A novel *Mycoplasma* sp. associated with phallus disease in goose breeders: pathological and bacteriological findings.

S. Carnaccini¹, N. M. Ferguson-Noel², R. P. Chin³, T. Santoro¹, P. Black², M. Bland⁴, A. A. Bickford¹ and C. G. Senties-Cué¹,⁵.

¹California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, University of California-Davis, Turlock branch, 1550 N. Soderquist Road, Turlock, CA 95380.

²Department of Population Health, The University of Georgia, 953 College Station Road, Athens, GA 30602-4875.

³California Animal Health and Food Safety Laboratory System, School of Veterinary Medicine, University of California-Davis, Tulare branch, 18830 Road 112, Tulare, CA 93274.

⁴Cutler Associates International, 3562 Jomar Drive, Napa, CA 94558.

⁵Corresponding author:

C. G. Senties-Cué,

1550 N. Soderquist Rd, Turlock, CA 95380.

Email: gsenties@cahfs.ucdavis.edu

Phone: (209) 634-5837
SUMMARY

In April 2014, poor fertility in a major commercial goose breeder operation in California triggered the submission of six live affected Toulouse ganders to the California Animal Health and Food Safety laboratory (CAHFS), Turlock branch (UC Davis). Toulouse were principally affected among all breeds and their egg fertility dropped from 65.7% to less than 33.9% in the first forty days of the 2014 breeding season. The flock consisted of 410 adult birds, 90 males and 320 females, between 2 and 5 years of age. Inspection of the flock revealed that 44.4% of the Toulouse ganders had severe phallic deformities which prevented them from mating. At necropsy, severe yellowish fibrocaseous exudate disrupted the architecture of the phallus and occasionally produced fistulating tracts through the wall of the organ. Microscopically, multifocal lymphoid nodules were noted in the mucosa and submucosa of the phallus and were associated with extensive granulomatous reaction, intra-lesional bacteria and spermatozoa. *Mycoplasma spp.* were isolated from the phallus of affected and non-affected birds and PCR protocols targeting the 16S-23S rRNA intergenic spacer (ISR) regions and the RNA polymerase beta subunit (rpoB) gene were performed to identify the isolates. Three distinct species were identified upon sequencing and analysis using NCBI BLAST: *Mycoplasma cloacale*, *Mycoplasma anseris*, and an unknown novel *Mycoplasma sp.* In addition, *Pasteurella multocida*, in combination with other bacteria, was also isolated from the phallic lesions and identified as serotype 3 with a DNA profile of 1511 (National Veterinary Service Laboratory). This is the first report of these *Mycoplasma spp.* and other bacteria associated with reproductive disease in ganders in the United States.
Keywords: fertility, gander, histology, *Mycoplasma, P. multocida*, phallus, venereal disease.

Abbreviations:

AVMA = American Veterinary Medical Association;

BHI = brain heart infusion broth;

BLAST = Basic Local Alignment Search Tool;

CAHFS = California Animal Health and Food Safety;

CO$_2$ = carbon dioxide

FMS = Frey’s medium with swine serum

HI = hemagglutination inhibion

ISR = intergenic spacer regions

M-ORT = Modified-Ortmeyer

NAHLN = National Animal Health Laboratory Network;

NCBI = National Center for Biotechnology Information;

NVSL = National Veterinary Services Laboratory;

OIE = Office International des Épizooties - World Organisation for Animal Health;

PAS = periodic acid schiff

PCR = polymerase chain reaction;

PPE = personal protective equipment;

rpoB = RNA polymerase beta subunit

rRNA = ribosomal ribonucleic acid;

RT-rPCR = real-time reverse transcription–polymerase chain reaction;

SP4 = Spiroplasma 4
INTRODUCTION

Diseases of the reproductive tract of the waterfowl were first reported by Wagener and Hams in 1948 and since then have been extensively studied (4, 7, 13, 25, 28, 29, 32, 43). Clinical and pathological presentation varies and may include reduced egg production, hatchability, fertility, cloacitis and inflammation of the reproductive tract and gonads (4, 14, 29, 35, 37, 38). Specifically, egg infertility may be as high as 80 to 90% in affected flocks, causing great economic losses in goose and duck farms (38). However, studies on the female reproductive tracts dominate the literature, giving little aid in identifying outbreaks of gander venereal diseases. Contagious diseases affecting male genitalia must be differentiated from bacterial infection secondary to mechanical injuries inflicted during copulation. The traumatic prolapse of the phallus is easily recognized by field veterinarians and should not exceed more than 0.5-1% of the drakes and ganders during the reproductive season, as it does not spread bird to bird (29, 38). Neisseria spp. (38), Candida albicans (3), P. multocida (12, 41), Acholeplasmas and Mycoplasmas including M. gallisepticum, M. synoviae, M. anseris, M. anatis, and the recent M. sp. 1220 (8, 11, 14, 25, 32, 33, 35, 40) are infectious agents that have been recovered from outbreaks of reproductive disease in geese, often aggravated by poor hygienic conditions and secondary bacterial infections. Recently, more attention was drawn by mycoplasmas as certain species have been identified as underlying cause of transmissible venereal disease in geese (8, 14, 34, 40). Originally isolated from geese by Kosovac and Djurisic (25), Mycoplasmas and Acholeplasmas were subsequently investigated by Stipkovits, El-Ebeedy, Kisary and Varga (33), who studied their incidence in geese. Since then, numerous species including M. gallinarum, M. anatis, M. cloacale, M. gallisepticum, M.
synoviae, M. anseris, M. imitans, and a novel Mycoplasma sp. strain 1220 were isolated from the reproductive tracts of male and female fowl (5, 6, 8, 11, 14, 18, 27, 32-36). Only a few of the mycoplasmas isolated were proven to be pathogenic for goose embryos and goslings, caused respiratory problems in adult birds and reduced egg production and hatchability. Naïve ganders introduced into infected flocks were shown to develop reproductive lesions and seroconverted to the mycoplasmas. However, experimental studies failed to reproduce phallic disease in ganders (34, 37, 40). Numerous questions are still unanswered about the pathogenesis of this disease, the transmission, and definition of contributing factors. Here we report the clinical, pathologic and microscopic findings combined with the laboratory results and characterization of the agents isolated during a reproductive disease outbreak in commercial ganders. To our knowledge, this is the first report of isolation of these Mycoplasma species (M. anseris, M. cloacale and novel Mycoplasma sp.) associated with disease in geese in the United States.
Case history – Farm and Clinical Observations

At the beginning of April 2014, eight live adult geese (6 sick Toulouse males, 1 Toulouse female and 1 Brown Chinese male) were submitted to the Turlock branch of the California Animal Health and Food Safety Laboratory System, for diagnostic evaluation. The healthy adult Brown Chinese gander from the same ranch was submitted at the same time for comparison. All live birds were humanely euthanized with carbon dioxide for post mortem examination following the AVMA guidelines. The diagnostic protocol consisted of clinical history, necropsy, histopathology, bacteriology, real time-reverse transcriptase polymerase chain reaction (RT-rPCR) for avian influenza, and serology.

The farm was a multi-age and multi-breed operation, with approximately 3,000 goose and 6,000 duck breeders in total. In 2014, all of the goose eggs were hatched and the birds were raised as breeder replacements or sold to individuals, feed stores or commercial growers throughout the United States. All the birds were brooded indoor on deep litter with access to pasture. At about 10 weeks of age, the goose breeders were moved to outdoor pens. Right before they reached sexual maturity, birds were divided into single-breed pens and remained as such throughout the breeding season (from January through July for the geese). Each pen consisted of a dirt floor with a wire fence perimeter and bird-proof netting covering the pens. During the laying period birds were provided with feed that was produced locally, and drinking water ad libitum. The affected Toulouse flock consisted of approximately 320 female and 90 male adult Toulouse geese. Additionally, there were several hundred goose breeders of other breeds, such as Buff, White Embden, Pilgrim and others of the same age in adjacent pens. The complaint was a
drastic reduction in fertility in the Toulouse flock from 65.7% to less than 33.9% within the first forty days of the 2014 breeding season, which was not accompanied by a drop in egg production. This particular Toulouse flock had a history of poor fertility, averaging about 70-65% in each previous laying season. At the beginning of January, in preparation for the 2014 breeding season all the gander genitalia were checked and no phallic abnormalities were noticed at this time. Egg production and hatch results, including numbers of infertile eggs and early and late embryo mortalities, were recorded. Eggs were candled on the 10th day of incubation and those without any signs of embryo development were classified as infertile. No unusual embryo mortality was noticed among the fertile eggs. During the 2014 breeding season, the weekly fertility from February 24 to March 24 was 70.7%, 65.4%, 51.6%, 40.6%, and 33.9%. The fertility in other goose breeds were within normal range, e.g., Embden (85.2%), White Chinese (92.4%), and Sebastopol (54%). Birds in these flocks did not show any other clinical signs. Following the fertility results, an unplanned check of all the Toulouse ganders was done at the beginning of April. Prominent abnormalities of the genitalia were found in 40 of 90 males which were removed from the flock. Hence, a representative sample of birds was sent to the diagnostic lab for further investigation. Treatment with both tylosin and chlortetracycline resulted in a moderate improvement in fertility, although the birds never returned to the initial production rates.

Sample Collection & Histopathology

Complete necropsies were performed in all birds. Tissue samples for histopathologic examination included testes, ovary, oviduct, epididymis, vas deferens, ejaculatory duct
and groove, vascular bodies, phallus, cloaca, brain, heart, liver, spleen, conjunctiva, sinus, trachea, lung, air sac, intestine, pancreas, cloaca, kidney, adrenal, gizzard, proventriculus, skin and skeletal muscle. All tissue sections collected during necropsy were fixed in 10% neutral buffered formalin, routinely processed, paraffin embedded, sectioned at 4 \( \mu m \) thickness, and stained with hematoxylin and eosin for microscopic examination. Gram and periodic acid schiff (PAS) stains were run specifically on phallic lesions to determine the presence of bacteria and fungi associated with the lesions.

**Bacteriology & Mycoplasma isolation and characterization**

Samples from livers (2/8), peritoneums (2/8), phalluses (7/7), cloaca (8/8), and cervix (1/1) were plated onto 5% sheep blood and MacConkey agars (Remel, Lenexa, Kansas, USA) and incubated at 37°C with 7% CO\(_2\) for a minimum of 48 hours. In addition, swabs from cloaca and phallus from each bird were inoculated separately in both Modified-Ortmeyer (M-ORT) and Frey’s broths and plates (Med Media Services, UCDavis, USA), incubated at 37°C with 7% CO\(_2\) for a minimum of 7 days and until growth was observed. Presumptive mycoplasma isolates were sent to the Poultry Diagnostic and Research Center of The University of Georgia for identification and characterization. Briefly, three types of broth media and corresponding agar plates were inoculated: Spiroplasma 4 (SP4), M-ORT, and Frey’s medium with swine serum (FMS) (PDRC, Georgia, USA). All broths and agar plates were incubated at 37 °C with 8% CO\(_2\) for a minimum of 48 hours. Mycoplasmas were separated by subbing colonies with different morphology and subsequent passages in the three different media-types.
Putative mycoplasma isolates were confirmed by PCR targeting the 16S-23S rRNA intergenic spacer (ISR) regions (30) and the RNA polymerase beta subunit (rpoB) gene (42). Nucleic acid was extracted from 200 µl of culture in modified Frey’s medium for nine isolates. Genomic DNA was extracted using the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA), following the manufacturer’s recommendations. DNA sequencing was performed as previously described (31). Each amplification product was sequenced in both directions with the forward and reverse amplification primers. Raw sequence data was analyzed with the EditSeq program (in Lasergene; DNASTAR, Inc.), and the complete overlapping of complementary sequences, editing, and consensus construction were produced with the SeqMan program (in Lasergene; DNASTAR, Inc.). Identification upon sequencing and analysis was done using the Basic Local Alignment Search Tool (BLAST) algorithm from the National Center for Biotechnology Information (NCBI) (2). Sequences from relevant *Mycoplasma* spp. were downloaded and alignments of individual target sequences were constructed by the Clustal W method using the MegAlign program (DNASTAR Lasergene, Madison, WI) (Fig.1).

*Additional tests*

Cloacal swabs from all birds submitted were collected in tubes of brain heart infusion broth (BHI) and tested for influenza A virus (AIV) matrix gene via real time-reverse transcriptase polymerase chain reaction (rRT-PCR) according the method described (NAHLN protocol, SOP-AV-0001).
Sera from the eight live geese were tested by hemagglutination inhibition (HI) for antibody detection against Newcastle disease, *Mycoplasma gallisepticum* and *M. synoviae*.

**RESULTS**

*Post-Mortem Findings*

All the birds submitted were in a good state of nutrition (8/8). Macroscopic lesions were confined to the lower reproductive tract of the Toulouse ganders (6/6) and severely compromising the anatomic integrity of their genitalia (Fig. 3-4). Most of the phalluses (4/6) had a partial prolapse through the cloaca and were severely enlarged, darkened or greyish in color. The spirals were unable to evert and the bodies of the phalluses were enlarged at the base, encumbering the lumen of the cloaca (Fig. 3). A firm, yellowish caseous core either dry or exuding viscous milky fluid was exposed by sectioning the body of the genitalia. In 4 of 6, the exudate was fistulating through the wall of the phallus (Fig. 4). In a few birds (3/6), vestiges of the prolapsed spiral were fused together in an indistinct mass of fibrotic adhesions. Multifocal yellowish, dry, irregular plaques (~ 0.5 to 1.0 cm in diameter) were noticed on the surface mucosa of the cloaca and coprodeum. Occasionally, the exudate at this level had fistulated through the cloacal wall, and reconnected internally with the *vasa deferentia*.

No lesions were found grossly in the Brown Chinese and female Toulouse reproductive tracts. The female was in production with more than six developed ovarian follicles. Testes varied in size among different birds and within the same bird as well. There was mild testicular atrophy, the right side being smaller and measuring ~1.8 cm longitudinally and ~1.5 cm in diameter compared with the left which was ~4.0 x 2.5 cm. No other
macroscopic lesions were observed. Left testes were slightly larger than their right pair, but consistently smaller compared to the Brown Chinese’s (~5.0 x 4.5 cm). No macroscopic lesions were noticed in the vasa deferentia of the ganders (7/7). All other organs were without visible lesions (such as ovary and oviduct, liver, spleen, sinus, trachea, lungs, air sac, intestine, pancreas, skin, muscles, and heart).

Histopathological Findings

Microscopic lesions in the phalluses could be grouped in two presentations depending on the progression of the disease. Severe multifocal lymphoid nodule formations were observed at several levels of the mucosa and submucosa of the phallus and cloaca. Specifically, severe diffuse lymphoplasmacytic infiltrations and nodules followed the blood vessels, ejaculatory ducts and groove, the lymph folds, vascular bodies, glandular part of the phallus and the sulcus spermaticus (Fig. 6-8). At the apex of the phallus, where the sulcus spermaticus joins the aditus of the cavity of the phallus, the mucosa of the blind ending tube was severely infiltrated by mononuclear cells and the lumen constricted by severe hyperplastic lymphoid nodules. At this level, there was also mild heterophilic infiltration of the inflamed mucosa and fibrinonecrotic exudation in the lumen intermingled with spermatozoa and cellular debris (Fig. 9-10). Severe extensive granulomatous inflammation was associated with ulceration the mucosa of the phallus and infiltration of the submucosa. Intermingled in the necrotic debris and inflammatory exudation were large numbers of bacteria and spermatozoa. The stratified squamous epithelium of the mucosa of the phallus was undergoing multifocal hypertrophy and acanthosis. Cellular changes included hydropic degeneration, vacuolation, cyst formation,
erosions of the superficial layers, necrosis and sloughing of necrotic debris in association with a diffuse infiltrative granulocytic reaction (Fig. 9). Granulomas were accompanied by lymphoid nodules in the deep connective tissue of the phallus. Special stains revealed the presence of rod-shaped Gram-negative bacteria associated within the granulomatous reaction (Fig. 10). PAS stain did not reveal any fungal structures. Moderate multifocal lymphoid nodule formation and lymphoplasmacytic infiltration were found in the wall of vasa deferentia and interstitium of the testis. Severe diffuse lymphoplasmacytic infiltration and occasional multifocal granulocytic and granulomatous reaction were seen in the mucosa of cloaca and coprodeum. Occasionally, the inflammatory reaction at these levels was associated with severe ulceration of the mucosa and penetration of the submucosa. Sections of the ovary revealed only mild multifocal lymphocytic infiltration. Few occasional germinal centers were noticed in splenic lymphoid follicles. No lesions or inflammatory reaction was noticed in heart, conjunctiva, sinus, trachea, lung, brain, adrenals, kidney, pancreas, skeletal muscles.

Bacteriologic Findings

Mycoplasma species were isolated from all (7/7) phallic and (8/8) cloacal cultures. They fermented glucose but did not hydrolyze arginine and were sensitive to digitonin (indicating sterol requirement). On agar the isolates produced typical mycoplasma colonies with “fried-egg” morphology presenting three slightly different phenotypic characteristics (22, 39). Direct immunofluorescence testing of the novel Mycoplasma sp. colonies on agar plates with conjugated antiserum specific for common avian mycoplasma species (including M. gallisepticum, M. synoviae, M. meleagridis, M.iowae, M.iners, M. gallopavonis, M. glyciphillum, M. cloacae, M. lipofaciens, M.
gallinarum, M. gallinaceum, M. anatis, and M. columborale) was negative. The novel
Mycoplasma sp. isolate grew relatively slowly in both modified Frey’s with swine serum
and SP-4.
Pasteurella multocida was also isolated from 4 of 6 phallic lesions and identified as
serotype 3 with a DNA profile of 1511 (NVSL). Escherichia coli was also isolated from
(7/7) phalluses and (1/1) cervix. No growth was observed from aerobic cultures of liver
(2/2) and peritoneum (2/2).

PCR results and sequencing
Ten Mycoplasma isolates were confirmed by polymerase chain reaction (PCR) (30, 42).
Three distinct species of Mycoplasma were identified upon sequencing and analysis using
NCBI BLAST: Mycoplasma cloacale (5/10), Mycoplasma anseris (1/10), and an
unknown novel Mycoplasma sp. (4/10) (Table 1). Sequencing and phylogenetic analysis
of the isolates are provided in figure 1. All novel isolates matched each other and their
sequences resulted in 89.5% and 83.5% similarity to M. spermatophilum and 87.5% and
82.5% similar to M. fermentans by ISR (30) and rpoB (42) respectively. One sample
(K6660G) that was confirmed by PCR was not included in the dendograms due to the
poor quality of the ISR sequences.

Additional tests result
All the samples for Avian Influenza tested negative by PCR. Sera from the eight live
geese tested negative for Newcastle disease, Mycoplasma gallisepticum and M. synoviae.
DISCUSSION

The severe inflammatory reaction associated with the isolation of Mycoplasmas, *P. multocida* and *E. coli*, without evidence of traumatic injury, is compatible with an infectious etiology for the phallic lesions. Subsequently, the severe phallic lesions were compromising the functionality of the organ, thus preventing mating which resulted in high infertility rates. Histology revealed the presence of severe lymphoplasmacytic infiltrates and hyperplastic lymphoid nodules in the mucosa and submucosa of the phallus, glandular part of the phallus, fibrolymphatic body, lymph folds, *sulcus spermaticus*, cloaca, vascular bodies, ejaculatory duct and groove, *vasa deferentia, vasa efferentia*, epididymis, and testes. Although not pathognomonic, the inflammatory reaction characterized by primarily mononuclear inflammatory cells and germinal center formations is suggestive of mycoplasma infection (16). Severe granulocytic inflammatory reaction and granulomas were also seen in the phallus associated with large, Gram-negative bacilli and coccobacillary bacteria. As heterophilic response is found more commonly as a result of *E. coli* and *P. multocida* infections (12, 19), this type of inflammatory reaction may be related to the isolation of one or both of these organisms.

These two types of inflammatory reactions were always combined in the phallus and its annexed structures. Although a minimal lymphocytic infiltration has been reported in healthy birds in the literature (1, 17, 23, 24, 26), the severity and distribution of the lesion were beyond what is expected in normal birds. Moreover, lymphoid nodule and
lymphoplasmacytic infiltrations appeared to compromise the anatomic integrity of these structures and were associated with degeneration, necrosis and sloughing of the superficial epithelium.

Anseriformes, (ducks, geese and swans), have an elaborate corkscrew-like intromittent phallus in the males and a spiral vagina in the females (9, 10, 15, 17, 20, 21, 26). At rest, the phallus is inverted within the phallic sac (saccus phalli) in the right ventral wall of the cloaca and the erectile mechanism is lymphatic rather than vascular as in mammals (10, 21). Semen formed in the testis runs internally through the epididymis to the vasa deferentia and then it is ejected by the ejaculatory ducts into the groove at the base of the phallus. Contrary to mammals, the semen is transported in an open channel (sulcus spermaticus or phallic sulcus) which runs on the dorsal surface of the phallus delimited by the fibrolymphatic bodies. In Anseriformes, the center of the phallus presents a blind-ending tubular cavity (cavum penis), which is surrounded by folds of mucus-secreting pseudostratified columnar epithelium (glandular part of the phallus) whose purpose is unclear. This deep blind-ending portion of the tube is non-erectile and is connected with the sulcus spermaticus through an opening at the apex of the phallus (9, 10, 15, 17, 20, 21, 26). In the affected birds, the presence of severe inflammatory reactions in the submucosa of the ejaculatory ducts, lymph folds and sulcus spermaticus may have limited the possibility for the semen and lymph to flow normally. This may also explain the atrophy and degenerative changes to the testicular epithelium. The formation of granulomatous cores in the phalluses may have been the consequence of an obstruction of the lumen of its cavity. The formation of a stagnant environment and the impossibility to purge the bacteria and necrotic debris may have aggravated the inflammatory reaction at
this level. This would explain the formation of abscesses at the center of the male genitalia and the fistulae of fibrinonecrotic exudate. Bacteria from the cloacal region may have taken advantage of the stagnant environment to proliferate.

Many strains of *P. multocida* are responsible for severe systemic disease in the fowl (19). Moreover, waterfowl are considered very susceptible to *P. multocida* infection (12, 19, 41). However, the lesions observed in these birds were localized and bacteria were not recovered from any of the other organs. Hence, the strain of *P. multocida*, which was ultimately identified as serotype 3 with a DNA profile of 1511, may have been of low pathogenicity. As the source of introduction of *P. multocida* is unknown, *Escherichia coli* are ubiquitous organisms responsible for severe secondary bacterial infection when allowed. Special stains did not reveal the presence of fungal components. Neither inclusion bodies nor lesions suggestive of a viral infection were seen. Although the lesions observed could not be considered pathognomonic, the severity of the lymphoid infiltrations supported the relevance of the mycoplasmas isolated. *M. cloacale* was isolated from affected and non-affected birds in high prevalence (Table 1), but is considered a commensal organism in the literature (4, 33, 36). Contrarily, *M. anseris* is a potential pathogen for geese possibly causing phallic abnormalities, infertility, reduced hatchability, respiratory morbidity and mortality (7, 8, 14, 34, 40). However, the low prevalence of *M. anseris* (1/6) among the birds may be attributed to the stage of the disease at which the culture samples were collected and/or to a reduced survival rate of this organism in the culture due to the competition with other bacteria. The problem observed in these birds was restricted to the male reproductive tract and no respiratory morbidity, mortality nor a drop in egg production and hatchability were noted. Therefore,
it seemed relevant that the novel *Mycoplasma sp.* was predominantly isolated from the birds presenting phallic deformities (4/6) and not from the ones that were unaffected. These findings however should be further confirmed by analyzing a wider representative sample of birds. The NCBI BLAST comparison highlighted a distant relationship of the novel *Mycoplasma sp.* isolated from these geese with the known genome of *M. fermentans* and *M. spermatophilum* (Fig. 1). The degree of similarity among the novel *Mycoplasma sp.* and *M. fermentans* and *M. spermatophilum* was relatively low (82-83% for rpoB and 87-90% for ISR), making it unlikely that these could be related. The *M. anseris* and *M. cloacale* similarities for the isolates identified as these species were all >95% which is consentient for intra-species variability (30). Treatment with both tylosin and chlortetracycline lead to an improvement in fertility, which indicates that these medications were successful in containing the infection. The fact that the birds never returned to the initial production rates had to be expected due to the irreversible deformities to the male genitalia.

An important factor to consider is transmission and breed susceptibility. Mycoplasmas are both vertically and horizontally transmitted (16). Therefore, the flock could have been infected through the admission of newly imported carrier birds and embryonated eggs from an infected breeder source or contact with migratory wild waterfowl. The Toulouse appeared to be the only breed affected. Though the different breeds commingle during the non-breeding season, there is not supposed to be any crossbreeding. Hence, this suggests that the principal transmission route may be venereal. These mycoplasmas were isolated from the cloacal swabs as well, suggesting that another possibility for infection is contact with contaminated fecal material. However, the relatively low resistance of mycoplasmas
in the outer environment (16) makes difficult indirect transmission. In addition, goose breeds may have different susceptibility in developing the disease. A future investigation has to be conducted to define the sites of replication of these *Mycoplasma* spp., such as the respiratory, enteric and reproductive tracts, to determine the principal shedding routes and the pathogenesis of this disease. As only one bird from another breed and a female were submitted in this case, more testing has to be done to determine the prevalence of these mycoplasma strains among breeds, in both genders and their pathogenicity. This is the first report of the isolation of *M. anseris*, *M. cloacale*, *P. multicida* and a novel *Mycoplasma sp.* associated with these reproductive disease features in ganders in the United States. In conclusion, more work is required to determine the pathogenicity of the novel *Mycoplasma sp.* and the other bacteria isolated and its role in the development of goose venereal disease.

**ACKNOWLEDGEMENTS**

The authors want to thank the staff of the CAHFS Turlock, Davis branches and Poultry Diagnostic Research Center for the outstanding professional support.

*Declaration of Conflicting interests*

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

*Funding*
The author(s) received no financial support for the research, authorship, and/or publication of this article.

References


Table 1. Bacteriology results are summarized in positive (+) and negative (-) for each individual bird. M = male; F = female; NA = Not affected; A = affected.

Figure 1. Phylogenetic tree based on (a) 16S/23S rRNA ISR and (b) rpoB sequences. M. anatis and M. sp. 1220 served as the out-group. Numbers of nucleotide substitutions are provided in the scale at the bottom of the tree. GeneBank accession numbers of sequences are given in parentheses. Mycoplasma isolate phylogenetic relationships are shown in the dendrograms based on nucleotide sequence data for the 16-23 intergenic
spacer and rpoB gene among species of the family *Mycoplasmataceae*. The names of the species that have not yet been fully described are referred to with individual identification number.

**Figure 2.** Normal phallic structure from the Chinese gander. s.s., *sulcus spermaticus*;

**Figure 3.** Affected Toulouse gander.

**Figure 4.** Severe caseonecrotic exudate fistulating through the wall of the phallus (arrow heads) and revealed by cross section (arrow);

**Figure 5.** Photomicrograph of a section of phallus from the unaffected Chinese gander. *sulcus spermaticus*, s.s.

**Figure 6.** Severe lymphocytic and lymphoid nodule infiltration (arrowhead) in the mucosa and lamina propria of the *aditus* of the cavity of the phallus. Severe granuloma formation in the submucosa (arrow).

**Figure 7.** Severe lymphocytic infiltration and multifocal lymphoid nodule formation in the submucosa at the opening of the *sulcus spermaticus* and *aditus* of cavity of the phallus.

**Figure 8.** Vascular body: severe multifocal lymphoid nodule formation and lymphocytic infiltration.

**Figure 9.** Necrosis and perforation of the mucosa of the phallus (arrowhead) and severe fibrinonecrotic exudate formation (arrow).

**Figure 10.** Severe granulomatous reaction associated with large numbers of bacteria (arrows) and spermatids (arrowhead).
<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Breed</th>
<th>Gender / status</th>
<th>M. cloacale</th>
<th>M. anseris</th>
<th>Mycoplasma sp. novel</th>
<th>P. multocida</th>
</tr>
</thead>
<tbody>
<tr>
<td>K16660A</td>
<td>Brown Chinese</td>
<td>M/NA</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K16660B</td>
<td>Toulouse</td>
<td>F/NA</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K16660C</td>
<td>Toulouse</td>
<td>M/A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>K16660D</td>
<td>Toulouse</td>
<td>M/A</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K16660E</td>
<td>Toulouse</td>
<td>M/A</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K16660F</td>
<td>Toulouse</td>
<td>M/A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>K16660G</td>
<td>Toulouse</td>
<td>M/A</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>K16660H</td>
<td>Toulouse</td>
<td>M/A</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1. Bacteriology results are summarized in positive (+) and negative (-) for each individual bird. M = male; F= female; NA = Not affected.
Table 1. Bacteriology results are summarized in positive (+) and negative (-) for each individual bird. M = male; F = female; NA = Not affected; A = affected.

*Escherichia coli*

+   
+   
+   
+   
+   
+   
+   
+   
+   
+   

ed; A = affected.