Swine conjunctivitis outbreaks associated with *Mycoplasma hyorhinis*

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Abstract. Conjunctivitis is an uncommon finding in commercial swine herds, and the etiology of the disease is rarely studied. We investigated cases of conjunctivitis in 3 wean-to-finish swine farms. Eye swabs and tissues were obtained from clinically affected pigs (8–22 wk of age), from unaffected pigs in contact with affected pen-mates, and from age-matched pigs from an unaffected herd. Real-time PCR (rtPCR) testing for *Mycoplasma hyorhinis* demonstrated consistent detection and high bacterial load in samples from affected herds (clinically affected animals and non-clinical pen-mates). Ct values in affected pigs were 18.9–25.3; values were 36.4–38.6 in unaffected pigs from unaffected herds. Additionally, *M. hyorhinis* was identified within inflamed palpebral conjunctivae by in situ hybridization. The association of rtPCR and in situ detection of *M. hyorhinis*, along with the lack of detection of other potential pathogens and noninfectious causes, suggests the involvement of *M. hyorhinis* in the etiology and pathogenesis of the reported swine conjunctivitis.

Key words: conjunctivitis; in situ hybridization; *Mycoplasma hyorhinis*; pigs.

Mycoplasma hyorhinis is a commensal bacterium that primarily colonizes the upper respiratory tract of swine. This bacterium has also been reported as the causative agent of polyserositis, polyarthritis, and otitis in pigs. A variety of infectious agents have been associated with conjunctivitis in pigs, including classical swine fever virus (CSFV; family *Flaviviridae*, genus *Pestivirus*, species *Pestivirus C*), African swine fever virus (ASFV; family *Asfarviridae*, genus *Asfivirus*), pseudorabies virus (PRV; family *Herpesviridae*, genus *Varicellovirus*, species *Suid alphaherpesvirus 1*), *Chlamydia* spp., porcine cytomegalovirus (PCMV; family *Herpesviridae, genus Roseolovirus*, species *Suid betaherpesvirus 2*), influenza A virus (IAV; family *Orthomyxoviridae*, genus *Alphainfluenzavirus*), and porcine reproductive and respiratory syndrome virus (PRRSV; family *Arteriviridae*, genus *Betaarterivirus*). Mycoplasma-like organisms have been shown to be involved in outbreaks of conjunctivitis in swine herds.[6,11] Even though *Mycoplasma* spp. are known to be causative agents of conjunctivitis in other animal species, such as cats, birds, sheep, and cattle,[2–5,8] the significance of mycoplasmas in the etiology of conjunctivitis in swine is unclear. We report herein investigation of an outbreak of conjunctivitis in commercial swine herds, demonstrating *M. hyorhinis* as the potential causative agent.

Conjunctivitis was clinically identified in 3 unrelated wean-to-finish swine farms in Minnesota, Ohio, and Oklahoma. Pigs from all farms were housed in standard nursery and/or finishing facilities. The onset of clinical signs was observed in pigs 8–10 wk old; clinical signs persisted until pigs reached 22 wk old. At the early stage of conjunctivitis, clinical signs consisted of conjunctival redness, palpebral edema, and serous ocular discharge (8–10 wk old; Fig. 1A). At the late stage, inflammatory signs of hyperemia and edema persisted; ocular discharges changed from serous to predominantly mucopurulent (22 wk old). The prevalence of the clinical signs was 30–60% within each herd. Anorexia and failure-to-thrive in affected pigs were the major concerns of producers and veterinarians. Noninfectious causes of conjunctivitis, such as lesions caused by foreign bodies, allergic reactions, or insect bites, which could be implicated in the clinical presentation, were not reported by the veterinary practitioners.

Eye swabs were collected from pigs at early and late stages of the clinical disease, as well as from contact pen-mates with no apparent clinical signs. Eye swab samples were also collected from pigs without a history of conjunctivitis (age-matched pigs) from a non-related herd. Tissue samples (eye globes along with their conjunctiva) from autopsied pigs were submitted to the Veterinary Diagnostic Laboratory at the University of Minnesota (UMN-VDL; Saint Paul, MN) for histologic evaluation and in situ hybridization (ISH) tests. In total, we obtained 12 tissue samples (3 from Minnesota, 3 from Ohio, 3 from Oklahoma, and 3 from an unaffected farm in South Dakota) and 36 eye swabs (11

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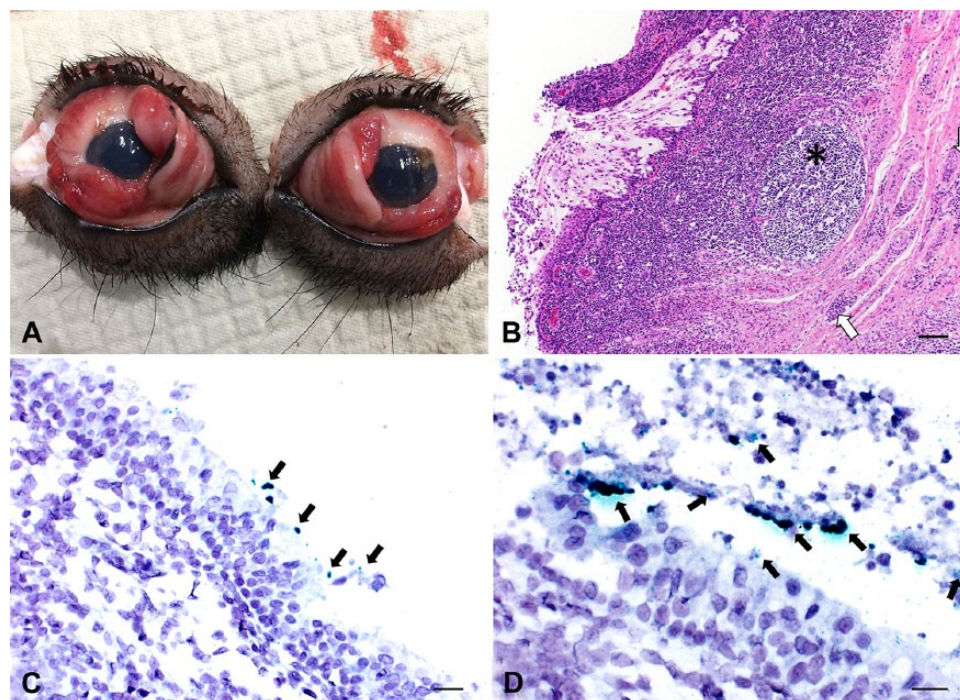
Figure 1. *Mycoplasma hyorhinis*–associated conjunctivitis is swine. **A.** The peak lesions occurred in 12-wk-old pigs. In this group, there was hyperemia and mild serous discharge with blepharedema. **B.** The epithelium of the ocular conjunctiva is eroded and ulcerated, with exposure of the lamina propria. The conjunctival fibrous layer is markedly expanded by a lymphoplasmacytic inflammatory infiltrate, with lymphofollicular hyperplasia (asterisk) and perivascular cuffing (arrows). H&E. Bar = 100 μm. **C.** Green *M. hyorhinis*–positive signals (arrows) detected in the epithelium of palpebral conjunctiva at the early clinical stage of conjunctivitis. In situ hybridization (ISH). Bar = 50 μm. **D.** Green *M. hyorhinis*–positive signals (arrows) detected within ulcerative regions of the palpebral conjunctiva at late clinical stage of conjunctivitis. ISH. Bar = 20 μm.

Table 1. Real-time PCR results for *Mycoplasma hyorhinis* from conjunctival swabs collected during an outbreak of conjunctivitis in commercial swine herds.

Pig clinical condition	No. of samples by state				Ct value (mean ± SD)
	MN	OH	OK	SD	
Affected					
Early stage (*n* = 11)	6	3	2	0	21.3 ± 2.4
Late stage (*n* = 11)	5	3	3	0	23.5 ± 1.8
Unaffected					
Contact with affected pen mates (*n* = 10)	4	6	0	0	29.7 ± 3.1
Non-contact (unaffected herd) (*n* = 6)	2	0	0	4	37.5 ± 1.1

OH = Ohio; OK = Oklahoma; MN = Minnesota; SD = South Dakota.

from affected pigs at early stage of the disease, 11 from affected pigs at late stage of the disease, 10 from contact pen-mates with no apparent clinical signs, and 4 from pigs housed in an unaffected herd) (Table 1).

The diagnostic investigation for potential pathogens was conducted at the UMN-VDL. Tissue samples were evaluated by histopathology, and eye swabs and tissue samples were screened for viral, bacterial, and fungal agents. Immunohisto-chemistry (IHC) and/or PCR were performed for CSFV, ASFV, PRV, *Chlamydia* spp., *M. hyopneumoniae, M. hyorhinis*,

PCMV, IAV, and PRRSV. A real-time PCR (rtPCR) was used to determine the presence and concentration of *M. hyorhinis* DNA in eye swabs. The rtPCR assay was developed and validated at the UMN-VDL. A hydrolysis probe (5'-/6FAM/CCG-GATATAGTTATTTATCGCATGA/MGBNFQ/-3'), and for-ward (5'-CGTACCTAACCTACCTTTAAGACTGGG-3') and reverse (5'-GTGAAGCTGTGAAGCTCCTTTCT-3') primer sequences, were developed targeting a specific region (position 131-182) of the 16S rRNA gene of the *M. hyorhinis* genome (NR_113686.1). An ISH assay was developed to

assess the expression of *M. hyorhinis* 16S rRNA within eye tissues. Briefly, unstained paraffin sections of tissue were deparaffinized in xylene and rehydrated through a series of alcohol washes. The rehydrated sections were tested using the RNAscope technique[12] (Advanced Cell Diagnostics, Newark, CA). Hydrogen peroxide was added over the tissue sections at room temperature for 10 min. Slides were then boiled in citrate buffer for 15 min, and incubated with protease at 40°C for 30 min. A *M. hyorhinis* probe was hybridized in tissue sections with the hybridization buffer at 40°C for 2 h. Then, according to the manufacturer's instructions, amplifiers were added for 15 or 30 min at 40°C in order to amplify the hybridization signal. Green colorimetric staining (alkaline phosphatase) detected the *M. hyorhinis* hybridization signal, and counterstaining was performed using hematoxylin. Microscopic assessment was conducted by 2 independent blinded pathologists.

At the early stage of the clinical disease (8–10 wk old), erosive lymphoplasmacytic conjunctivitis associated with lymphofollicular hyperplasia was observed predominantly in the palpebral conjunctiva with minimal or no extension to the bulbar conjunctiva (Fig. 1B). Tissue samples collected at the late stage of the clinical course revealed ulceration associated with lymphoplasmacytic and neutrophilic infiltration distributed throughout the palpebral and bulbar conjunctiva, and lymphofollicular hyperplasia.

CSFV, ASFV, PRRSV, IAV, PRV, and *M. hyopneumoniae* infections were ruled out by their absence on PCR assays. Samples were negative for *Chlamydia* spp. by culture and capture ELISA (QuickVue; Quidel, San Diego, CA). PCMV was inconsistently detected by PCR in affected and unaffected pigs. Inclusion bodies suggestive of rhinitis caused by PCMV were not observed during histologic examination. *Staphylococcus chromogenes* and *S. aureus* were also isolated from eye swabs of affected and unaffected animals. Tissue samples from unaffected pigs did not show evidence of histopathologic change.

Eye swabs and conjunctival tissues from clinically affected animals and unaffected contact pen-mates were consistently positive for *M. hyorhinis* by rtPCR (Table 1). ISH-positive signals for *M. hyorhinis* were observed associated with histologic lesions of the conjunctival epithelium and connective tissue, and where the substantia propria was exposed as a result of ulceration (Figs. 1C, D). Bacterial isolation of *M. hyorhinis* was attempted from samples of all herds. However, overgrowth of *S. aureus* limited isolation of *M. hyorhinis*, despite indication of significant bacterial concentration, as evidenced by low cycle threshold (Ct) values of rtPCR in broth. ISH-positive signals were not observed in tissue samples from unaffected pigs (data not shown).

Conjunctivitis is not usually considered a disease with significant economic impact in intensive pig production. Sporadic cases are usually related to the presence of irritating materials in the environment (dust, gases, and chemicals), and/or presence of foreign bodies in the eye region. *Chlamydia* spp. have been common infectious agents associated with conjunctivitis outbreaks in pig herds; however, there are limited reports in the literature that describe diagnostic investigation of conjunctivitis in pigs, and incidence of pathogens causing conjunctivitis may be higher. *M. hyorhinis* has been isolated from the conjunctivae of pigs with and without clinical disease[6]; based on serologic evaluation, the author suggested the involvement of a distinct variant species of *M. hyorhinis*. Mycoplasma-like particles have been described by ultrastructural evaluation without association with other pathogens[11]; unfortunately, these investigators did not perform experimental infection of *M. hyorhinis* isolates to verify the reproducibility of clinical signs.

Several animal species are susceptible to conjunctivitis caused by *Mycoplasma* spp. *M. gallisepticum* has been associated with outbreaks of conjunctivitis in birds.[3] *M. felis* has been isolated from cats exhibiting clinical conjunctivitis. Cats inoculated with *M. felis* isolates showed moderate-to-severe conjunctival hyperemia.[7] Conjunctivitis and keratoconjunctivitis in small ruminants associated with *M. conjunctivae* have been consistently reported in the literature.[4,10] Cattle can also suffer from conjunctivitis caused by *M. bovoculi* and *M. bovis*.[1,8,9] Finally, *M. genitalium* was reported to be associated with a case of chronic conjunctivitis in a human patient affected by mild urethritis caused by the same agent.

Real-time PCR coupled with ISH is a valuable tool for outbreak investigations to identify the potential causative agent in association with histologic lesions, especially when there are commensal bacterial organisms involved and lack of antibodies available to perform immunohistochemistry assays. Although we did not fulfill Koch's postulates in our investigation, certain factors indicate *M. hyorhinis* as the primary agent in our cases. First, Ct values detected by rtPCR were lower in affected pigs compared with unaffected pen-mates, indicating that although *M. hyorhinis* was detected in healthy pigs as part of the eye microbiota, in conjunctivitis-affected pigs, higher bacterial loads of *M. hyorhinis* were present. Additionally, samples from unaffected, non-contact pigs had high Ct values. Although *M. hyorhinis* was not isolated from conjunctival swabs, *M. hyorhinis* was detected with the developed probe for the ISH assay when targeting mRNA of the 16S gene. Therefore, positive signals corresponded to metabolically active bacteria present in the tissue. The extensive number of *Mycoplasma* species that can cause conjunctivitis and keratoconjunctivitis in other animal species also supports *M. hyorhinis* as the etiology of the swine conjunctivitis in our study. Comprehensive studies involving whole genome comparison among *M. hyorhinis* strains isolated from clinical cases of conjunctivitis, polyserositis, and asymptomatic carriers are warranted to clarify potential genotypic differences associated with a specific disease phenotype.

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