IMPORTANCE OF SEROLOGICAL DIAGNOSTICS IN OVINE EPIDIDYMITIS CAUSED BY BRUCELLA OVIS

DANKA KOVÁČOVÁ, PAVOL ZUBRICKÝ, MARIANA BABINČÁKOVÁ, AND MILAN TRÁVNIČEK

State Veterinary and Food Institute, 080 01 Prešov, Slovakia
kovacovasvpupo.sk

University of Veterinary Medicine, 041 81 Košice, Slovakia
travnicekuvm.sk

Received for publication September 13, 2006.

Abstract

The aim of the study was the presentation and evaluation of the results of serological monitoring of ovine epididymitis in Slovakia in 1995–2005. The study was based on the results of serological examinations for Brucella ovis antibodies carried out in 6 veterinary institutes (Bratislava, Nitra, Dolný Kubín, Zvolen, Košice, Prešov, Žilina, and Michalovce. During 1995–2004, the laboratory in Zvolen used the complement fixation test (CFT) in diagnostics of Brucella ovis infection, while other laboratories used mainly the double agar gel immunodiffusion (AGID) test. In 2005, almost 56% of samples were examined by ELISA, 15% by CFT, and 29% by AGID test. Although CFT is the prescribed test for international trade, we regard AGID test as the most practical serological method because of its sensitivity, simplicity, and easy interpretation. Totally, 91 926 blood sera of rams and ewes were examined. Ewes were examined in statistically non-significant number (up to 3%). Out of this number, 7 160 were positive for Brucella ovis, that represents 7.78% seropositivity. We propose to carry out serological diagnostics for ovine epididymitis consisting of AGID+ELISA methods in breeding rams before mating season, including young rams. Negative results of the tests would guarantee economic profitability of sheep breeding.

Key words: sheep, epididymitis, Brucella ovis, immunodiagnosis.

Ovine epididymitis is clinical or subclinical disease, where Brucella ovis is a causative agent. This disease negatively influences reproduction in sheep breeding. The course of the disease is mostly chronic and is manifested by changes in the genital organs in rams (epididymitis, orchitis) and by inflammation of the placenta in ewes (placentitis). Ewes may also abort. Disease can result in reduced fertility of rams and in increased perinatal mortality. Asymptomatic course of the disease is frequent as well. Infection is an infected ram that spreads Brucella ovis with semen. Brucella penetrate to the organism mainly orally and through genitalia. The pathogen causes inflammatory necrotic changes in sexual organs, chiefly in the epididymis. The infection may have a latent course as well. Infection is spread in flocks with higher number of rams in different age categories. Young rams are more sensitive at the time of sexual maturation. Additionally, ewes fertilised by infected rams help to spread the infection. Sheep are in hazard only during one mating period, they recover after finishing it.

The presence of genital changes (epididymitis occurring at one side and occasionally at both sides), diagnosed by palpation, can indicate Brucella ovis infection in a herd. Clinical examination discloses only about 50 % of rams infected by Brucella ovis (6). Clinical diagnostics is also non-specific, because clinical epididymitis can be caused also by other microorganisms: Actinobacillus seminis, Actinobacillus actinomycetemcomitans, Histophilus ovis, Haemophilus sp., Corynebacterium pseudotuberculosis, Brucella melitensis, and Chlamydia psittaci (5). The disease is rarely clinically manifested by swelling, painfulness, and increased temperature of the reproductive organs, especially the epididymis. The formation of cysts and various adhesions, and gravidity disorders with following complications in ewes are observed during a chronic course. The following clinical signs have been exceptionally found at auction markets of breeding rams: asymmetry of the scrotum (Fig. 1), palpable thickening of the deferent ducts, and enlargement of the epididymis head (Fig. 2), but mainly the epididymis tail with a size of walnut up to the size of chicken egg. Frequently, only one epididymis is affected. The tests in this case is enlarged or, in the case of excessive enlargement of the epididymis, atrophied. Latent course of the infection without clinical signs is the most frequent at present.
The presented paper summarises the results of serological monitoring of ovine epididymitis in Slovakia in 1995-2005 performed by the veterinary laboratories.

Material and Methods

Serological diagnostics of *Brucella ovis* infection in sheep in 1995-2005 was done in the State Veterinary Institute in Zvolen and in the State Veterinary and Food Institutes in Bratislava, Nitra, Dolný Kubín, Košice, and Prešov. Veterinary laboratories in Žilina and Michalovce performed the diagnostics only in 1995–1999. The majority of samples being examined were blood sera of rams. Ewes were examined in statistically non-significant number (up to 3%).

During 1995–2004, the laboratory in Zvolen used the complement fixation test (CFT), other laboratories used mainly the double agar gel immunodiffusion (AGID) test. In 2005, almost 56% of samples were examined by ELISA, 15% by CFT, and 29% by AGID test. The ELISA was used in the laboratories in Bratislava, Dolný Kubín, Zvolen, Prešov, CFT was used in all laboratories, AGID was used in the laboratories in Bratislava, Dolný Kubín, and Prešov in 2005.

In AGID test, *Brucella ovis* antigen, prepared in the State Veterinary and Food Institute in Prešov, was applied. CFT method was based on antigen and positive serum from the VLA Weybridge, United Kingdom, and from the National Veterinary Research Institute in Pulawy, Poland. In ELISA, a commercial kit (Bommeli, Switzerland) was used.

Results

Totally, 91,926 blood sera of rams and ewes were examined in the monitoring period 1995 – 2005. Out of this number, 7,160 were positive for *Brucella ovis* antibodies, that represents 7.78% seropositivity (Table 1).

The percentage of serologically positive sheep for ovine epididymitis, according to individual years, is clearly illustrated in Fig.3. The highest percentage of positive results was recorded in 2005 (24.10%) and the lowest one in 2001 (2.94%).

The highest rate of samples for ovine epididymitis, out of totally examined blood sera in the monitored period, was demonstrated at the laboratory in Prešov – 25%, Dolný Kubín – 18%, and Zvolen – 22% (Fig.4). The least number of animals positive for *Brucella ovis* antibodies was in the regions of Nitra and Bratislava, where sheep breeding is not so important.

The percentage of serologically positive sheep for *Brucella ovis* according to regions is presented in Fig.5. The Fig. 5 points out a relatively high prevalence of sheep with *Brucella ovis* antibodies in regions of Prešov – 11.82% and Zvolen – 11.32%. The lowest prevalence of *Brucella ovis* antibodies was recorded in the region of Nitra – 3.07%.

As it results from Fig. 6, in the period of 2002–2005, we recorded the stability in the number of serologically positive animals for infectious epididymitis (about 8,000 sheep per year).

In 2004, parallel serological examinations for contagious epididymitis were carried out in the Veterinary Institute in Prešov, using CFT and AGID methods (about 500 sheep blood samples). There was 90% correlation between results of both methods. Eighty blood samples were examined altogether by ELISA. Positive results in CFT or AGID method were always confirmed by ELISA. Monitoring proved great significance of antibodies for the diagnostics of the disease. However, the results cannot be coincidental because AGID method detects precipitating antibodies and CFT method complement fixation antibodies. There are different immunoglobulins (Ig) that despite similar space structure form in each individual a mixture of
molecules with different physical, chemical, antigenic, and biological properties. The most represented in blood sera of sheep is immunoglobulin G (IgG) which also binds the highest percentage of antibody activity. Its important characteristic is that it penetrates through placenta from mother to foetus; it has precipitating properties and binds complement.

In 2005, 8352 blood samples of rams were examined. Out of them, 2013 animals manifested specific antibodies against *Brucella ovis* (Table 1). Substantial part of samples (89%) was examined at the institutes in Prešov, Dolný Kubín, and Zvolen. Infectious epididymitis was confirmed by three methods. All the institutes, with the exception of Dolný Kubín, used CFT as an obligatory serological method. ELISA was used in laboratories of Dolný Kubín, Zvolen, Prešov, and Košice where almost 56% of samples were examined by the test. Area of utilisation of ELISA explains extremely high seropositivity of ram blood samples to *Brucella ovis* (24.1%). Only institute in Prešov commonly used three tests in their routine laboratory work – AGID test, as the most practical for diagnostics of ovine epididymitis, CFT for a quantitative expression of results and for international trade purposes, and ELISA as confirmation method.

![Fig. 3. The percentage of serologically positive sheep for *Brucella ovis* in 1995–2005.](image)

![Fig. 4. The rate of samples tested for antibodies against *Brucella ovis* by different veterinary institutes.](image)
Fig. 5. Percentage of serologically positive sheep for *Brucella ovis* in particular regions.

Fig. 6. Number of serologically examined sheep for *Brucella ovis* antibodies in Slovakia in 1995 - 2005.
for infectious epididymitis thus includes an obligation of immunological examination of breeding rams before mating season. It is undoubtedly a very important preventive measure. Therefore, we propose to carry out serological diagnostics for ovine epididymitis consisting AGID+ELISA methods once a year, including young rams. Negative results of the tests would guarantee economic profitability of sheep breeding. This consideration results from the fact that good correlation of AGID and ELISA results was confirmed, but absolute conformity in antibody detection determined by different methods does not exist. Even in using ELISA method, at interpretation of results certain sample results are evaluated as dubious and in that case ELISA producer recommends other suitable method.

The fact, that presence of specific antibodies against Brucella ovis signalises carrier state of infectious agent in reproductive organs, emphasises the following prevention measures:

1) introducing rams into breeding from non-infected herds (status of the herd without Brucella ovis occurrence)
2) control of serological diagnostics results for infectious epididymitis of rams in all transfers
3) evaluating rams only with negative serological result at auction markets
4) implying a suitable eliminating method in the case of confirmation of positive serological finding (exclusion of a ram from the breeding or according to regulation of the veterinary office)

Discussion

OIE Manual of diagnostic tests and vaccines recommends the use of CFT, AGID, and ELISA for the diagnosis of Brucella ovis infection (6). The sensitivity of AGID and ELISA is similar, sometimes higher than CFT (2), and therefore exactly the combination of AGID and ELISA shows the best results in diagnostics of infectious epididymitis (4). The fact that AGID is the most practical serological method because of its sensitivity, simplicity, and easy interpretation is underlined in the OIE Manual (6). CFT stays further an imposed serological test for diagnostics of infectious epididymitis in rams in the international trade. Standardisation of CFT results, based on the usage of international standard antiserum Brucella ovis (1 000 IU/mL), has been elaborated. It is generally accepted that blood sera reacting in CFT positively at the titre 10 and higher or blood sera containing 50 IU/mL or more are considered as positive for infectious epididymitis in the EU states (6).

In December 2004, the State Veterinary and Food Administration of the Slovak Republic issued new regulation concerning infectious epididymitis of rams, that includes an obligation of repeated serological examination of positive or dubious rams on day 21 after the first blood sample taking, using ELISA. In the first screening examination, one of the recommended serological methods can be used: CFT, AGID or ELISA. The regulation determines also conditions in the transport of rams from the place of breeding and in the trade with other member states of the EU. Recovery plan

### Table 1
Serological examination of sheep for ovine epididymitis in 1995–2005 by veterinary institutes

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1995</td>
<td>278/13</td>
<td>1301/132</td>
<td>728/25</td>
<td>0/0</td>
<td>1356/163</td>
<td>136/174</td>
<td>3148/0</td>
<td>8117 / 907</td>
<td>6.24%</td>
<td></td>
</tr>
<tr>
<td>1996</td>
<td>221/5</td>
<td>873/87</td>
<td>912/44</td>
<td>12/1</td>
<td>1961/141</td>
<td>1574/201</td>
<td>1351/75</td>
<td>6904 / 554</td>
<td>8.02%</td>
<td></td>
</tr>
<tr>
<td>1997</td>
<td>342/6</td>
<td>2811/214</td>
<td>939/32</td>
<td>24/4</td>
<td>3513/42</td>
<td>2393/168</td>
<td>2530/54</td>
<td>12552 / 520</td>
<td>4.14%</td>
<td></td>
</tr>
<tr>
<td>1998</td>
<td>192/13</td>
<td>1337/147</td>
<td>1279/1</td>
<td>163/19</td>
<td>3959/59</td>
<td>1953/94</td>
<td>2245/0</td>
<td>11128 / 333</td>
<td>2.99%</td>
<td></td>
</tr>
<tr>
<td>1999</td>
<td>121/20</td>
<td>2237/185</td>
<td>839/0</td>
<td>119/0</td>
<td>2952/0</td>
<td>1819/6</td>
<td>1225/51</td>
<td>9312 / 262</td>
<td>2.81%</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>157/4</td>
<td>2067/340</td>
<td>1452/13</td>
<td>124/3</td>
<td>2276/0</td>
<td>1833/85</td>
<td>0</td>
<td>7909 / 445</td>
<td>5.62%</td>
<td></td>
</tr>
<tr>
<td>2001</td>
<td>82/1</td>
<td>1106/80</td>
<td>1102/0</td>
<td>109/0</td>
<td>448/0</td>
<td>513 / 18</td>
<td>0</td>
<td>3360 / 99</td>
<td>2.94%</td>
<td></td>
</tr>
<tr>
<td>2002</td>
<td>180/0</td>
<td>2427/305</td>
<td>2266/0</td>
<td>153 / 3</td>
<td>1134 / 101</td>
<td>2018 / 6</td>
<td>0</td>
<td>8178 / 415</td>
<td>5.07%</td>
<td></td>
</tr>
<tr>
<td>2003</td>
<td>150/0</td>
<td>2050/238</td>
<td>2229/0</td>
<td>127 / 3</td>
<td>1121 / 58</td>
<td>2224 / 220</td>
<td>0</td>
<td>7901 / 519</td>
<td>6.57%</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>143/0</td>
<td>2841/384</td>
<td>2353/291</td>
<td>54 / 3</td>
<td>70 / 6</td>
<td>2752 / 809</td>
<td>0</td>
<td>8213 / 493</td>
<td>18.17%</td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>152/0</td>
<td>2565/443</td>
<td>2611 / 963</td>
<td>117 / 6</td>
<td>617 / 40</td>
<td>2290 / 561</td>
<td>0</td>
<td>8352 / 2013</td>
<td>24.10%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>samples/pos</th>
<th>samples/pos</th>
<th>samples/pos</th>
<th>samples/pos</th>
<th>samples/pos</th>
<th>samples/pos</th>
<th>samples/pos</th>
<th>samples/pos</th>
<th>samples/pos</th>
<th>samples/pos</th>
</tr>
</thead>
<tbody>
<tr>
<td>2018</td>
<td>21615/2555</td>
<td>16710/1369</td>
<td>1002/42</td>
<td>19407/610</td>
<td>20675/2342</td>
<td>10499/180</td>
<td>91926/7160</td>
<td>7.78%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Total positivity: | 3.07% | 11.82% | 8.19% | 4.19% | 3.14% | 11.32% | 1.71% | 7.78% |
5) regular serological examinations of rams for *Brucella ovis* before auction markets, mating season, and transfer, and in quarantine
6) performing disinfection of stable spaces before buying rams
7) keeping exact record in registers of breeds and to secure compulsory report of infection
8) thorough realisation of measures for suppression of infectious epididymitis in herds
9) studying also health heredity, because the reason of various results at infection transmission is diversity of sheep race
10) in a flock of ewes, where a ram with *Brucella ovis* operated, there is necessary at least 4 months pause in breeding

The aim of these preventive measures is the recovery of sheep breeding and reduction of economic losses because of disease occurrence. Qualitative and regular preventive serological diagnostics of infectious epididymitis is the most important in the achievement of successful recovery process. It also contributes to fulfilment of Government regulation of the SR No. 47, 2005 concerning the requirements for animal health during transport of sheep and goats and their exchanges with member states. That means that valid legislation of the EU has already been transposed into law of the Slovak Republic. It is legislation concerning the requirements for sheep breeding including infectious epididymitis control.

### References