated in Lonjsko polje Park, where the traditional method of breeding both horses and pigs in the pastureland environment enables contacts between domestic and wild animals, which probably also permits the spread of brucellosis and other diseases. This is partially confirmed by a previous isolation of biovar 3 from horses in the same micro-location: the first evidence of this biovar in Europe (7). The isolation of *B. suis* biovar 2 from pigs and wild boar in this area has already been described (6), but this is the first evidence of *B. suis* biovar 3 infection in pigs and wild boar in Europe.

Gagrcin et al. (13) described viral infections in wild boar in the former Yugoslavia, mentioning the importation of wild boar from Iraq. This may be the source of biovar 3 in Croatia, since this biovar is classically known to be enzootic in Asia. In the present study, *B. suis* biovar 2 was confirmed as the main cause of brucellosis in pigs and wild boar in Croatia, as it is in all other European countries where the infection has been reported. However, the concomitant presence of biovar 3 in such animal populations is of great concern in terms of public health. While biovar 2 of *B. suis* has very rarely been reported as a cause of human brucellosis and seems restricted to highly exposed and immuno-compromised hunters or breeders (16, 27), biovar 3 is known to be highly pathogenic to humans in Asia. Its presence in a wild population in Europe, a population whose size has increased exponentially in the last 20 years, suggests its potential...
spread throughout Europe, with serious consequences for medical and veterinary public health. Human infection caused by \( B. \) suis biovar 3 has not been reported in Croatia or the rest of Europe, in either rural populations or among hunters, despite the widespread practices of farming pigs and hunting wild boar throughout Europe. However, in most cases, the \( Brucella \) strain is rarely isolated from infected patients and, when it is isolated, it is very rarely typed.

This study found that infections in swine are relatively limited and mostly concentrated on free-range pig farms. It may be assumed that, as is the case for biovar 2 in other European countries, the infection of domestic pigs is due to direct contact between the pig population and the wild boar population; in particular, through breeding (15). \( Brucella \) suis biovar 3 has been isolated from horses in Croatia (7), but only two cases were identified among 6,015 serosurveyed animals. Horses are rarely tested for brucellosis in Europe. Apparently, \( B. \) suis infection has not spread in ruminants (Croatia has been free of bovine, ovine and caprine brucellosis since the 1990s) and, in fact, brucellosis was very rarely reported in small ruminants until the 1990 introduction of \( B. \) melitensis from a neighbouring infected country (29). Therefore, up until now, no argument has been made that \( B. \) suis biovar 3 infection is likely to have spread in the area.

In the absence of (i) an anti-\( Brucella \) vaccine which is efficient in pigs and/or easily applicable to wild boar, and (ii) eradication strategies that are easily applied to wild fauna, health authorities should promote the isolation and typing of \( Brucella \) in infected patients. Moreover, they should encourage the wildlife and game authorities to decrease wild boar populations until their density is sufficiently low to reduce the transmission of infection to domestic pigs and the risk to humans. Veterinary authorities should recommend or, even better, make compulsory the adequate fencing of free-range pig farms, to prevent the cross-breeding of wild boar with domestic sows, since this is, at present, thought to be the main route of infection.

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Infection à \( Brucella \) suis chez le porc domestique et le sanglier en Croatie


Résumé

Les auteurs présentent les résultats d’une étude conduite en Croatie entre 2000 et 2004, au cours de laquelle 93 107 sérums porcins issus de 1 997 élevages de 11 régions du pays ont été analysés pour la recherche d’anticorps dirigés contre la brucellose. Des résultats positifs ont été observés dans 67 élevages de 7 régions (prévalence moyenne de 1 % environ à l’échelle individuelle et de 3,4 % à l’échelle des troupeaux). Tous les élevages infectés, sauf deux, étaient des élevages en plein air, très probablement en contact avec la faune sauvage. Des analyses ont également été réalisées sur 424 sérums prélevés de manière aléatoire sur des sangliers tués à la chasse en 2003 et 2004 ; la séroprévalence moyenne était de 27,6 %. \( Brucella \) spp. a pu être isolée de 88 des 151 porcs à sérologie positive (58,3 %) ainsi que de 7 des 93 sangliers (7,5 %) sélectionnés de manière aléatoire pour l’analyse bactériologique. Toutes les souches isolées se sont avérées être du biovar 2 de \( Brucella \) suis, à l’exception de trois d’entre elles qui appartaient au biovar 3.
Infección de cerdos domésticos y jabalíes de Croacia por *Brucella suis*


Resumen
Entre 2000 y 2004 se analizaron 93.107 muestras de suero procedentes de 1.997 explotaciones porcinas de once regiones de Croacia para detectar la presencia de anticuerpos contra la brucelosis. Se obtuvieron resultados positivos en 67 piares de siete regiones (prevalencia mediana individual: 1% aproximadamente; prevalencia de pia: 3,4%). Todas las piares infectadas, excepto dos, eran explotaciones al aire libre, probablemente en contacto con animales salvajes. De 2003 a 2004 también se analizaron 424 muestras séricas extraídas aleatoriamente de jabalíes abatidos por cazadores, en que se observó una seroprevalencia mediana de 27,6%. Se aisló *Brucella* spp. en 88 de los 151 cerdos seropositivos (58,3%) y en 7 de los 93 jabalíes sometidos a examen bacteriológico aleatorio (7,5%). Todas las cepas aisladas pertenecían al biovar 2 de *Brucella suis*, excepto tres que correspondieron al biovar 3.

De estos resultados se deduce que la brucelosis es enzoótica en las poblaciones de jabalíes de Croacia; estas poblaciones pueden constituir un reservorio a partir del cual la enfermedad se extendería a granjas de cerdos al aire libre, como ocurre en otros países de Europa Central y Occidental. Es la primera vez que se notifica la presencia del biovar 3 de *B. suis* en cerdos y jabalíes europeos, hecho que constituye un grave motivo de preocupación desde el punto de vista de la salud pública.

Palabras clave

Ces résultats indiquent que la brucellose est enzootique dans les populations de sangliers de Croatie. Ces populations constituent donc un réservoir potentiel de l’infection pour les porcs élevés en plein air, comme c’est déjà le cas dans d’autres pays d’Europe centrale et occidentale. C’est la première fois que *B. suis* biovar 3 est isolée chez des porcs et des sangliers en Europe ; cette découverte est préoccupante en termes de santé publique.

**Mots-clés**

Ces résultats indiquent que la brucellose est enzootique dans les populations de sangliers de Croatie. Ces populations constituent donc un réservoir potentiel de l’infection pour les porcs élevés en plein air, comme c’est déjà le cas dans d’autres pays d’Europe centrale et occidentale. C’est la première fois que *B. suis* biovar 3 est isolée chez des porcs et des sangliers en Europe ; cette découverte est préoccupante en termes de santé publique.

**Mots-clés**
References


