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CASE REPORT

Mycoplasma hyopharyngis isolated from the joint of a weaner: A case report

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ABSTRACT

Background: Mycoplasma hyopharyngis is a commensal bacterium in the upper respiratory tract of swine. As it is recognized to be apathogenic, examinations regarding this species are scarce, compared to other swine mycoplasmas. However, in a few cases, *M. hyopharyngis* was detected in lesions of different organs. This report presents a case study in which *M. hyopharyngis* (along with other bacteria) was isolated from the joint of a pig showing lameness. *Case presentation:* A Hungarian farm was repopulated with 250 gilts and 1,700 finishers after undergoing a complete depopulation and disinfection. Two days later, cases of diarrhoea and septicaemia caused by *Salmonella enterica* serovar typhimurium were seen in the finishers. At the same time, following the first farrowing, swollen joints were observed in 21–25 days old piglets. Joint samples were collected, and isolation of *Mycoplasma* sp. and other bacteria was attempted. Analysis of the joint samples revealed the presence of *Staphylococcus haemolyticus, Staphylococcus hyicus, Aerococcus viridans, Trueperella pyogenes, Streptococcus agalactiae* and *M. hyopharyngis*. *Conclusions:* This is the second isolation of *M. hyopharyngis* from joints, which highlights the necessity of a better understanding the biology of this often-overlooked species, and its role in the progress of arthritis or other lesions.

KEYWORDS

isolation, joint, *Mycoplasma hyopharyngis*, mycoplasmas, pig, swine

BACKGROUND

Arthritis is commonly recognized in swine of all ages and leads to significant economic losses due to reduced growth rates and lower performance of adult breeding stock and lactating sows (Ross and Bailey, 1978). The pathogenesis of arthritis is multifactorial, including infectious or degenerative processes (Faria et al., 2011). The major predisposing factors are inadequate management and housing conditions, rapid muscle development, circulatory or endocrine disorders, as well as skin abrasions (Zoric, 2008; Faria et al., 2011).

Infectious arthritis in swine is commonly associated with various bacterial agents (Faria et al., 2011). Among suckling pigs, some of the primary pathogens responsible for arthritis are *Staphylococcus (Staph.) hyicus* (causative agent of exudative dermatitis) and *Staph. aureus*

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(Frana, 2012; Van Der Wolf et al., 2012; Dimitrova and Yordanov, 2022). *Streptococcus* spp. (notably *Streptococcus (Strep.) suis*) and *Glaesserella (G.) parasuis* can also be observed in suckling and nursery pigs, contributing to arthritis cases (Bagus Oka Winaya et al., 2022; Silva et al., 2023). Pigs aged between 3 and 10 weeks are susceptible to *Mycoplasma (M.) hyorhinis*, which causes symptoms such as arthritis and polyserositis (Salogni et al., 2022). *Actinobacillus suis* can manifest primarily during the grow-finishing phase (10–16 weeks of age), but it can also be observed in pre-weaning piglets (MacInnes et al., 2008; Silva et al., 2023). Similarly, *Mycoplasma hyosynoviae* is detectable in pigs aged between 12 and 22 weeks, causing arthritis (Nielsen et al., 2001). *Erysipelothrix (Er.) rhusiopathiae* predominantly affects growing and adult pigs, leading to clinical signs such as fever, arthritis, skin lesions and sudden death (Habte et al., 2021).

Trueperella (T.) pyogenes, an opportunistic pathogen with a widespread prevalence, is associated with purulent infections (Jarosz et al., 2014; Rzewuska et al., 2019). Moreover, *Brucella suis* is implicated in porcine arthritis and reproductive losses in swine (European Food Safety Authority (EFSA), 2009; Olsen and Tatum, 2016).

Lately, both the number and significance of arthritic lesions due to mycoplasmas (*Mycoplasma hyorhinis* and *M. hyosynoviae*) increased (Lin et al., 2006; Šperling, 2011; Neto et al., 2012). *M. hyorhinis* and *M. hyosynoviae* are common inhabitants of the upper respiratory tract of swine, similarly to *Mycoplasma hyopharyngis* (Pieters and Maes, 2019).

M. hyopharyngis was first isolated from oral and nasal swabs taken from two distinct pig herds in the United States of America (Erickson et al., 1986). Subsequent research resulted in a successful cultivation of the bacterium from tonsillar scrapings (Friis et al., 2003), lung samples of pigs with chronic respiratory illness (Luehrs et al., 2017), lesions of porcine ear necrosis (Malik et al., 2023), arthritic joints and subcutaneous abscesses (Bradbury et al., 1994). However, information about the incidence rates and pathogenic potential of *M. hyopharyngis* remains limited (Kobisch and Friis, 1996; Pieters and Maes, 2019), as currently this microorganism is considered to be apathogenic (Kobisch and Friis, 1996). Our aim was to present a case when *M. hyopharyngis* was isolated from a swine's joint.

FARM DESCRIPTION

The investigated pig farm is in Hungary, approximately one kilometre away from a minor road, and there are no other facilities within a ten-kilometre radius. The farm has designated biosecurity areas classified according to their hygiene levels. Vehicular traffic is restricted on the farm, except in emergencies. The livestock loading platform aligns with the fence, and other materials (such as sperm or medicine) are stored in a UV-sterilized area before entering the farm. Notably, the entire facility underwent reconstruction in 2014, incorporating cutting-edge technologies.

CASE HISTORY

In 2021, a decision was made to improve the animal health status of the farm by adopting the all-in-all-out system and introducing a stock with modern genetics. In January 2022, the farm underwent a thorough process of depopulation, meticulous cleaning and sterilization. During the cleaning process, first a basic (Anti-germ foam B-25; Kersia Group, Dinard, France), then an acidic foam (Perfect acid, AlphaVet Kft. Székesfehérvár, Hungary) were used, followed by a two-step sterilization process [p-chlorophenol (Perfect Kombicid, AphaVet) and peracetic acid (Deptil APM, Kersia)]. Finally, a mist decontamination step was applied using a disinfectant with wide antimicrobial spectrum (Fumagri OPP, Kersia). All equipment was dismantled, cleaned, dried in the sun, then reassembled and disinfected again. The feed towers were disinfected with mist decontamination (Fumagri OPP, Kersia) and the water system was disinfected with hydrogen peroxide (Dewasil, Dinax Kft, Budapest, Hungary).

Three months later, the farm was restocked with 253 DanBred F1 gilts and 1,700 male fattening pigs from the same farrowing site but different fattening farms. The animals were in good health upon arrival without any known health issues. The prior vaccination status of these animals was unknown. Two days after the arrival of the fattening pigs, diarrhoea was observed, therefore organs (spleen, small intestine, mesenteric lymph node) and anal swab samples were collected for laboratory examination. *Salmonella (S.) enterica* serovar typhimurium was detected in the anal swab samples (this pathogen was not detected before in this farm) and antibiotic susceptibility testing was carried out. Before receiving the results of the susceptibility test, the fatteners were treated with two courses of antibiotics: 5 $mg^{*}kg^{-1}$ gentamicin-sulphate (Neogent 200 mg g^{-1}, Kela N. V., Hoogstraten, Belgium); 25 $mg^{*}kg^{-1}$ trimethoprim-sulfamethoxazole (Methoxasol-T 20/100 $mg^{*}mL^{-1}$, Tolnagro Ltd., Szekszárd, Hungary). As both proved to be ineffective, based on the antibiotic susceptibility test results, 100,000 $NE^{*}kg^{-1}$colistin (Hidrocol 4,000,000 $NE^{*}mL^{-1}$, SP Veterinaria SA, Riudoms, Spain) was applied. Even though the last course of treatment reduced the clinical signs, septicaemia and circulatory disorders were also detected in the affected animals, leading to inflammation and necrosis of the ears and tails with subsequent cannibalism. Upon reaching 40–45 kg, many the pigs exhibited joint swelling. Overall, the farm experienced a final loss of approximately 10% among the fattening pigs. During slaughter of the same fattening stock, 7% of the lungs showed lesions, whereas epicarditis and pleuritis were observed in 15% and 4% of the cases, respectively. The clinical signs of S. typhimurium were only present among the fattening pigs.

Regarding the breeding stock, problems were only observed after moving the sows to the farrowing room. During this period, a total of 13 out of 158 sows died due to ulcers in the stomach. Nevertheless, during the first farrowing process, a 94% farrowing rate was achieved and the

number of live pigs born per litter was approximately 15.5 piglets. The piglets' mortality rate by weaning was about 11.7%. From an aborted foetus, the presence of the following pathogens was determined by qPCR: porcine reproductive and respiratory syndrome virus (virotype® PRRSV RT_PCR Kit, Qiagen Leipzig GmbH, Leipzig, Germany), porcine circovirus 2 (Brunborg et al., 2004) and 3 (Palinski et al., 2017), porcine cytomegalovirus (Hamel et al., 1999), porcine parvovirus 1 (Streck et al., 2015) and *Leptospira* spp. (ingenetix BactoReal Leptospira spp. (lip32), ingenetix GmbH, Vienna Austria).

When the piglets reached 20–21 days of age, several cases of swollen joints combined with diarrhoea were detected in many weaners (Fig. 1). By 45–50 days of age, the mortality rate increased to 25–30%. During this period, a total of six piglet carcasses (five carcasses around 2.5–3 kg, one around 5 kg) with joint lesions were sent for gross pathological examination and bacterial culture to a diagnostic laboratory. *Escherichia (E.) coli* in small intestinal content and S. typhimurium in the large intestine content were detected in three pigs. *Staphylococcus* sp. and *Streptococcus* sp. were cultured from the affected joints (all carcasses) and enlarged spleens (two cases). For additional bacteriology examinations (including *Mycoplasma* sp.), tissue samples of the spleen, mesenteric lymph nodes, intestine and joints from another animal presenting clinical signs of arthritis and diarrhoea were sent to our laboratories.

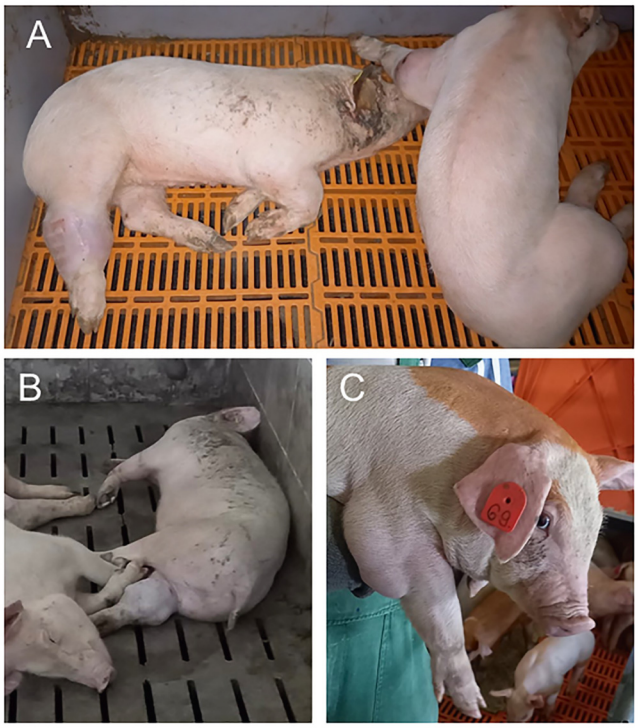

Fig. 1. Clinical presentation of joint swelling among the weaners. In picture (A), ear necrosis and joint swelling can be seen while (B) and (C) show severe joint swelling cases

BACTERIOLOGY AND *MYCOPLASMA* SP. DETECTION

The collected samples (spleen, mesenteric lymph node and four joints: tarsi and carpi from both sides) were inoculated onto blood and chocolate agar plates, incubated at 37 °C in the presence of 5% CO_2 for 48 h. Intestine samples were examined using Rappaport-Vassiliadis enrichment broth (Scharlau Rappaport-Vassiliadis broth, Scharlab Hungary Ltd., Debrecen, Hungary), which was further inoculated onto Rambach medium (Rambach™ agar, Merck, Rahway, NJ, USA). Identification of cultured pathogens relied on morphological, biochemical and growth characteristics combined with mass spectrometry (Bruker Corporation, Billerica, MA, USA).

After the joints were opened, two swab samples were taken from each joint (eight in total). One swab from each joint was analysed by qPCR to detect the presence of *M. hyorhinis* (Földi et al., 2023) and *M. hyosynoviae* (Martinson et al., 2018). The other swab from each joint was inoculated into MolliScience General Mycoplasma (GM) liquid media (MolliScience, Veterinary Medical Research Institute, Budapest Hungary) and subsequently filtered through a 0.45 µm syringe filter. The broths were incubated at 37 °C until colour change was detected. Following this, streak cultures were made on MolliScience GM solid media (MolliScience) and incubated at 37 °C with a 5% CO_2-supply. Pure cultures were achieved by picking colonies from the solid media and inoculating those into liquid media (one-colony broths), then the one-colony broths were incubated until colour change, indication of growth of the isolates. The identification of the one-colony broths was performed by PCR. First, species-specific PCRs were performed for *M. hyorhinis* and *M. hyosynoviae*, as these are the common pathogenic mycoplasmas in the joints of swine. The samples proved to be negative by these qPCRs, a *Mycoplasma* genus-specific PCR (Lauerman et al., 1995) was carried out, targeting the intergenic spacer region of the 16 and 23S rRNA genes. This assay can detect a wide range of *Mycoplasma* species. However, species cannot be identified without sequencing. Therefore, the detected product was sent for sequencing on an ABI Prism 3500XL-automated DNA sequencer (Applied Biosystems, Waltham, MA, USA). The quality of the obtained sequence (GenBank accession number: PP953499) was analysed, then a BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed. The BLAST search revealed high similarity (percent of identity: 98.24–98.74%) to three partial sequences from the NCBI database, all originated from *M. hyopharyngis* (sequence IDs: AY762639.1; AY816344.1; AY816345.1).

No bacteria were detected in the spleen or mesenteric lymph node. From the examined intestine sample, mixed saprophytes with *E. coli*-dominance were cultured and the sample was also positive for *S. enterica* (not serotyped). In the joints, the following bacteria were found: a high number of *Staph. haemolyticus* colonies (one joint); a high number of *Staph. hyicus* colonies (another joint); a high number of

Strep. agalactiae and *Trueperella pyogenes* colonies with high number of *M. hyopharyngis* colonies (still another joint); a few *Aerococcus viridians* colonies (in a fourth joint). The presence of other mycoplasmas in the joint, such as *M. hyosynoviae* or *M. hyorhinis*, was not found by PCR examination or isolation.

CLINICAL OUTCOME

The health issues re-emerged among the piglets and the fattening stock approximately three months after the first epidemic, thus it was decided to empty the farm again in 2023, applying the all-in-all-out system. After depopulation, the farm was thoroughly cleaned and decontaminated as described above. Approximately 78 days were planned for the cleaning and decontamination of the farm before the next herd arrived.

DISCUSSION AND CONCLUSIONS

M. hyopharyngis is a neglected *Mycoplasma* sp., considered an apathogenic member of the microbiota of the upper respiratory tract (Kobisch and Friis, 1996; Pieters and Maes, 2019). However, there are a few reports identifying this bacterium from lesions like subcutaneous abscesses, arthritis (Bradbury et al., 1994) or lungs of animals with chronic or recurrent respiratory disease (Luehrs et al., 2017) and ear necrosis (Malik et al., 2023). As of today, there is no clear understanding of whether *M. hyopharyngis* can induce these lesions, given the rarity of studies on the prevalence and pathogenicity of this species.

In the presented case, the diagnostic procedures were not exhaustive, as the farm did not conduct a histopathological examination and the detection of bacterial pathogens could be influenced by the applied antibiotic treatment. Although S. typhimurium is presumed to be the primary cause of the clinical signs in the herd, the swollen joints are likely the result of a mixed secondary bacterial infection.

A S. typhimurium infection can also disrupt the immune function through an inflammation in the gut, disintegrating the gut microbiota and barrier, which is known to increase susceptibility to other pathogens (Drumo et al., 2016). Also, the consequences of septicaemia caused by salmonellosis, namely the tail and ear necrosis, as well as biting, could facilitate the introduction of minor pathogens, such as *M. hyopharyngis*, into the bloodstream, which may then colonize other organs or body parts (Zoric, 2008; Malik et al., 2023).

Based on the current study, a direct association between swollen joints and the presence of *M. hyopharyngis* cannot be confirmed. However, as the second study to isolate this pathogen from joints with lesions, it underscores the importance of gaining a more comprehensive understanding of the prevalence and potential pathogenicity of this *Mycoplasma* species. Concerning other commensal mycoplasmas, there are several reports indicating that human mycoplasmas, such as *Mycoplasma hominis* (Steuer et al., 1996), *Mycoplasma orale* (Paessler et al., 2002) or *Mycoplasma salivarium* (Totten et al., 2021), may cause arthritis in immunocompromised patients. Also, there is one report of *Mycoplasma pulmonis* (a respiratory pathogen) causing arthritis in immunodeficient mice (Evengård et al., 2008).

Ethics approval and consent to participate: Ethical approval was not required for the study, as the samples were taken during routine diagnostic examinations with the written consent of the owner.

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