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Analysis of hygienic critical control points in boar semen production

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ABSTRACT

The present study addresses the microbiological results of a quality control audit in artificial insemination (AI) boar studs in Germany and Austria. The raw and processed semen of 344 boars in 24 AI boar studs were analyzed. Bacteria were found in 26% (88 of 344) of the extended ejaculates and 66.7% (18 of 24) of the boar studs. The bacterial species found in the AI dose were not cultured from the respective raw semen in 95.5% (84 of 88) of the positive samples. These data, together with the fact that in most cases all the samples from one stud were contaminated with identical bacteria (species and resistance profile), indicate contamination during processing. Microbiological investigations of the equipment and the laboratory environment during semen processing in 21 AI boar studs revealed nine hygienic critical control points (HCCP), which were addressed after the first audit. On the basis of the analysis of the contamination rates of the ejaculate samples, improvements in the hygiene status were already present in the second audit ($P = 0.0343$, F-test). Significant differences were observed for heating cabinets (improvement, $P = 0.0388$) and manual operating elements (improvement, $P = 0.0002$). The odds ratio of finding contaminated ejaculates in the first and second audit was 1.68 (with the 95% confidence interval ranging from 1.04 to 2.69). Furthermore, an overall good hygienic status was shown for extenders, the inner face of dilution tank lids, dyes, and ultrapure water treatment plants. Among the nine HCCP considered, the most heavily contaminated samples, as assessed by the median scores throughout all the studs, were found in the sinks and/or drains. High numbers ($>10^3$ colony-forming units/cm^2) of bacteria were found in the heating cabinets, ejaculate transfer, manual operating elements, and laboratory surfaces. In conclusion, the present study emphasizes the need for both training of the laboratory staff in monitoring HCCP in routine semen production and audits in such AI centers for the external control of hygiene parameters.

1. Introduction

Raw boar ejaculates commonly contain bacterial contamination [1]. Bacteria may affect *in vitro* quality and the longevity of preserved boar semen during storage, resulting in lower fertility rates [2] and economic losses for the boar studs [3]. The spermicidal effect appears to be dependent on the concentration of bacteria [4,5].

To control bacterial growth, antimicrobial agents are essential components of semen extenders [6]. The respective epizootic departments regulate the addition of antibiotics and their minimum concentrations. In the European Union, these regulations are outlined in Council Directive 90/429/EEC; however, creative leeway still exists.

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Traditionally, penicillin and streptomycin were the common antimicrobial combination added to the extenders [7]. Today, the most popular preservative antimicrobial class used in porcine semen extenders is aminoglycosides, especially gentamicin [8]. Although these agents are added in high concentrations, studies revealed bacteriospermia in 14.7% to 31.2% of the extended porcine semen [8,9]. Bacteria from the families Enterobacteriaceae, Alcaligenaceae, and Xanthomonadaceae were frequently identified. Most of these bacteria are associated with the environment but are also opportunistic pathogens commonly related to nosocomial infections in human and animal medicine [10].

The high number of environmentally associated species raises the question of the source of these contaminant bacteria. Through field investigation, multiple contamination points of extended ejaculates have been identified. Sources can be simply classified into animal or nonanimal origin [11]. Unfortunately, the evaluation of contamination points was limited because of the lack of comparable data.

Thus, we conducted a thorough artificial insemination (AI) boar stud benchmark study to locate and evaluate hygienic critical control points (HCCP) according to their significance in semen processing. The present article will compare the microbiological results of an external quality control program of 24 AI boar studs, including the types and sources of contaminants and their resistance to antimicrobial agents.

2. Materials and methods

2.1. Animals, semen processing, and boar studs

Field investigations were performed over a 4-year period (2010–2013), excluding the summer, in 24 AI boar studs (size, 80–470 boars) as part of an external quality control program. The boar studs were located throughout Germany and Austria. Seven AI centers held fewer than 120 boars, 11 were middle sized (120–240 boars), and six centers held more than 240 boars. The boar replacement rates were 40% to 60% depending on the AI center and interfered with genetic progress, semen quality, libido, and physical soundness. All the studs used solid flooring. Six AI studs used deep litter with sawdust (25%), and 18 AI studs used straw (75%). All the boars were routinely used for AI, received a commercial feed (pellets) for AI boars, and were housed in individual pens equipped with nipple drinkers according to the European Commission Directive for Pigs Welfare.

From 24 boar studs in 2010/11, an average of 14.3 ± 2.4 raw and corresponding extended ejaculates were analyzed (data set 1, n = 344). The extended ejaculates were analyzed on Day 2 of semen storage. Contaminant bacteria were quantified and characterized (species and/or resistance profile) as described in the following. In 2012/13, during a follow-up investigation, an average of 15.0 ± 0.5 extended ejaculates were analyzed from 21 boar studs (data set 2, n = 314). The age distribution (average ± standard deviation) of the boars included in data sets 1 and 2 was 25.0 ± 4.9 months.

Ejaculates of randomly selected boars were collected weekly by the gloved-hand method. The gel fraction of the semen was removed by gauze filtration. After collection, the semen volume and sperm concentration were determined to produce the insemination dose (90-mL aliquot) containing 2×10^9 spermatozoa. Only ejaculates that passed the thresholds for commercial use in AI were used. The selection criteria were a minimum of 75% morphologically normal spermatozoa, sperm motility of at least 70%, and a total amount of 20 billion or greater spermatozoa per ejaculate. Because microbiological analyses were performed in a different laboratory than the spermatological analyses, the samples were stored in liquid nitrogen for transport and until further processing. For cryoprotection of the bacteria, 1 mL of each raw ejaculate was supplemented with 25% glycerol and frozen immediately after semen collection [12].

The semen samples were extended in a one-step (n = 8 studs) or two-step (n = 16 studs) dilution procedure with Beltsville thawing solution supplemented with 250 μg/mL gentamicin (BTS; Minitube, Tiefenbach, Germany). The one-step dilution was performed at 32 ± 1 °C. In the two-step dilution procedure, an initial dilution was performed within 15 minutes after semen collection with a preheated extender (32 ± 1 °C) approximately 1:1 (v:v), followed by a second dilution (24 ± 3 °C) within 1 hour after predilution [13].

The samples were transported to the laboratory of the Institute for the Reproduction of Farm Animals Schönow (IFN, Bernau, Germany) in insulated boxes with controlled temperature (17 ± 2 °C) for further storage and analysis of semen quality. The semen samples remained at 17 ± 2 °C during storage in the laboratory. On Day 2 of semen storage, an aliquot of the extended semen was frozen with glycerol 5:1 (v:v) [12] and transported in liquid nitrogen to the laboratory of the Leibniz Institute for Zoo and Wildlife Research (IZW, Berlin, Germany) for microbial analyses.

2.2. Microbial analysis

2.2.1. Bacterial isolation and identification

Ten microliters of each semen samples (raw and extended) was streaked onto the plate set routinely used in the bacteriologic diagnostics at the IZW (Columbia Agar with sheep blood, Gassner and UTI Clarity agar [all Oxoid Thermo Fisher, Wesel, Germany]). Additionally, 100 μL of the extended samples was pipetted into 5 mL of Nutrient Broth (Oxoid Thermo Fisher). The plates and broths were incubated at 37 ± 2 °C under aerobic conditions. After 24 hours, the plates were visually inspected for growth; if no growth was present, the corresponding broths were streaked onto Columbia Agar with sheep blood. Then, the plates were incubated at room temperature for five additional days to promote environmental bacteria, during which time they were checked for additional growth every 24 hours. Each distinct bacterial colony type was subcultured onto Columbia Agar with sheep blood and identified by either classical biochemical assays [14] or the respective commercial Api Test system (BioMerieux, France).

2.2.2. Total aerobic cell count

A dilution series of 10^{-2} to 10^{-6} with 0.9% saline solution was performed for each sperm sample to determine