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Case Report

Myonecrosis In 3-Day Old Suckling Piglets Following Intramuscular Administration of an Antimicrobial and a Mycoplasma Hyopneumoniae Vaccine Using a Contaminated Automatic Syringe

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Abstract

This case report describes an acute mortality outbreak in 3-day-old suckling piglets following routine intramuscular injection of ampicillin and intramuscular vaccination against Mycoplasma hyopneumoniae. On a 1275-sow farm, piglets were vaccinated at two days of age using an automatic syringe. Shortly after, multiple piglets exhibited clinical signs including lateral decubitus, convulsions, and severe swelling with red discoloration in the cervical-thoracal region.

Macroscopic necropsy of affected piglets (n=5) revealed fibrinous necrotising inflammation of the muscular and connective tissues at the injection site, alongside splenomegaly indicative of septicemia. While bacteriological examination of the vaccine fluid was negative, analysis of the disassembled automatic syringes identified significant contamination within the internal injection chambers and pestles. The isolated pathogens included *Clostridium perfringens, Pseudomonas aeruginosa*, and Aero coccus viridians. The synergy of these pathogens - specifically the myotoxic effects of *C. perfringens* and *P. aeruginosa* and the potential for *A. viridans* to cause meningitis - created a "deadly pathogenic cocktail" that led to rapid, irreversible tissue damage and systemic failure. This case highlights that even when vaccine fluid remains sterile, improperly cleaned multi-dose injection equipment can serve as a primary source of fatal polymicrobial infections. The findings underscore the critical necessity for stringent hygiene and sterilization protocols for automated delivery systems in swine production to prevent iatrogenic outbreaks.

Keywords

3-day old piglets, IM injection, *Mycoplasma hyopneumoniae* vaccine, Ampicillin Injection, Myonecrosis, meningitis, *Clostridium perfringens, Pseudomonas aeruginosa, Aerococcus viridans*

Introduction

A 1275-sow farm with Topigs-Norsvin TN70 sows (Topigs-Norsvin, Vught, The Netherlands) operating in a 1-week batch management system. Piglets are crossbred Topigs TN70 x Tempo. At day 2 of life, piglets are treated with 0.5 ml of ampicillin IM

(Albipen; Intervet International, Boxmeer, Netherlands) and vaccinated against Mycoplasma hyopneumoniae with 2.0 ml Stellamune One IM (Elanco Animal Health, Indianapolis, IN, USA) using an automatic syringe. After weekly use, the syringes are rinsed with water from the tap and stored under cleaned and dust-free conditions until the next week. Following these injections, an episode of acute to subacute mortality occurred on three occasions with typical clinical signs of lateral decubitus with convulsions (Figure 1A). Macroscopic lesions were situated in the cervico-thoracal region with red discoloration and swelling (Figure 1B).

Figure 1A: Typical clinical symptoms of lateral decubitus and convulsions.

Figure 1B: Macroscopic lesions were situated in the cervico-thoracal region with red discoloration and swelling.

Diagnostic Approach

Macroscopic necropsy findings

Several suckling piglets (n = 5) that acutely died following the intramuscular injection were submitted to a diagnostic laboratory (GD Deventer, Deventer, The Netherlands) for further macroscopic examination. Upon necropsy, a fibrinous necrotising inflammation was observed in the cervico-thoracal region of all submitted piglets. The inflammation affected both the muscular structures and the loose connective tissue in this region. This observation was combined with a clear swelling of the spleen, indicating a septicemic infection. No other abnormalities were observed in any of the necropsied piglets.

Bacteriological examination of vaccine injection fluid and syringes

Both the vaccine injection fluid (Stellamune One; Elanco Animal Health, Indianapolis, IA, USA) and two syringes used for intramuscular injection of the suckling piglets were submitted to a diagnostic laboratory (Animal Health Care-Flanders, Torhout, Belgium) for further bacteriological examination. To obtain a complete picture of the potential source of infection, both aerobic and

anaerobic microbial analysis was carried out. Subsequent positive cultures were further type using MALDI-TOF analysis. A total of 50 µl of the vaccine injection fluid was plated out on a standard blood agar and incubated both under aerobic and anaerobic conditions at 20°C for 24h. Both culture plates were negative for growth after 24h. The syringes (syryinge 1, grey (S1); syringe 2, colored (S2)) were disassembled under sterile conditions and a swab was taken from the internal injection chamber and pistle (Figure 1) and plated out on a standard blood agar and incubated both under aerobic and anaerobic conditions at 20°C for 24h. The results of the analysis of both syringes are given in (Table 1).

Table 1. Microbiological results of injection syringes for both aerobic and anaerobic culture.

	Aerobic culture	Anaerobic culture
S1 – grey	Negative	*Clostridium perfringens*
S2 - colored	*Aerococcus viridans*	*Clostridium perfringens*
	Pseudomonas aeruginosa	

(Figure 2). Automatic 2 ml injection syringe. Samples were collected within the injection chamber (A) and the injection pestle (B) for microbial analysis.

Figure 2. Automatic 2 ml injection syringe. Samples were collected within the injection chamber (A) and the injection pestle (B) for microbial analysis.

Discussion

Clostridium perfringens-an anaerobic bacterium - causes gas gangrene characterized by myonecrosis, shock, multiple organ failure and finally death of the patient [1,2]. The pathogenesis involves the production of several toxins (α-toxin, θ-toxin) and enzymes (collagenase, hyaluronidase, sialidases and cysteine protease α-clostripain [3]. Following intramuscular injection of C. perfringens type A severe edema, contraction of the muscle fiber diameter and plasma creatine kinase activity were observed, which indicated that the infection induced skeletal muscle destruction [3]. The α-toxin, which is a cytotoxic bacterial phospholipase C, plays a key role in the pathogenesis of myonecrosis [4]. The infection progresses so rapidly that death precedes diagnosis in some patients and, thus, an effective therapy for treating patients with C. perfringens-mediated myonecrosis is in most cases too late. In the present case report, we could isolate *C. perfringens* from injection chamber / pestle of both injection syringes, indicating that these devices were contaminated with C. perfringens at the location that comes into intensive contact with the sterile vaccine injection fluid. Therefore, it might be possible that suckling piglets injected with this potentially contaminated injection fluid, following passage through the contaminated injection chamber and pistle. Gas gangrene or clostridial myonecrosis frequently occurs after

contamination of a deep surgical or traumatic lesion [5]. This could perfectly explain the pathogenesis under the current case report, since the vaccine injection fluid might have been contaminated during passage and in contact with the injection chamber and pestle, and subsequently being injected intramuscularly, which resembles a deep traumatic lesion. If not controlled, clostridial myonecrosis results in multiorgan failure, shock and death. This was the case under the reported conditions, where was observed two episodes of suckling piglet mortality following the standard preventive vaccination procedure for the early protection against Mycoplasma hyopneumoniae.

The other pathogen identified in one of the contaminated injection syringes was Pseudomonas aeruginosa - a Gram-negative bacterium first isolated from wounds in 1882 [6,7]. Pseudomonas aeruginosa is a functionally versatile organism that allows the bacterium to adapt to different habitats, resulting in a bacterium that can range from a harmless saprophyte and commensal organism to a deadly broad-host-range pathogen. The bacterium has been reported to cause acute and chronic infections in humans, particularly patients suffering from immune deficiencies. Acute infections are often spread rapidly and can cause tissue damage and sepsis with high mortality rates [7]. These infections can include the bloodstream, urinary tract, eye and soft-tissue wounds. At 3 days of age, the immune system of the suckling piglets involved in this case report might still not be fully developed [6]. Therefore, these animals are also at risk of a Pseudomonas aeruginosa infection when injected intramuscularly with a contaminated vaccine injection fluid, resulting in soft-tissue (loose connective tissue and muscle) inflammation. Moreover, P. aeruginosa infections are notably difficult to treat due to their high intrinsic antimicrobial tolerance or resistance, the production of several intracellular and extracellular virulence factors and its rapid adaptation to the infection [6]. Recently, an increase in multidrug-resistant, extensively drug-resistant and pandrug-resistant strains has been reported leading to higher mortality rates [6].

Aerococcus viridans are microaerophilic Gram-positive non-motile bacteria species that are rarely associated with infections such as arthritis, bacteremia, endocarditis, and meningitis. The bacteria are also fastidious and often confused with Streptococci species or treated as a contaminant [8]. Besides C. perfringens and P. aeruginosa, these specific bacteria were also isolated from the contaminated syringe injection chamber and pestle of the automatic syringe used to administer the vaccine. The presence of A. viridans might explain the clinical signs of lateral decubitus and convulsions, as illustrated in (Figure 1A), since the bacteria can be associated with meningitis, and therefore the clinical signs cannot be distinguished from the meningitis variant of *Streptococcus suis (S. suis*). Moreover, typical meningitis due to S. suis is mostly observed post-weaning in swine farms under Dutch production conditions, whereas in this case the convulsions were already present at day 3 of age, which would be very atypical for a meningitis due to S. suis. The presence of *P. aeruginosa, C. perfringens* and *A. viridans* in the contaminated syringe injection chamber and pestle might be considered a deadly pathogenic cocktail once intramuscularly injected into the cervical muscles and loose connective tissue of the 3-day old suckling piglets. Both pathogens - *P. aeruginosa* and *C. perfringens* - produce several toxins and virulence factors that provoke irreversible damage to the muscle tissue, and the infection progresses so rapidly that adequate treatment is impossible. The concurrent infection with A. viridans adds another complicating factor to this already detrimental pathology. Moreover, as for P. aeruginosa, the presence of multidrug-resistance among many of the circulating strains makes it impossible to perform an effective treatment, even if the farmer would be in-time to identify the inflammatory conditions in the affected piglets.

Conclusions

The current case report describes an acute outbreak of an intramuscular inflammation caused by *C. perfringens, P. aeruginosa* and *A. viridans* leading to mortality in several 3-day old suckling piglets injected with a vaccine injection fluid that was contaminated using an automated injection syringe. This case highlights that even when vaccine fluid remains sterile, improperly cleaned multi-dose injection equipment can serve as a primary source of fatal polymicrobial infections. The findings underscore the critical necessity for stringent hygiene and sterilization protocols for automated delivery systems in swine production to prevent iatrogenic outbreaks.

Conflict of Interest

None.

Acknowledgments

None.

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