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Short communication

Outbreak of *Pseudomonas aeruginosa* mastitis in a dairy cow herd in northern Greece and its control with an autogenous vaccine

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Abstract

In a dairy cow herd consisted of herd of 200 lactating Holstein-Friesian cows and heifers, clinical signs of mastitis in 40 out of 170 animals were observed. Treatments with antibiotics were proved ineffective. Milk bacterial cultures from 15 affected animals revealed *Pseudomonas aeruginosa*. An autogenous vaccine was administered subcutaneously, twice in a month period, to all adults. Cases of clinical mastitis declined significantly ($p < 0.0001$) during next 3 months.

Key words: dairy cows, *Pseudomonas aeruginosa*, clinical mastitis, control, autogenous vaccine

Introduction

Outbreaks of *Pseudomonas aeruginosa* mastitis have been reported in dairy cows (Osborne et al. 1981) and sheep (Las Heras et al. 1999). In such cases, infection usually stems from a point source, such as contaminated dry-off preparations, water, or teat dips. The present paper reports a *P. aeruginosa* mastitis outbreak in a dairy cow herd in northern Greece. Furthermore, preparation and administration of an autogenous vaccine to control the outbreak is reported for the first time.

Materials and Methods

Our study refers to a herd of 200 lactating Holstein-Friesian cows and heifers. Clinical signs of mastitis, mostly in one quarter, 30-50 days post-partum in 40 out of 170 animals were observed. Empirical treatments with antibiotics administered intramammarily or systemically, as penicillin-streptomycin (Vetricillin®-CEVA, Tetra-Delta®-VETERIN), enrofloxacin (Baytril®-BAYER) and ceftiofur (Eficur®-HIPRA) were proved ineffective and 12 cows were slaughtered.

Table 1. Clinical mastitis cases and animals slaughtered before and after vaccination.

	Before vaccination	After vaccination
Mastitis cases	40	45
Animals slaughtered	12	-

The cows were milked twice daily in a herring-bone parlor. In general, the level of hygiene in the farm was considered by the authors as relatively high. Dry-off treatment was applied to all the animals in the herd using antimicrobial suspension containing benethamine penicillin, penethamatehydriodide and framycetin sulphate (Benestermycin®, Boehringer Ingelheim GmbH), following all instructed guidelines.

The first clinical sign of the affected animals was milk drop, accompanied by distinctively abnormal and watery mammary secretion. No systemic clinical findings were observed. Mild enlargement and increased sensitivity of the mammary parenchyma were observed. Cultures of the mammary secretions were performed before application of antimicrobial therapy. Milk samples separately obtained from each quarter from 15 affected animals (60 samples in total) were collected in sterile tubes after discarding first squirts. Three (3) milk samples from non-affected cows were also collected. All isolates were biochemically identified using standard microbiological techniques (Barrow and Feltham 1993). Cultures were also conducted for the water in the farm, the parlor, the wipes for dry cow treatment, as well as the preparation used for dipping.

Results and Discussion

All milk samples from the affected animals were found positive for *P. aeruginosa*. Coagulase negative *Staphylococci* (CNS) were identified in 3 cases of mixed infections, while the milk samples of the 3 non-affected cows were found negative to culture as well as the water of the farm, the parlor and the preparations used for the udder hygiene. Antibioqram was not conducted in any of the above cases.

An autogenous vaccine for *P. aeruginosa* was prepared in the Laboratory of Microbiology and Infectious Diseases of the Veterinary Faculty in Thessaloniki and administered subcutaneously twice in a month period, to all adult female animals. During the first 10 days after vaccination 3 new cases of *P. aeruginosa* mastitis were observed. In general, cases of clinical mastitis declined significantly ($p < 0.0001$; Chi-square test) after this point and only 5 new cases of clinical mastitis occurred during the next 3 months (Table 1).

In general, environmental bacteria are isolated from less than 10% of clinical cases in dairy herds (Smith

and Hogan 2008). Intensive preventive measures against contagious pathogens in dairy cows have lowered respective incidence rates, whilst environmental microorganisms remain difficult yet to eradicate, despite systemic application of high hygiene standards (Oliveira and Ruegg 2014). Verbeke et al. (2014) estimated low incidence (5.4%) of non-specific pathogens among which *P. aeruginosa*.

In the present study an outbreak of massive mastitis in a dairy cow herd is described, attributed to *P. aeruginosa*, that led many animals to the slaughterhouse. *Pseudomonas aeruginosa* thrives on high temperatures, high humidity, organic bedding materials as well as overcrowded and poorly ventilated facilities (Divers and Peek 2008). The infection route in the present study was not possible to be established, as examinations of the water, of the parlor and the equipment of udder hygiene were found negative. Usually, the equipment for udder hygiene is the most possible infection source (Daly et al. 1999), as well as the water of the farm (Divers and Peek 2008).

In this case, clinical findings were restricted to the udder, in contrast to the literature data describing additionally systemic signs found in *P. aeruginosa* mastitis outbreaks (Osborne et al. 1981). In regard to treatment, mastitis caused by *P. aeruginosa* is known not to respond to antimicrobial therapy (Osborne et al. 1981). Similar were our conclusions in the present study. As the hygienic measures were also ineffective in the present case, an autogenous vaccine for the control and prevention of the outbreak was used. Autogenous vaccines against *P. aeruginosa* have been reported effective in dogs, cats, and rabbits (Weiss et al. 1998). Commercial vaccines against *E. coli*, the other important environmental agent, are used in Europe (Bradley et al. 2015). Vaccinations seem promising for the control of cow mastitis, having as an advantage the decreased use of antibiotics.

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