CASE REPORT

An Outbreak of *Pseudomonas* Mastitis in Dairy Cows

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Summary

An outbreak of mastitis in a dairy herd is described in which the causative organism was *Pseudomonas aeruginosa*. Cases occurred either in dry cows or in animals which had very recently calved. The fact that all four quarters were involved is a very strong indication that the bacteria had been introduced in the dry cow therapy.

Résumé

Une éruption de mammite à *Pseudomonas*, chez des vaches laitières

Les auteurs décrivent une éruption de mammite, imputable à *Pseudomonas aeruginosa*, dans un troupeau laitier. Des cas se produisirent, tant chez des vaches taries que chez des fraîche vêlées. L'implication de tous les quartiers permit de présumer que le traitement au tarissement était à l'origine du problème.

History

A herd of 45 milking cows and 15 heifers of the Holstein and Brown Swiss breeds and crosses was involved in a mastitis problem which affected 12 cows in all mainly before or soon after calving. The animals were kept at pasture during the summer months but were brought in to a large barn during the winter. Lactating and nonlactating cows occupied the same building which had a roof 25 feet (7.6 m) high. During the winter months the walls remained damp despite the use of fans. Individual stalls were well bedded with sand and straw but remained wet because of a poor floor drainage slope. At one end of the building in which calving stalls were situated drainage was particularly poor and the central passage also held a considerable amount of water in spite of daily cleaning. Stalls were cleaned infrequently.

Cows were milked in a 4:4 herringbone parlor. Udders were washed prior to milking and partially dried with paper towels. Dry cow treatment was only carried out on cows which showed evidence of infection when quarter samples were taken at the time of drying off. The syringe used for intrammamary injection was not sterilized after use and the temperature of water used in washing was not greater than 30°C.

Clinical Findings

In September 1979 a five year old animal (No. 1), in good physical condition, which had calved the previous day was found in sternal recumbency showing clinical signs of severe depression and reduced respiratory rate. All four quarters of the udder were swollen and the secretion was blood tinged. Because of a possibility of hypocalcemia treatment with a calcium, magnesium, potassium preparation intravenously[1] was commenced but the animal died as a result of cardiac arrest after administration of 50 mL.

A field postmortem examination showed no specific lesions but a yeast was cultured from the milk and a diagnosis of acute yeast mastitis was made.

During October and November acute mastitis occurred in animals no. 2, 3 and 4 two to three weeks prior to calving. All three animals had been treated with a dry cow preparation containing cloxacillin[2] from a multidose vial, 8.5 mL quantity of the infusion (500 mg benzathine cloxacillin) into each quarter. After use the syringe used to infuse the drug was washed in hot water but not sterilized. The antibiotic preparation was usually stored in a refrigerator at a temperature of 2-4°C.

Similar signs were shown by each of the animals; all four quarters became swollen and secreted a serous or serohemorrhagic secretion. Respiratory rate was increased to 70-80 per minute and the temperature was elevated to 39.4°C up to 40.3°C. Severe depression followed within two to three days and two of the three animals died despite treatment. The third cow, no. 4, recovered after treatment but was sent for slaughter.

During the next six weeks mastitis occurred in eight animals which had calved within the previous two to three weeks (cows nos. 5-12). All showed signs which persisted for several days similar to those described above. Following treatment the animals showed an improvement in systemic signs of illness with return of appetite but the udder secretion remained abnormal.

Laboratory Findings

Pure cultures of *Pseudomonas aeruginosa* were obtained from milk of all affected animals and from udder tissue sampled from the fatal cases. The organism was also recovered from the spleen of animal no. 3.

[1]Cal. Mag. K., M.T.C. Pharmaceuticals, Brampton, Ontario.
[2]Orbenin Dry Cow, Beecham (Ayerst), St. Laurent, Quebec.

Treatment

Cows were treated with oxytetracycline,[3] 10 mg/kg twice a day (B.I.D.) I.M., chloramphenicol[4] 20 mg/kg B.I.D. I.M., trimethoprim-sulfadoxine[5] 3 mL/100 lb (45.5 kg) once a day (S.I.D.), I.M. None of these agents produced improvement in the general condition of the animal or the condition of the udder.

The severely affected animal no.4 was given neomycin[6] intravenously, steroids and antihistamines. This animal survived and was sent for slaughter.

Following submission of samples of milk for culture and antibiotic sensitivity testing of the isolate, gentamycin[7] was used parenterally (2 mg/kg S.I.D., I.M.). Despite improvement in the general condition of the animal the quarters remained indurated and secretion did not return to normal. Intramammary procaine penicillin, dihydrostreptomycin and prednisone acetate[8] (100 000 I.U. procaine pencillin, 300 mg dihydrostreptomycin sulphate and 4 mg prednisone acetate) was of no value.

A preparation containing lincomycin and spectinomycin,[9] (25 mL B.I.D., I.M. of a mixture containing 50 mg lincomycin HCl and 100 mg spectinomycin sulfate) given intramuscularly, once daily, produced considerable improvement both in general health and condition of the udder. However following cessation of treatment clinical signs recurred and treatment had to be reinstituted. No further deaths occurred following treatment with lincomycin-spectinomycin.

On December 18, 1979 a complete bacteriological herd milk test was carried out to ascertain the *Pseudomonas* carrier state within the herd. This revealed infection in all four quarters of two cows and two quarters of another animal, none of which had calved but were being milked following development of mastitis. One lactating animal showed infection in three quarters and three other animals were excreting *P. aeruginosa* from one quarter. The owner, advised to dispose of these animals to avoid further spread of the pathogen, was unwilling to do so. Continued treatment of the affected animals failed to eliminate the infection, and over the following five months all seven animals were disposed of for slaughter. During this period the number of cows in the milking herd was reduced to 27 and new cases occurred in lactating animals. In these cows abnormal milk was first noted producing a 3 + reaction on the California mastitis test (C.M.T.) of the secretion from one to four quarters. On culture of milk from affected quarters at this stage no pathogen could be isolated but subsequent cultures yielded a heavy growth of *P. aeruginosa* sensitive only to gentamycin and polymixin B. Some animals showed systemic disease which was controlled by the injection of lincomycin and spectinomycin at the same dosage as indicated above.

Over the next four months further possible *Pseudomonas* mastitis occurred in lactating and dry cows. The organism was not obtained on culture, but recovery followed lincomycin-spectinomycin treatment. Over this period there has been a general improvement in herd milk yield concurrent with a marked inprovement in hygiene.

Discussion

Circumstantial evidence of involvement of a contaminated dry cow therapy antibiotic is very strong. The fact that in the majority of cases several quarters of the udder were affected synchronously and that all had been given intramammary cloxacillin from the same multidose vial supports the suspicion of the direct introduction of a pathogen into the udder rather than chance contamination. If the latter had been responsible one would expect only one or two quarters to be affected in most animals. Also the syringe used for intramammary injection was not sterilized after use. Unfortunately the vial of antibiotic preparation used for dry cow therapy had been emptied and was not available for culture. For this reason an attempt to assess the survival potential of *P. aeruginosa* in the dry cow therapy preparation was made. Two vials containing 20 mL of the cloxacillin preparation were seeded with a culture of the organisms to give a concentration of 176 000 organisms/mL and 800 organisms/mL respectively. The mixture was kept at 4°C for a period of eight weeks and sampled at approximately weekly intervals using a modified Miles and Misra technique (4). Counts of organisms fluctuated downwards to 44 000/mL in the first vial and 400/mL in the second vial during this period. A parallel experiment in which the antibiotic suspension was stored at room temperature, a common occurrence on many dairy farms, showed a 30X increase in numbers of organisms over the same period. A control culture of *Staphylococcus aureus* was not reisolated at any time after a sampling immediately following addition to the antibiotic.

Pseudomonas infection of the bovine udder associated with contamination of the antibiotic preparation used in treatment has been described previously (7). An outbreak in a herd of 50 cows in which the dairyman had used penicillin incorporated in a water in oil emulsion vehicle for routine treatment of lactating and dry cows was described. *Pseudomonas* was isolated from the used penicillin and vehicle vials and from the "sterile" saline solution used to dissolve the penicillin.

More recent reports (1,5) describe an extensive outbreak of *P. aeruginosa* mastitis which resulted from the use of dry cow therapy containing neomycin which had been contaminated at the time of manufacture. The more detailed report (5) gives a clinical history very similar to that described above. Where preparations are used as

[3]Liquamycin LP(R), Rogar STB., Div. B.T.I. Products Inc., London, Ontario.
[4]Zoomycetine 250(R), PVU Inc., Victoriaville, Quebec.
[5]Trivetrin(R), Wellcome Veterinary Division Ltd., La Salle, Quebec.
[6]Biosol Liquid(R), TUCO Products Co., Orangeville, Ontario.
[7]Gentocin(R), Schering Canada Inc., Pointe Claire, Quebec.
[8]Metibiotic(R), Mastitis Foam, Schering Canada Inc., Pointe Claire, Quebec.
[9]Linco Spectin(R), TUCO Products Co., Orangeville, Ontario.

for dry cow therapy all four quarters are normally inoculated. This explains why several of the animals were affected in all quarters. Later cases in the outbreak described probably resulted from spread of infection from infected cows via the equipment or from the contaminated environment in the damp barns. Once established, *Pseudomonas* mastitis is extremely resistant to treatment and, even where an antibiotic to which the organism is sensitive *in vitro* is used, complete resolution is rare (2). For this reason culling of infected cows is usually recommended since otherwise they will remain as a source of contamination. We believe that the failure to do this after the completion of the herd test, combined with the unsatisfactory environmental conditions, explains the persistence of the problem in this herd for several months after the use of the dry cow therapy from a multidose vial was discontinued.

An outbreak probably associated with contaminated washing water has been described by Malmo *et al* (3) and it has been demonstrated by Schalm *et al* (6) that the level of contamination need not be great for mastitis to occur. They have suggested that small numbers of organisms, which enter the gland and do not immediately stimulate a massive inflitration of leukocytes, are more likely to establish infection than large numbers. They emphasize that *Pseudomonas* is no more likely to establish itself in a gland showing a higher neutrophil count than *Escherichia coli.* This underlines the necessity of avoiding contamination of antibiotic preparations used in dry cow therapy by resistant organisms.

Although the suspicion of contamination of the dry cow therapy material was not proven, the experimental evidence of survival of *P. aeruginosa* in the preparation is a very good reason for deploring the use of such a preparation in the form employed in this herd. The advent of antibiotics in small disposable tubes brought to an end the danger previously encountered of spread of resistant bacteria on teat siphons, etc. It would be unfortunate if the small monetary saving in using bulk preparations of antibiotics should reintroduce a hazard so easily and cheaply avoided.

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LETTER TO THE EDITOR

Ampicillin Toxicity in Rabbits

DEAR SIR:

There are, at the present time, conflicting reports in the literature on the use of ampicillin in rabbits. Several recent publications have recommended the use of ampicillin in rabbits for the treatment of *Pasteurella* infections (3,4). Wood (5), on the other hand, states that "ampicillin is specifically contraindicated in the rabbit."

There is some evidence that ampicillin is indeed toxic to rabbits. The experiment reported by Milhaud *et al* (2) indicates that oral administration of ampicillin for three consecutive days, at dosage rates of 5, 15, 50 mg/kg resulted in mortalities of 63%, 45% and 100% respectively. The majority of the deaths occurred three to six days after treatment had ended, with signs of diarrhea before death. The rabbits had lesions in the digestive tract and the cause of death was presumed to be a disturbance of the intestinal microflora, leading to enteritis and a fatal diarrhea.

The therapeutic doses of ampicillin recommended by Rusell (4) at 22 to 44 mg/kg orally in divided doses, and by Raphael (3) at 10 to 25 mg/kg every eight hours intramuscularly are in the same range where Milhaud *et al.* (2) reported mortalities of 50% to 100% in treated rabbits. The toxicity of penicillin in rabbits has also been reported (1).

Therefore it would seem prudent to avoid the use of ampicillin in the rabbit, and rely on other broad spectrum antibiotics known to be safe. Also, the recommendation of ampicillin as a therapeutic agent for pasteurellosis in rabbits should be withdrawn until its safety (or toxicity) is adequately established.

Sincerely,

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