DETECTION OF BOVINE GENITAL CAMPYLOBACTERIOSIS IN POPULATION OF HEIFERS IN POLAND

MONIKA SZYMAŃSKA-CZERWIŃSKA AND KRZYSZTOF NIEMCZUK

Department of Cattle and Sheep Diseases, National Veterinary Research Institute, 24-100 Pulawy, Poland
monika.szymanska@piwet.pulawy.pl

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Abstract

The main aim of this study was to evaluate the prevalence of campylobacteriosis in population of heifers in Poland. An additional objective was to compare two independent methods used in diagnosis of the disease: culture and PCR. The 1,600 vaginal swabs from heifer origin from different farms of Poland were investigated using both methods. The results showed that the percentage of positive samples was 0.75% when the culture method was used, while the application of PCR method has shown 1.81% positive results.

Key words: heifers, Campylobacter foetus venerealis, campylobacteriosis, laboratory diagnosis, Poland.

Campylobacteriosis is OIE notifiable disease. It is considered to have socioeconomic and/or public health implications and is significant in the international trade of animals (8). Causative agent of the disease - Campylobacter foetus is divided into two subspecies: C. foetus subsp. foetus (CFF) and C. foetus subsp. venerealis (CFV) (13). CFV is isolated from the bovine reproductive tract of both sexes. The reservoir of CFV is the penile prepuce of infected bulls not showing clinical signs, but being carriers and infecting females at service (1, 10, 14). Females are most often asymptomatic carriers of the pathogen. Infection occurs most commonly during mating or artificial insemination. It seems that litter, fertiliser, equipment for animal cleaning, semen, and environmental conditions may be also important in the transmission of the agent (9). With the introduction of artificial insemination and testing of bulls for the presence of CFV, the disease is rarely recorded (10).

The aim of this study was to assess the prevalence of genital bovine campylobacteriosis in the population of heifers in Poland using culture and PCR techniques. The additional target of this study was to compare the results obtained by both methods.

Material and Methods

Materials. A total of 1,600 vaginal swabs from heifers originating from different farms of Poland were examined during 2008-2009. The swabs were taken from farms involved in the export of heifers. The samples were put into the transport medium (Cary-Blair).

Diagnostic culture. 200 μl of the transport medium was plated onto Campylobacter blood free selective Agar Base (Oxoid) supplemented by antibiotics SR 155E (Oxoid) and Bartlett medium (10). Next, the samples were incubated at 37°C in a microaerobic environment that was generated by using an anaerobic jar and a Campygen sachet (Oxoid). The presence of C. foetus subsp. venerealis was indicated by the presence of smooth, translucent colonies arising after 48 to 72 h, followed by microscopic confirmation of Campylobacter morphology and motility. Additionally, the glycine tolerance was tested for positive samples. The medium contained enriched broth, fibrous agar, and 1% glicyne, its pH was 7.0. A few drops of culture were added to this medium and the medium was incubated at 37°C in a microaerobic environment. A positive result was accepted if there was a significant multiplication of bacteria.

DNA extraction: The genomic DNA from swabs was extracted using a commercial kit (QIAamp DNA mini kit, Qiagen), according the manufacturer’s protocol for swabs DNA extraction. The positive control of genomic DNA was obtained from reference strain of Campylobacter foetus subsp. venerealis (ATCC19438) using commercial DNeasy Blood & Tissue Kit (Qiagen), following the manufacturer’s instructions.

PCR. The PCR was done in a final volume of 50 μl of reaction mixture containing: 5 μl 10 x PCR buffer with MgCl₂, 2 μl of 2 mM dNTP, 1 μl of 10 pmol primer VenSF (5’-CTTAGCAGTTTGGGATA TTGCCATT-3’), 1 μl of 10 pmol primer VenSR (5’-
GCTTTTGAGATAACAATAAGAGCTT-3’), 0.2 μl of 5 U/μL thermostable polymerase DNA, 39.8 μl of sterile water and 1 μl of chromosomal DNA solution. The following condition were applied: 40 cycles, initial denaturation at 96°C – 60 s, denaturation at 96°C – 15 s, annealing at 60°C – 60 s, elongation at 72°C – 60 s, final elongation at 72°C – 120 s. Amplification was carried out in a Tepersonal termocycler (Whatman Biometra). PCR reactions were analysed by electrophoresis of 8 μl of PCR product in 1% agarose gel in 1 x TAE buffer and visualised by staining with ethidium bromide and ultraviolet transillumination. The molecular weight of the obtained product was determined on the basis of molecular weight marker, which was GeneRuler™ 100 bp DNA Ladder (Fermentas) and positive control. A sample was considered positive when amplified DNA of the same size as the positive control DNA (142 bp) was present in the PCR reaction (5).

Results

The results of the study are presented in Table 1. The percentage of positive sample was 0.75% when culture method was used. In case of a positive glycine tolerance test, the isolates were designed as glycine tolerant *C. foetus* subsp. *venerealis*. This test allowed distinguishing the CFV of *Campylobacter foetus foetus*, which is glycine intolerant. All isolates were glycine tolerant and designated as CFV. This was subsequently confirmed by the PCR study. When the PCR was used, the percentage of positive results was higher and amounted to 1.81%. The results of PCR are presented in the Fig. 1. All samples positive in subculture were confirmed as positive by PCR. Out of 1,588 negative samples in culture method, 17 samples were found to be positive by PCR.

Discussion

Although the incidences of bovine venereal disease have decreased in countries where artificial insemination, effective vaccination, and control programmes are extensively practised, the distribution of BVC is still worldwide (3, 10). There is a little information about *Campylobacter foetus venerealis* infections in the world and therefore little data on the economic impact of BVC is available. Campylobacteriosis is still a significant problem in Australia, the United Kingdom, Nigeria, Argentina, Jamaica, and some parts of the United States (2, 6, 12). For example, in a study of cattle herd in New South Wales, Australia (1981 to 1991), BVC was present in 35% of farms and was suspected to be present in 11% of other farms. A total of 25% of herds were endemic and 5% of new herds were infected each year (12). The first cases of the disease of cattle have been described in Poland in 1950. Since that time, the disease has been diagnosed in both heifers and bulls. The last literature data on the prevalence of the bovine genital campylobacteriosis in Poland came from the 80s of the last century (9). Comparing our results with those studies, it should be noted that the percentage of BVC cases was much higher in the past than today.

Table 1

Comparison of culture and PCR assays results from vaginal swabs from heifers

<table>
<thead>
<tr>
<th>Method</th>
<th>Positive sample (n)</th>
<th>Negative sample (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>12</td>
<td>1,588</td>
</tr>
<tr>
<td>PCR</td>
<td>29</td>
<td>1,571</td>
</tr>
</tbody>
</table>

Fig. 1. Electrophoretical separation of amplification products.

Lines: 1 - positive control, 2 - negative control, 3-5, 7-9 – negative sample, 6 – positive sample.
The introduction of artificial insemination and testing bulls for the presence of CFV reduced the incidence of this disease. However, it was shown that the pathogen is present in the environment, but the infection rate is not high and amounts to 1.81%. The diagnosis of BVC is difficult because of the low survival rate of the bacteria with conventional sampling procedures (1). The pathogen is very sensitive to atmospheric oxygen and it limits its survival during transport. Moreover, slow growth of CFV allows a rapid overgrowth by more vigorously multiplying contaminating microorganisms. This may be the reason for obtaining false-negative results for infected animals (7). In our study, Campylobacter fetus subsp. venerealis was isolated from 12 (0.75%) swabs when the culture method was used. When the same material was tested by PCR this pathogen was found in 29 (1.81%) tested samples. This indicates that PCR is more sensitive in comparison to the culture method, which can give false negative results.

References