BRUCELLOSIS OF RUMINANTS IN BOSNIA AND HERZEGOVINA: DISEASE STATUS, PAST EXPERIENCES AND INITIATION OF A NEW SURVEILLANCE STRATEGY

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ABSTRACT

The current animal health situation in Bosnia and Herzegovina requires the prioritization of diseases for the application of control measures. One of the diseases requiring high priority is brucellosis of ruminants. Brucellosis is a zoonotic infectious disease and one of the most important zoonoses in the world. Brucellosis has been recognized during the past five decades as an important infectious disease in ruminants in Bosnia and Herzegovina. Control and eradication of brucellosis in animals is based on test and slaughter control policy. When the existing brucellosis control program was instituted, the veterinary and animal production sector was almost exclusively owned by the government, an arrangement that promoted compliance with the program and resulted in the successful control of the disease.

This paper provides an overview of the current institutional and legislative framework for brucellosis control including the laboratory detection system and the epidemiological status of brucellosis in ruminants in Bosnia and Herzegovina. Relevant data were collected during the period spanning from the beginning of 2004 until the middle of 2007.

Data we collected reveal an increase in the number of reported outbreaks in ruminants as well as a related increase in the number of human cases.

This has brought serious consequences to public health, animal health and production and international trade.

KEY WORDS: brucellosis, ruminants, surveillance
INTRODUCTION

Brucellosis is a infectious disease with a long history in the Mediterranean region (1). Brucella agents are highly pathogenic for humans, causing one of the most serious zoonoses known (2). Since disease is generally not transmissible from human to human (3,4), prevention of human cases generally depends on the control of the disease in animals (5). For example, in Spain, from 1993 to 2003, 5 fold decrease of brucellosis prevalence was recorded along with concurrent sharp decline in the number of reported human cases, what was direct result of the national ruminant brucellosis eradication program initiated in early 90-ties (6). Brucellosis poses many challenges in designing effective surveillance system; the infection is chronic in both humans and animals, symptomatology and incubation periods are variable and microbiological confirmation is the only fully credible mean of final diagnosis. Brucellosis has been recognized during the past five decades as an important infectious disease in ruminants in Bosnia and Herzegovina (B&H). A government program designed in 1989 is currently in effect to control the disease. The aims of this paper are: to provide an overview of the currently applied brucellosis detection and control measures, to demonstrate the disease patterns based on available data collected for the last seven years, and to propose a surveillance strategy for disease control.

MATERIALS AND METHODS

This paper provides an overview of the current institutional and legislative framework for animal disease control including the laboratory detection system and the epidemiological status of brucellosis in ruminants in B&H. Relevant data were collected during the period spanning from the beginning of 2001 until the end of June, 2007. A presentation and descriptive analysis of current measures used for detection, control and prevention of brucellosis in ruminants is provided. This includes detailing protocols existing in the animal health laboratory network and providing the current scheme of disease information distribution among involved government veterinary agencies and animal health laboratories. The analysis is accomplished primarily through a retrospective assessment of relevant legal acts and their consecutive changes over time. Data on methods, frequency and extent of serum sampling were collected through interviews with representatives of animal health laboratories. Disease levels and trends were obtained using data from the national infectious diseases data base, published scientific papers and an investigation into the numbers and origins of samples submitted for routine laboratory testing. The data on disease occurrence in animals and humans were presented in respect to their temporal and spatial distribution in order to establish epidemiological trends. Established trends for brucellosis in animals were presented using the frequency of reported outbreaks, while a trend of brucellosis in humans was presented using frequency of reported cases. Definition of an outbreak of brucellosis in animals is adopted from the official Regulations on measures for control and eradication of brucellosis in cattle, sheep, goats and swine, were an outbreak represents occurrence of one or more brucellosis cases among animals of same species owned by the same farmer (7). Univariable linear regression models were constructed separately for cattle and sheep in order to quantitatively describe observed temporal trends in brucellosis occurrence in animals (8). Same regression analysis was used to describe temporal trend of brucellosis occurrence in humans. Statistical significance of the regression models was assessed using Student t test for significance of regression coefficients and F test for significance of regression model, at 5% level of statistical significance. In order to establish relationship between brucellosis occurrence in ruminants and humans we used correlation coefficients (9). Correlation coefficients were calculated for every pair of variables; number of outbreaks in cattle vs. number of outbreaks in small ruminants, number of outbreaks in cattle vs. number of cases in humans and number of outbreaks in small ruminants vs. number of cases in humans. Calculated correlation coefficients were statistically assessed using Student t test at 5% level of statistical significance for two sided test (9). Regression analysis, calculation of correlation coefficients and all statistical testing were accomplished using MINITAB 14 (Statistical software for Windows, ©2005 Thomson learning). Lack of reliable estimates on ruminant population in the country and inconsistent and incomplete data on the number of serum samples collected for brucellosis testing among animals had excluded possibility of reliable estimates of disease rates. We also identified constraints and limitations for the interpretation of the results provided.

Detection and control measures for brucellosis in animals in Bosnia and Herzegovina

Control measures for brucellosis have undergone several revisions since they were first instituted in 1948 (10-13). Almost two decades have passed since the last revision of brucellosis detection and control measures.
When the existing brucellosis control program was instituted, the veterinary and animal production sector was exclusively owned by the government, an arrangement that promoted compliance with the program and resulted in the successful control of the disease (14). Sporadic local outbreaks were reported in small ruminants (mainly sheep) after the introduction of new animals originating from within the former Yugoslavia into unexposed herds (1, 14). Human cases of brucellosis were rare and principally occurred in veterinarians and farmers as a consequence of occupational exposure. Currently, brucellosis detection is provided through serological screening of: all imported ruminants during quarantine, all dairy cattle (once a year) and all clinically suspect ruminant animals (7). The primary cause for clinical suspicion of brucellosis is the occurrence of abortion in late pregnancy. After laboratory confirmation, positive reactors are slaughtered and their remains buried. Quarantine measures are imposed on the herd/flock of origin. During quarantine, all animals in the herd/flock are sampled and tested using reference laboratory procedures at least twice within a 60 day period after identification of the first case. If new cases of brucellosis are established, diseased animals are removed and quarantine measures continued. There are no requirements for adjacent and contact herd/flock testing. There is no requirement to trace movement into and out of an infected herd/flock. This is partly due to the lack of a uniform animal identification system. There are seven veterinary laboratories in B&H. Six of these laboratories provide screening test for brucellosis (Rose Bengal) on serum samples submitted by field veterinarians or veterinary inspectors. When positive or inconclusive results occur, samples are submitted for confirmation testing at reference veterinary laboratories, where the complement fixation test is used. Both tests are done according to testing procedures for detection of brucellosis in animals prescribed by the OIE and relevant national legislation (2,7). Confirmed positive cases are reported by the regional official veterinarian to the entity Ministry of Agriculture/Veterinary service and the State Veterinary Office (Figure 1). Laboratories are not obliged to report the number of samples submitted for testing.

RESULTS AND DISCUSSION

Occurrence of brucellosis in ruminants in Bosnia and Herzegovina

Data on brucellosis occurrence we collected indicate an increase in the number of established outbreaks in ruminants, especially sheep and goats, as well as a disturbing increase in the number of human cases (Figure 2).
The summary statistics for the regression models that describe observed increase is provided in Table 1. According to results of the regression analysis, each year the number of recorded cases in humans is increased for about 72 cases more than previous year, while the number of outbreaks in sheep is for 58 outbreaks larger than year before. A significant increase in the numbers of human cases, followed by the resultant public outcry, triggered a more rigorous testing policy, and increased government funds were allocated for brucellosis control during the past three years. An expanded testing program had presumably contributed to an increased number of detected brucellosis cases in animals during this period. Therefore, regression models established using data predominantly from official sources as it is case in this paper, can not reliably quantify increase of ruminant brucellosis occurrence since it is not clear what portion of this increase is contributed by the expanded testing program and what is the direct consequence of disease spread. The spatial pattern of disease indicates a spreading trend (Map 1). The most prominent clusters of outbreaks of animal brucellosis over the last seven years period are seen in areas of Banjaluka, Bugojno, Konjic, Nevesinje, Novi Travnik, Sarajevo, Tomislavgrad, Travnik and Zenica. The absence of cases in some parts of the country cannot be interpreted as absence of disease, because of the small numbers or lack of samples submitted for testing from those areas during the past seven years (absence of evidence is not evidence of absence). The north-south pathway of disease spread coincides with the seasonal movement of sheep towards mountain pastures in the central south region in spring and towards valley pastures in the northern region in autumn.

The seasonal trend of brucellosis occurrence over the last seven years also demonstrates an increase in the numbers of identified outbreaks in ruminants. Within a year the largest number of brucellosis cases in ruminants is observed during the second and third quarters of the year (Figure 3). At first glance, it appears that the identified pattern of brucellosis occurrence does not coincide with the increased disease occurrence usually seen during the lambing/calving season (first quarter of the year). The lambing/calving season is quite extended in B&H, due to relatively significant climate variations in some regions. Also, the time period from sampling to laboratory confirmation and identification of an outbreak can be, in some instances, over one month. Therefore, at least some of the infections that occurred during the first quarter of the year were accounted for in the second quarter. This assumption is supported by data from years 2005-2007, when a more extensive and rigorous sampling campaign were undertaken. The spatial trend of spreading ruminant brucellosis in the country correlates with the observed pattern of increase in the number of reported brucellosis outbreaks in animals over the last seven years. Thus, it can be claimed that the current brucellosis detection system in B&H can detect trends, but it fails to provide reliable

<table>
<thead>
<tr>
<th>Model</th>
<th>Intercept</th>
<th>p value for β0</th>
<th>Slope</th>
<th>p value for β1</th>
<th>R²</th>
<th>p value for the model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporal trend for brucellosis occurrence in cattle</td>
<td>-3486</td>
<td>0.414</td>
<td>1.7</td>
<td>0.412</td>
<td>13.8%</td>
<td>0.412</td>
</tr>
<tr>
<td>Temporal trend for brucellosis occurrence in sheep</td>
<td>-5074.7</td>
<td>&lt;0.001</td>
<td>25.4</td>
<td>&lt;0.001</td>
<td>93.1%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Temporal trend for brucellosis occurrence in humans</td>
<td>-9550.9</td>
<td>0.003</td>
<td>47.7</td>
<td>0.003</td>
<td>86.2%</td>
<td>0.003</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Variables</th>
<th>Annual frequency of human cases</th>
<th>Annual frequency of outbreaks in cattle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual frequency of outbreaks in cattle</td>
<td>0.439 0.324</td>
<td></td>
</tr>
<tr>
<td>Annual frequency of outbreaks in small ruminants</td>
<td>0.924 0.003</td>
<td>0.57 10179</td>
</tr>
</tbody>
</table>

TABLE 2: The correlation coefficients with corresponding p values for every pair of assessed variables (the contents of cells represents Pearson's correlation coefficient and corresponding p value)

MAP 1. Spatial distribution of ruminant brucellosis outbreaks by reporting district during the last seven years
estimates of brucellosis levels in ruminant population. The reported outbreaks and cases shown here provide limited input for precise quantification of disease levels. Due to the limitations of our data sources (national disease data base contains only confirmed positive cases/outbreaks), disease proportions and rates could not been calculated.

**Evaluation of the current brucellosis control measures**
Compliance with animal brucellosis control requirements is currently lower than when these control measures were first instituted, due to the economic transition that has occurred. The primary components of the disease reporting system (farms, dairies, veterinary practices and laboratories) are no longer exclusively under government control. The effectiveness of the implementation of regulations is also compromised by the small ruminant management system (free range pasture, and transhumance), and the lack of coordination between veterinary service agencies and veterinary laboratories. Additionally, established high correlation between increase of the number of reported brucellosis cases humans and outbreaks in small ruminants, indicates that small ruminants, rather than cattle are the primarily source of infection in humans. Screening of imported animals is mandatory and is applied to all legally imported ruminants. Animals whose milk is bought by milk processing plants are tested once a year, and their milk is pasteurized. According to reported experiences of other countries, widespread application of pasteurization during milk processing significantly reduces the probability of indirect transmission of brucellosis from animals to humans (5). Bosnian Dairies generally process milk from cattle only. Therefore, pasteurization is almost exclusively applied to cow’s milk. Large quantities of dairy products, especially those of small ruminants (sheep), are frequently home-produced by farmers and sold on local markets. Consumption of these products is widespread, due to the public perception that they are organically produced. Inconsistent, and in some instances inadequate, processing of these products, traditional dietary habits and lack of education on methods of disease transmission to humans represent significant public health risk factors (1). Current detection of brucellosis in sheep is based on testing sporadic and unsystematically collected serum samples. Emphasis is placed more on disease detection in individual animals, contrary to general recommendations that brucellosis be treated as a flock/herd problem (15). Field veterinarians submit samples from clinically suspect animals only if they are notified by animal owners that an abortion has occurred. Due to the poor financial compensation available for testing and removal of diseased animals, the number of submitted samples from clinically suspect cases is generally very low. Since a nomadic and semi-nomadic sheep management system predominates, sheep flocks are often pastured in minimally accessible mountainous areas. Consequently, most animal health problems are handled by a shepherd. It is not uncommon for nomadic flocks to be the product of a merger between several flocks, thus promoting intermingling of flocks. These animals spend more time on communal pastures and therefore have a greater opportunity for contact with other, potentially infected, flocks.
(16). The lack of motivation by farmers to report suspect cases to the local veterinarian, aside from financial and practical reasons, can also be explained by community judgment that occurs towards owners of flocks in which infected animals are detected. This arises primarily from poor education of the public about this disease. The application of Rose Bengal and complement fixation tests in series is the prescribed testing protocol in current brucellosis detection requirements and it is also recommended by relevant international authorities such as OIE and EU. Nevertheless, many studies have demonstrated substantial inadequacies in this testing regime when applied in the detection of brucellosis in small ruminants (17). As a result of the above mentioned limitations, the official data on disease occurrence are often unreliable, incomplete, and unrepresentative.

CONCLUSION

The approach to control, prevention or eradication of brucellosis in a country or region will depend on many factors, such as the level of infection in the herds or flocks, type of husbandry, economic resources, public health impacts, and potential international trade implications. Decision-making by those charged with policy making is likely to be intuitive unless accurate and current epidemiological information is available. Even though the brucellosis detection system in Bosnia and Herzegovina indicates an increasing trend for disease in ruminants, the numbers of cases/outbreaks that have been reported are still low relatively to the estimates for the overall ruminant population. However, the evaluation of the brucellosis detection system provided in this paper indicates available data on brucellosis occurrence are not reliable.

An assumption that large numbers of brucellosis cases in animals are not reported is likely valid. This is supported by reports of increased numbers of brucellosis cases in humans. This finding would not be possible if disease levels among animals were low and sporadic as official data indicate. Also, the spatial distribution of brucellosis outbreaks in animals tracked over time presents an indirect verification of this underreporting. These data indicate ruminant brucellosis spreads to wider areas from year to year. Brucellosis persistence in some areas, despite the implementation of control measures following initial outbreaks, raises questions about the efficiency of measures used. Based on this, our conclusions are:

- The implemented brucellosis control measures have not halted its spread,
- Human health in this country is seriously threatened by brucellosis and
- The current brucellosis detection system cannot be used to provide reliable estimates of disease prevalence and incidence and cannot be used to establish a scientifically based surveillance program. A well-functioning surveillance system, fed by valid data collected from the field, must be established before a brucellosis control program can be designed and implemented.
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