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All communications should be addressed to:

The Editor-in-Chief
Philippine Journal of Veterinary Medicine
College of Veterinary Medicine
University of the Philippines Los Baños
Laguna, Philippines 4031
Telefax Nos. +63-49-536-2727, +63-49-536-2730
Email: pjvm1964@gmail.com, pjvm.uplb@up.edu.ph

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CASE REPORT

MASTITIS IN A HOLSTEIN X SAHIWAL COW CAUSED BY STREPTOMYCIN-RESISTANT *Pasteurella multocida*

Russel Denny O. Manzanilla, DVM
Flor Marie Immanuelle R. Pilapil-Amante, DVM, MSc

Department of Veterinary Clinical Sciences, College of Veterinary Medicine
University of the Philippines Los Baños, 4031, Laguna, Philippines

ABSTRACT

Mastitis, the most economically relevant disease in the dairy industry, can be caused by a wide array of bacterial species. Although scarcely reported, *Pasteurella multocida* is included as a contagious etiologic agent causing mastitis. Targeting the pathogens is the treatment approach when dealing with udder infection and with bacteria being the chief causative agents, antibiotics is prominently utilized for therapy. This dairy farm was previously treating its mastitis cases using a Penicillin-Streptomycin solution for intramuscular injection without veterinary supervision. The collection of milk samples from infected quarters was prompted by non-response of their mastitis cases to antibiotic treatment. The selection of quarters for sample collection was based on the severity of clinical signs present. Bacterial isolation was performed using the milk samples afterward, the isolated bacteria, *Staphylococcus aureus* and *P. multocida*, were subjected to antibiotic sensitivity tests. The two isolates shared a resistance against streptomycin, an antibiotic previously used in the farm in combination with benzylpenicillin. Thus, a different antimicrobial drug, oxytetracycline, was prescribed to which the pathogens responded positively.

Keywords: *antimicrobial resistance, bovine mastitis, Pasteurella multocida, streptomycin*

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INTRODUCTION

With a decrease in milk production, an increase in veterinary expenses, and culling rates, bovine mastitis remains to be the most economically relevant disease in the dairy industry (Gomes, 2016). It is also considered as one of the most difficult veterinary diseases to control since over a hundred different microorganisms serve as the etiologic agent (Bradley, 2002). Mastitis may be identified by measuring the cell count in milk. An increase in the number of somatic cells in the milk is a result of an influx of polymorphonuclear (PMN) leucocytes from the mammary capillaries into the teat cistern as a response to bacterial proliferation (Blowey and Edmonson, 2010). Toxin production also contributes to mammary tissue damage and with increased permeability, hematologic elements are allowed to escape into the milk (Bekuma and Galmessa, 2018).

The pathogens responsible for mastitis include viruses, algae, and fungi but among these organisms, bacteria are reported to be the primary cause of bovine mastitis (Viguier, 2009). Contagious mastitis pathogens are transferred from an infected cow to a non-infected one usually during milking (Blowey and Edmonson, 2010). *Staphylococcus aureus* was identified to be the most commonly involved mastitis organism as reported in most countries (Hossain, 2017). Environmental mastitis pathogens, on the other hand, originate from the surroundings with their infection rates vastly influenced by management practices (Blowey and Edmonson, 2010). As reported by Gomes *et al* (2016), *Streptococcus uberis* is the most frequently isolated environmental pathogen in bovine mammary infections. While there are new species becoming more commonly isolated, certain species remain to be uncommon causes of mastitis (Hamadani *et al.,* 2013).

Wilson and Ho (2013) describe *Pasteurella multocida* as a small, Gram-negative, non-flagellated coccobacillus. Isolates are preferably grown on 5% sheep's blood in dextrose-starch, casein-sucrose-yeast, chocolate, Mueller-Hinton, or brain-heart infusion agar and no growth can

***FOR CORRESPONDENCE:**
(e-mail: frpilapil@up.edu.ph)

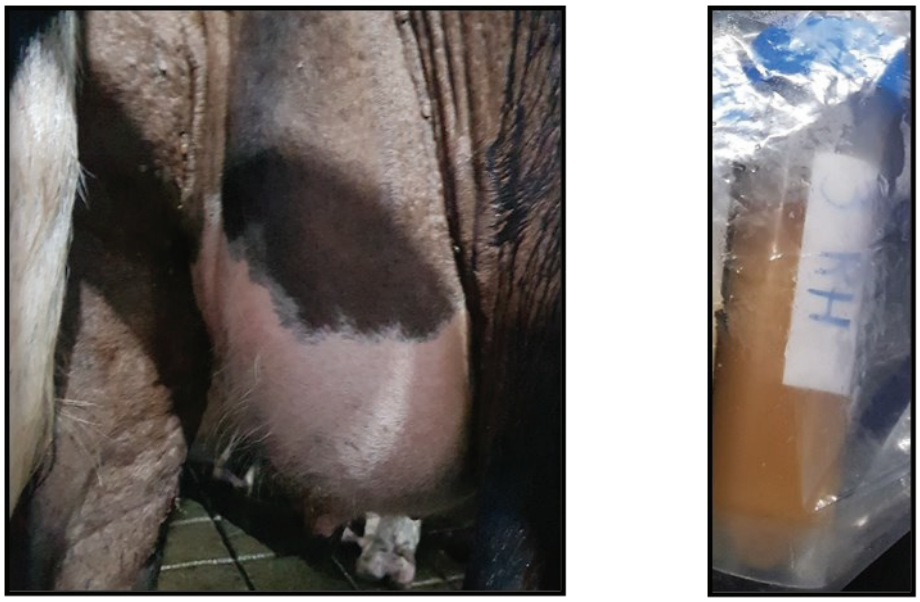
be observed on MacConkey agar. *Pasteurella multocida* is the main causative agent in Hemorrhagic septicemia, in addition to its association with bovine pneumonia (Dabo *et al.,* 2007).

Among mastitis-causing pathogens, antimicrobial resistance is predominant in *Escherichia coli* and *Staphylococcus aureus*. It has been reported that generally, clinical mastitis cases exhibit resistance against penicillin, amoxicillin, oxytetracycline, and methicillin (Chandrasekaran *et al.,* 2014).

CASE PRESENTATION

In early January 2019, a dairy cattle farm comprised of a Holstein-Sahiwal milking herd with an average body condition score of 3/5 and located in Batangas, Philippines reported difficulties in the treatment of their mastitis cases. The animals that exhibited clinical signs were previously treated with Benzylpenicillin procaine-Benzylpenicillin benzathine-Dihydrostreptomycin sulphate combination (Pendistrep L.A., 120,000 IU/ 80,000 IU/ 200mg per ml solution; Phenix, Anupco Ltd., Essex, England) at a dose of 10 mL per 100 kg body weight through intramuscular injection once a day for 3 days without any supervision from a veterinarian. Upon consultation, the owner was advised to have milk samples from their mastitis-infected cows be collected and undergo bacterial isolation. Physical examination and sample collection were performed on February 13, 2019, a month after the reported non-response to treatment. The caretakers emphasized that based from their California Mastitis Test (CMT), the number of their infected cows went up from five to thirteen. The said thirteen cows had at least one udder quarter with a grade of 3 from the CMT results based on standard scoring (Ruegg, 2005) which corresponds to >5,000,000 cells/ml and interpreted as severe mastitis infection. Sampling population was further reduced as only five cows, as shown in Figure 1A, exhibited mastitis clinical signs (hard and hot udder, coagulations in milk).

The yellowish milk samples (Fig. 1B) collected were taken to the Animal Disease Diagnostics Laboratory in the University of the Philippines Veterinary Teaching Hospital – Los Baños. Bacterial isolation was performed on the milk samples using Blood Agar Plate (BAP) and two bacterial species were isolated and cultured, *Staphylococcus aureus* and *Pasteurella multocida* (Table 1). Following bacterial growth after 48 hours, antibiotic sensitivity testing (against Norfloxacin, Florfenicol, Trimethoprim, Doxycycline, Tetracycline, Amoxicillin, Spectinomycin, and Streptomycin by Kirby-Bauer disk diffusion) was performed using Muller-Hinton agar and Blood Agar Plate, respectively. Results showed that the two bacterial species had developed intermediate resistance against



(A) **(B)**

Fig 1. (A) Swollen appearance of cow # 3's mammary glands during milk sample collection; (B) Yellow milk sample collected from cow # 3.

Table 1. Bacterial isolation and Antibiotic Sensitivity Testing results (CLSI, 2013).

Sample ID	Results
Cow #1 LF	No growth after 48 hours of incubation
Cow #2 RH	No growth after 48 hours of incubation
Cow #3 RH	*Pasteurella multocida* Sensitive: Norfloxacin, Florfenicol, Trimethoprim, Doxycycline, Tetracycline Intermediate: Amoxicillin, Spectinomycin Resistant: Streptomycin
Cow #4 LF/RF	*Staphylococcus aureus* Sensitive: Doxycycline, Florfenicol, Tetracycline Intermediate: Spectinomycin, Enrofloxacin, Norfloxacin Resistant: Streptomycin, Amoxicillin, Trimethoprim
Cow #5 RF	No growth after 48 hours of incubation

Mammary Gland Quarters: LF Left front, RF Right front, LH Left hind, RH Right hind

Streptomycin. *Pasteurella multocida* had also developed intermediate resistance against Amoxicillin while *Staphylococcus aureus* had developed the same against Enrofloxacin and Norfloxacin and full resistance against Amoxicillin and Trimethoprim. Upon examining these results, the veterinarian prescribed a different class of antibiotic, oxytetracycline (Oxitetraciclina 200 L.A., 200mg per ml solution for intramuscular injection; Invesa, Livisto, Barcelona, Spain) at 1ml per 10 kg body weight once a day and to be repeated after three days as recommended by the manufacturer. Clusters of the milking machines were also examined. After identifying that the isolated pathogens are contagious in nature, samples using sterile swabs were acquired on February 15, 2019 from five randomly selected clusters. No bacteria were isolated from the swab samples, further suggesting that the isolated samples from the milk may have been transmitted by other means such as the milker's hands or the flies. The farm owner complied with the change in antibiotic treatment and had positive results such as recovery from inflammation and reduction of CMT score to negative (N) the following week leaving only Cow # 3 with clinical mastitis. Second dosing of Oxytetracycline was then advised which resulted to a better response to treatment and patient recovery.

DISCUSSION

Mastitis continues to plague the dairy industry as its most costly disease causing economic losses through different factors: treatment costs, untimely culling, and preventive expenditures. In addition to a declining milk production, milk quality is also negatively affected. Reduced milk components such as lactose, milk fat, and casein lessens product viability resulting to more economic losses (DeGraves *et al.,* 1993).

Bovine mastitis usually manifests in a clinical form, displaying symptoms both systemically and locally in the udder of the infected animal and milk produced, which was apparent in this farm. Subclinical mastitis also exists in the form of increased somatic cell count (SCC) subsequently diminishing milk produced (Franca *et al.,* 2017). Infection is primarily a result of bacterial proliferation in the mammary gland. Its broad diversity of microorganisms involved can be categorized as either 'contagious' or 'environmental' pathogens (Bradley, 2002). Contagious pathogens contribute to majority of mastitis cases generally causing subclinical form of udder infection. In contrast, infection by environmental pathogens normally manifest as clinical mastitis (Blowey and Edmonson, 2010). However, at the time of evaluation in this farm,

majority of the mastitis cases exhibited subclinical forms with more than half of the infected cows having quarters with a high SCC despite the absence of clinical signs.

One of the isolated organisms in this case is *P. multocida*, an opportunistic pathogen normally found in the oral, nasopharyngeal, and upper respiratory tract (Davies *et al*., 2004). In a previous case report by Milanov *et al* (2017), it was summarized that mastitis cases caused by *P. multocida* have a low incidence rate of not more than 1.25%; and data was retrieved only from a handful of studies. Immunosuppression, brought about by environmental stressors or nutritional deficiency, of the animal would allow *P. multocida* to proliferate and cause diseases in bovine such as Hemorrhagic Septicemia and Pneumonic Pasteurellosis (Davies *et al.* 2004). However, in cases of mastitis, the route of infection continues to be unclear, but several courses have been hypothesized. From the respiratory tract, organisms may be distributed via hematogenous or lymphogenic vessels into the udder. Suckling calves that have been infected by *P. multocida* may also serve as vectors as they may transfer the organism from their oropharynx to the udder while feeding (Milanov *et al*., 2017).

Preventive measures against mastitis such as teat disinfection before and after milking and thorough cleaning of the milking system are practiced in this farm however, even with such control procedures, cows still develop mastitis eventually necessitating antibiotic treatment (Oliver and Murinda, 2012). Antibiotic therapy in mastitis cases is applied in two approaches: treatment of clinical infections during milking usually through intramammary administration and in severe cases, systemic antibiotics are used to supplement the treatment prior to drying off which is roughly six weeks before the next calving and the other technique, referred to as dry cow therapy, is the infusion of the udder with antibiotics (Krömker and Leimbach, 2017).

A 2008 study on antimicrobial susceptibility against *P. multocida* by Yoshimura *et al* used minimum inhibitory concentrations (MIC) to compare the effectiveness of certain antibiotics against the said organism. The study included three antibiotics mentioned in this case and among them, the article reported that benzylpenicillin appeared to be the most active against *P. multocida*. Between the two other drugs, oxytetracycline performed better than dihydrostreptomycin with the latter having 19.4% of the isolates already resistant against the aminoglycoside. Even if penicillin was not included in the antibiotic sensitivity testing of this case, another antibiotic, amoxicillin, belonging to the same drug family, was tested to which *P. multocida* had developed intermediate resistance. Although the study used a different method, their results agree with the pathogen resistance against streptomycin of this case.

Antimicrobial usage is considered as the principal source for pathogens' development of drug resistance, reinforced by administration of suboptimal concentrations and extra-label use of antibiotics (Sharma *et al.,* 2018). In addition to grower farms using antibiotics as growth promotants, non-adherence to the prescribed protocol, and excessive use of antibiotics to treat diseases (Pilapil-Amante *et al*., 2020), the presented case may serve as a prime example of inappropriate use of antimicrobials as the dairy farm was previously treating its mastitis cases using an antibiotic in the absence of veterinary consultation thus, contributing to the budding problem about antimicrobial resistance. In practice today, antibiotic sensitivity testing is primarily done to affirm if the current therapy is appropriate or adjustments are necessary (Kromker and Leimbach, 2017). Further recommendations to the farm to improve the mastitis prevention, control, and management would include adopting a better cleaning and disposal program applicable to all farm areas to reduce and control the fly population (Levesque, 2004), practicing dipping of the milkers' hands in a disinfectant solution in between milking cows, and availability of written treatment plans, treatment, and disinfection records (Pilapil-Amante *et al*., 2020).

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STATEMENT OF COMPETING INTEREST

The authors have no competing interests to declare.

AUTHOR'S CONTRIBUTION

The primary author contributed to the investigation, data curation and analysis, resources, visualization and original manuscript writing. The second author contributed to the conceptualization, investigation, methodology, data analysis, resources, project administration, supervision, visualization and manuscript review and editing.

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