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PCR DETECTION OF COXIELLA BURNETII FROM BULL SEMEN SAMPLES USED FOR ARTIFICIAL INSEMINATION

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ABSTRACT

444 samples of bull frozen semen used for artificial insemination from local and foreign breeding centers were investigated by PCR for the presence of DNA of *Coxiella burnetii*. *C. burnetii* DNA was detected in 32 semen samples from US and was not found in semen straws from local breeding centers and breeding centers of Netherlands and Great Britain. According to the results, it is evident that despite the restrictions and controls on the international movement of semen, bull semen is still a potential source of pathogen transmission.

KEY WORDS

Artificial insemination, *Coxiella burnetii*, bull semen, PCR.

Coxiella burnetii is an obligate intracellular parasite, a polymorphic gram-negative rod-shaped bacterium that causes human Q fever and animal coxiellosis. In animals coxiellosis is often asymptomatic. The pathogen has a tropism for reproductive organs. It plays a role in infertility of cattle and may cause metritis, abortions, prematurity of the fetus. In sick animals, the pathogen is actively released into the external environment with urine, placenta and feces [1].

Outbreaks and sporadic cases of coxiellosis are reported everywhere, especially in countries with developed livestock production. Endemic Areas are the western part of the USA, Australia, Africa, Great Britain, the Mediterranean countries, and the Central Asian republics of the former USSR. In the Russian Federation the disease is registered in more than 50 administrative units. In recent years, a significant increase in the incidence of coxiellosis has been recorded in many countries of the world. The acute form of Q fever in humans is manifested in the form of a flu-like febrile illness, SARS.

C. burnetii is very resistant to environmental influences. Low temperatures (from –4 to –70 degrees Celsius) create particularly favorable conditions for the conservation of bacteria. At the same time, the virulent properties of *C. burnetii* do not change, or, decreasing during storage, are quickly restored when passaged in laboratory animals. Even a single infective particle can initiate an infection in the animal model [2].

In the 90s, cases of the detection of *C. burnetii* in semen of seropositive bulls were described [3].

Artificial insemination (AI) is a successful technique that is used for the breeding of cattle around the world. Modern technologies for the collection of semen for AI involve deep freezing and using diluents and cryo-protectant in order to stay sperm viable. Other substances that can be added to the semen are antibiotics, and an extender, which makes it possible to use the amount of sperm for a greater number of inseminations. However, the widespread sale of doses of sperm increases the potential risk of the spread of infectious diseases.

Cell culture is still used as a sensitive tool for routine detection of *C. burnetii*, but this method is laborious and time-consuming. Isolation and handling of the Q fever agent requires rigorous compliance requirements due to pathogen can potentially be used in bioterrorism and its handling is federally regulated.

PCR is a highly sensitive and specific detection method that has been used in different countries for *C. burnetii* detection in milk, blood and animal tissue samples [4-10].

The aim of our work was to investigate the prevalence of *C. burnetii* in frozen bull semen samples of Russian origin and imported semen straws used for artificial insemination.

MATERIALS AND METHODS OF RESEARCH

Semen straws were collected from local (n=211) and foreign (Netherlands, Great Britain and the USA) (n=233) AI centers. Semen sample was diluted 1:3 in 0.9% sodium chloride. DNA was extracted from 100 µl suspension with RIBO-prep extraction DNA/RNA kit (AmpliSens, Russia). PCR for *C. burnetii* detection was performed using LSI VetMAX™ Triplex *Coxiella burnetii* and *Chlamydomphila* spp. kit (Life Technologies Corporation, France) according to the manufacturer's instructions. All real-time PCR assays were performed on a RotorGene Q (Qiagen, Germany) real-time PCR instrument. Positive samples were re-tested using conventional PCR with primers *cocF* and *cocR* [10], which amplifies the 16S rRNA gene of *C. burnetii*. The amplification was performed in a total volume of 25 µL containing 10 µl of DNA sample, 5 µl PCR-mix 1 (0.2 mM dNTPs, 10 µM each primer), 10 µl PCR-mix blue (AmpliSens, Russia). The thermal program was carried out on "Tercyc" Multi-block Thermocycler (DNA-technology, Russia) under the following conditions: 94°C for 5 min and then for 42 cycles of 94°C for 10 sec, 60°C for 10 sec, 72°C for 10 sec and a final extension at 72°C for 3 min. The PCR-amplification products were examined by electrophoresis in a 1.8% agarose gel, visualized under UV and photographed by gel documentation system. Four positive samples were confirmed by sequencing of the 16S rRNA PCR products using specific primers and the Big Dye® Terminator v1.1 Cycle Sequencing Kit on a GeneAmp PCR System 2720 PCR instrument (Applied Biosystem, USA) and ABI PRISM 3130 Genetic Analyzer automatic sequencer.

RESULTS AND DISCUSSION

We studied total 444 semen samples of bulls of different meat and dairy breeds, including 211 samples from bulls from Russian breeding centers, 233 samples from foreign breeding centers. No DNA of *C. burnetii* was found in semen samples from local breeding centers and breeding centers of Netherlands and Great Britain. In our study *C. burnetii* DNA was detected in 32 semen samples from US breeding centers. The presence of pathogen DNA was further confirmed by amplification and sequencing of the 16S rRNA gene using specific primers.

C. burnetii is widespread among domestic animals. Nevertheless, information about the pathogen transmission through the bull semen is still insufficient. Manufacturers of cryoconserved semen for AI use special media for sperm dilution and storage. They increase the volume of sperm, which is of great practical importance for the intensive use of bulls, as well as to protect sperm and support its biological properties. One of the medium often used in US breeding farms includes milk. It should be noted that it is well known about the distribution of *C. burnetii* with milk [7-9]. It was shown that *C. burnetii* was found in 1.42% of milk samples studied in Turkey [10], the frequency of *C. burnetii* in milk samples studied in Iran using different PCR assays was more than 10% [11]. *C. burnetii* was found in >94% samples of bulk tank milk from U.S. dairy herds tested during 2001-2003 [9] and 45% of composite milk samples of lactating cows tested in the U.S. in 2007 [12]. Possibly, the high frequency of DNA *C. burnetii* detection in semen samples in our study is associated with *C. burnetii* contaminated milk used in US breeding centers to dilute the sperm. But the cases of the pathogen detection in semen of seropositive bulls indicate the need for additional control of semen production used for AI.

CONCLUSION

According to the results, it is evident that despite the restrictions and controls on the international movement of semen, bull semen is still a potential source of pathogen transmission. The results of this study are limited to the PCR-based methods for detection of *C. burnetii* in the semen samples, so we cannot confirm the viability of the pathogen. In subsequent studies it is better to compare results of PCR methods for *C. burnetii* detection with results of other methods. However, our results suggest that the risk of transmitting *C. burnetii* via semen exists and that it would be valuable to test semen batches for the presence of pathogen.

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