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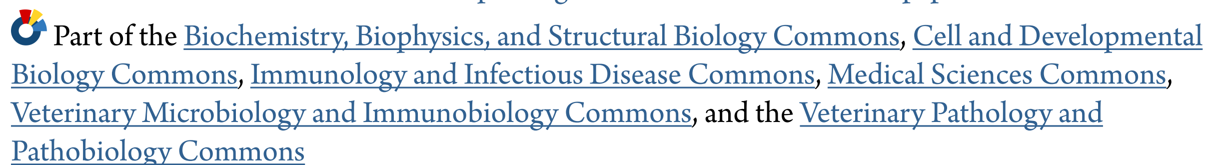
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Moraxella spp. isolated from field outbreaks of infectious bovine keratoconjunctivitis: a retrospective study of case submissions from 2010 to 2013

John Dustin Loy,[1] Bruce W. Brodersen

Abstract. Infectious bovine keratoconjunctivitis (IBK), also known as pinkeye, is the most costly eye disease of cattle. The principal etiologic agent of IBK is the Gram-negative bacterium *Moraxella bovis*. However, there have been reports of IBK outbreaks associated with *Moraxella bovoculi*. A retrospective study of IBK diagnostic cases submitted from July 1, 2010 through October 31, 2013 was conducted. Included in the study were 1,042 *Moraxella* isolates from 1,538 swabs of lacrimal secretions collected from 282 herds from 30 U.S. states. *Moraxella* isolates were identified to the species level and were composed of *M. bovoculi* (701 isolates), *M. bovis* (295 isolates), *Moraxella ovis* (5 isolates), and other *Moraxella* spp. (41). Minimum inhibitory concentrations required for 90% growth inhibition (MIC_{90}) was calculated for representative isolates. The MIC_{90} values for both *M. bovis* and *M. bovoculi* were as follows: ampicillin and ceftiofur: ≤0.25 μg/ml; clindamycin: 2 μg/ml; danofloxacin and enrofloxacin: ≤0.12 μg/ml; florfenicol: 0.5 μg/ml; gentamicin: 1 μg/ml; neomycin: 4 μg/ml; tulathromycin: 2 μg/ml; and tylosin: 8 μg/ml. The MIC_{90} values for *M. bovoculi* included the following: chlortetracycline: ≤0.5 μg/ml; oxytetracycline: 4 μg/ml; penicillin: 0.25 μg/ml; spectinomycin: 32 μg/ml; sulfadimethoxine: >256 μg/ml; tiamulin: 1 μg/ml; and trimethoprim–sulfamethoxazole: 4 μg/ml. For *M. bovis*, MIC_{90} values included the following: chlortetracycline and oxytetracycline: 1 μg/ml; penicillin: ≤0.12 μg/ml; spectinomycin: 16 μg/ml; sulfadimethoxine: ≤256 μg/ml; tiamulin: ≤0.5 μg/ml; and trimethoprim–sulfamethoxazole: ≤2 μg/ml. The current work describes the frequency of isolation and differences in antimicrobial sensitivity observed among *Moraxella* isolates from case submissions.

Key words: Infectious bovine keratoconjunctivitis; *Moraxella bovis*; *Moraxella bovoculi*.

Introduction

Infectious bovine keratoconjunctivitis (IBK), also known as pinkeye, is a common and costly eye disease of domestic cattle.[28,29] Cattle with IBK demonstrate a variety of clinical signs, including increased tear production, sensitivity to light, and ultimately corneal swelling that progresses into corneal ulceration and possible blindness.[2] Very little is understood about the impacts of IBK on animal welfare[30]; however, pain assessment studies demonstrate that it is a painful and irritating condition.[13] Economic losses in the beef industry can be tremendous, where a 15.9-kg loss in weaning weight per head has been estimated.[20] Midwestern U.S. beef herds report that IBK is endemic in nearly 50% of herds with a prevalence of 8.75 out of 100 cattle affected.[29] Adequate and timely treatment of acute IBK with antimicrobial therapy is frequently challenging due to many animals being remotely pastured or grazed during peak occurrence in the summer months.[26]

The principal etiologic agent for IBK is the Gram-negative bacterium *Moraxella bovis*.[19] One of the major virulence factors of *M. bovis* is a secreted cytotoxin that has been shown to reproduce IBK lesions in calves.[10] This toxin is a repeats-in toxin (RTX), which forms pores in the membranes of target host cells causing lysis and cell death.[11] *Moraxella bovoculi*, a newly described species in the genus *Moraxella*, has been isolated and characterized in association with IBK in the absence of *M. bovis*.[9] Additional evidence for the role of *M. bovoculi* in the causation of IBK includes secretion of a RTX class cytotoxin similar to that of *M. bovis*.[6] However, studies evaluating vaccine efficacy show a decrease in incidence of IBK neither when *M. bovoculi* was included in an autogenous vaccine formulation[15] nor when subunit *M. bovoculi* cytotoxin was used as a vaccine in calves.[8] Direct inoculation of *M. bovoculi* into scarified calf corneas does not cause IBK lesions, making the direct role of *M. bovoculi* in IBK pathogenesis uncertain.[18] The data indicates that

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although *M. bovoculi* is isolated in cases of IBK, there is no direct evidence supporting a causal role, and temporal associations of *M. bovoculi* and correlation of eye colonization with disease have been difficult to determine.[21] In order to further characterize the microbial populations present in bovine eyes during outbreaks of IBK, a retrospective study was undertaken to assess the composition of *Moraxella* spp. in a large number of diagnostic submissions, as well as to assess the antimicrobial susceptibility patterns to selected antimicrobial agents of representative isolates from diagnostic submissions.

Materials and methods

Samples included in the current study were comprised of diagnostic laboratory submissions received from July 1, 2010 to October 31, 2013 that had been submitted as diagnostic cases to the Nebraska Veterinary Diagnostic Center (Lincoln, Nebraska). Data was extracted from a laboratory information management software system (LIMS) that includes all diagnostic submissions over the time period. A subset of data was extracted into spreadsheet software including case submissions of swabs of lacrimal secretions collected from bovine eyes during cases of IBK in beef or dairy herds. For some diagnostic submissions, a specific state or geographic region where the herd was located in which the sample was taken was not included on the submission information. Herd data was based on unique accession numbers assigned to each case. Individual data was determined based on a unique individual identification number present on a case submission form. Isolation of *Moraxella* spp. on eye swabs was conducted by trained technicians following standard operating procedures in an American Association of Veterinary Laboratory Diagnosticians (AAVLD) fully accredited laboratory. Data entry into LIMS was performed by the technician, and results were reviewed by a laboratory manager and a case coordinator for accuracy. The protocol used by all trained technicians in the laboratory at the time period, to determine if viable *Moraxella* spp. were present in the samples, was as follows: Swabs were streaked onto tryptic soy agar containing 5% sheep's blood and incubated for 18–24 hr at 37°C with 5% CO_2 supplementation. Bacterial colonies with morphology consistent with members of the genus *Moraxella* were further screened for oxidase production. Colonies positive for oxidase production were then subjected to Gram staining and were subcultured for purity. All subcultured organisms that were characterized as Gram-negative rods or coccobacilli by Gram stain were subjected to molecular speciation. Isolates were subsequently identified as *M. bovis*, *M. bovoculi*, or *Moraxella ovis* based on a polymerase chain reaction (PCR) assay and a subsequent restriction fragment length polymorphism analysis that targets the 16S-23S intergenic spacer region in combination with phenotypic tests as needed.[4] Some isolates could not be definitively identified based on PCR data and phenotypic data, and were categorized as *Moraxella* sp.

For PCR testing, genomic DNA was extracted from 24-hr subculture growth by picking out several well-isolated colonies with a sterile stick and then resuspending the colonies into 100 µl of nuclease-free water to a 1–2 McFarland standard turbidity. Cell suspensions were boiled at 100°C for 10 min to lyse bacterial cells. Cell debris was clarified by centrifugation at 15,700 × *g* for 2 min. Extracted nucleic acid was subjected to PCR using primers[a] ISRdown (5′-GTGAAGTCGTAACAAGGTAGCCGT-3′) and ISRup (5′-ACCGACGCTTATCGCAGGCTATCA-3′).[4] The PCR master mix[b] consisted of 50 µl, which contained nuclease-free H_2O (32.1 µl), 5.0 µl of 10× reaction buffer, 4.0 µl of $MgCl_2$ (50 mM), 0.5 µl of deoxyribonucleotide triphosphates (100 mM), 2.5 µl of ISRup (10 µM), 2.5 µl of ISRdown (10 µM), 0.4 µl of *Taq* polymerase (5 U/µl), and 3.0 µl of template DNA. The PCR reactions were subjected to thermocycling under the following conditions; 95°C for 60 sec, then 40 cycles of 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec and a final extension of 72°C for 7 min. Amplicons were then digested with restriction endonuclease *Afa*I[c] and subjected to a 60-min digestion at 37°C. Digested DNA (15 µl) was resolved using capillary gel electrophoresis.[d] Interpretation of molecular weight fragments corresponding to *M. bovoculi*, *M. ovis*, and *M. bovis* was 450- and 150-bp, 600-bp, and 650-bp fragments, respectively.

As part of the diagnostic workup of each case submission, representative isolates were selected on a herd level based on similar colony growth characteristics and PCR speciation. The representative isolates were then subjected to antimicrobial sensitivity testing. Because of the small number of *M. ovis* isolates and possible diversity within the *Moraxella* sp. isolated, these organisms were excluded from the antimicrobial susceptibility analysis. To conduct in vitro antimicrobial sensitivity, a broth microdilution system was used following Clinical Laboratory Standards Institute (CLSI) guidelines.[12] Several colonies of pure culture were suspended into 10 ml of sterile demineralized water to a 0.5 McFarland standard and vortexed to ensure uniform resuspension. Inoculation density was confirmed using a calibrated nephelometer. A 10-µl aliquot of the resuspended organism was then inoculated into 11 ml of sterile inoculation media and vortexed to ensure uniform resuspension. A 100-µl aliquot of culture per well was then inoculated into bovine and/or porcine antimicrobial susceptibility panels[e] with an autoinoculator. Samples were incubated at 35°C for 18 hr without carbon dioxide supplementation and were read automatically with the minimal inhibitory concentration (MIC) determined using an automated system.[f] The auto-read values were confirmed manually by observation as necessary. Quality control organisms used for assays include *Staphylococcus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212), and *Escherichia coli* (ATCC 25922). No specific CLSI-approved interpretative criteria exist for *Moraxella* spp. in cattle, therefore interpretive criteria established for bovine respiratory disease or other Gram-negative veterinary isolates as available were used, with organisms classified as susceptible (S),

Table 1. Selected antimicrobial drugs utilized in broth microdilution testing for minimum inhibitory concentrations, the range of concentrations evaluated for each antimicrobial, and interpretive criteria (susceptible, intermediate, or resistant) applied to each antimicrobial tested.

Antimicrobial	Concentration(s) tested (µg/ml)	Susceptible	Intermediate	Resistant
Ampicillin	0.25–16	≤0.5	1	≥2
Ceftiofur	0.25–8	≤2	4	≥8
Chlortetracycline	0.5–8	≤2	4	≥8
Clindamycin	0.25–16	≤0.5	1–2	≥4
Danofloxacin	0.12–1	≤0.25	*	*
Enrofloxacin	0.12–2	≤0.25	0.5–1	≥2
Florfenicol	0.25–8	≤2	4	≥8
Gentamicin	1–16	≤2	4	≥8
Neomycin	4–32	*	*	*
Oxytetracycline	0.5–8	≤2	4	≥8
Penicillin	0.12–8	≤0.25	0.5	≥1
Spectinomycin	8–64	≤32	64	≥128
Sulfadimethoxine	256	≤256		>256
Tiamulin	0.5–32	≤16		≥32
Tilmicosin	4–64	≤8	16	≥32
Trimethoprim–sulfamethoxazole	2/38†	<2/38		≥2/38
Tulathromycin	1–64	≤16	32	≥64
Tylosin	0.5–32	*	*	*

* No interpretive criteria available.
† A single concentration of 2 µg/ml trimethoprim in combination with 38 µg/ml sulfamethoxazole (2/38) was tested.

intermediate (I), or resistant (R; Table 1).[12] Breakpoints were not available for neomycin and tylosin. For trimethoprim–sulfamethoxazole and sulfadimethoxine only a single drug concentration was tested. Percent susceptibility (% susceptible) was determined by dividing the number of organisms with MIC values that fell into a susceptible MIC breakpoint (numerator) by the total tested (denominator) and multiplying by 100. The MIC_{50} and MIC_{90} values were defined as the concentration of antibiotic that was capable of inhibiting growth of 50% and 90% of total tested isolates.

Results

A total of 1,538 samples were included in the data set. Of these, 600 had no growth of *Moraxella* spp. or were too contaminated with overgrowth to determine if there were *Moraxella* organisms present in the specimen. Out of this total, 938 swabs had growth of 1 or more *Moraxella* spp., and the total number of isolates subjected to identification was 1,042 as some had multiple species present. Submitted samples included case submissions from cases of IBK in 30 states. Submissions from 29 out of 30 states had at least 1 *Moraxella* sp. isolated. The number of submitted samples on a state-by-state basis that contained at least 1 *Moraxella* spp. isolated as well as the number of herds from which these samples originated, is indicated (Fig. 1). For 261 herds, geographic information was sufficient to determine the state of origin for the submission. On individual animal level 938 submissions were identified. The frequency of isolation of *Moraxella* in individuals included: *M. bovis*: 193; *M. bovoculi*: 600; *M. ovis*: 4; *Moraxella* sp.: 37; *M. bovis* and *M. bovoculi*: 99; *M. bovoculi* and *Moraxella* sp.: 1; *M. bovoculi* and *M. ovis*: 1; *M. bovis* and *Moraxella* sp.: 3 (Table 2).

On a herd level, 282 total herds were identified. The frequency of isolation of *Moraxella* spp. in herds included: *M. bovis*: 18; *M. bovoculi*: 140; *M. ovis*: 2; *Moraxella* sp.: 7; *M. bovis* and *M. bovoculi*: 102; *M. bovoculi* and *Moraxella* sp.: 2; *M. bovoculi* and *M. ovis*: 1; *M. bovoculi* and *M. ovis*: 1; *M. bovis* and *Moraxella* sp.: 1; *M. bovis, M. bovoculi*, and *M. ovis*: 1 (Table 2).

For *M. bovoculi*, 213 total isolates were subjected to MIC testing against 18 antimicrobials with a range of concentrations (Table 1). MIC_{90} values were as follows: ampicillin, ≤0.25 µg/ml; ceftiofur, ≤0.25 µg/ml; chlortetracycline, ≤0.5 µg/ml; clindamycin, 2 µg/ml; danofloxacin and enrofloxacin, ≤0.12 µg/ml; florfenicol, 0.5 µg/ml; gentamicin, 1 µg/ml; neomycin and oxytetracycline, 4 µg/ml; penicillin, 0.25 µg/ml; spectinomycin, 32 µg/ml; sulfadimethoxine, >256 µg/ml; tiamulin, 1 µg/ml; trimethoprim–sulfamethoxazole, 4 µg/ml; tulathromycin, 2 µg/ml; and tylosin, 8 µg/ml (Table 3). The total susceptible phenotypes observed for *M. bovoculi* were as follows: ampicillin, 99%; ceftiofur, 100%; chlortetracycline, 93%; clindamycin, 11%; danofloxacin, 98%; enrofloxacin, 98%; florfenicol, 92%; gentamicin, 98%; neomycin, not determined (ND); oxytetracycline, 85%; penicillin, 96%; spectinomycin, 86%; sulfadimethoxine, 86%;

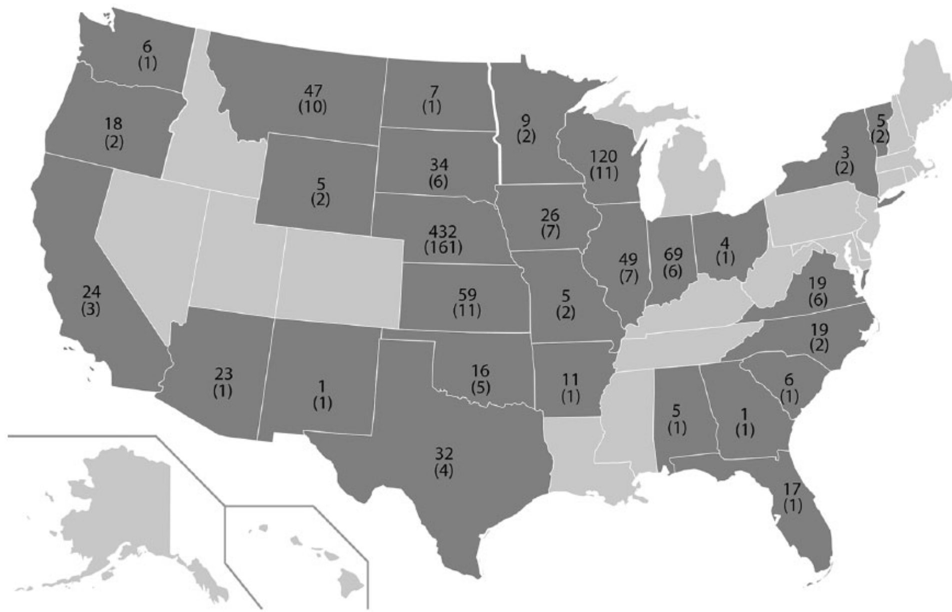

Figure 1. Number and state-by-state distribution of case submissions that at least 1 *Moraxella* spp. was isolated from bovine ocular swabs. States with at least 1 *Moraxella* isolate included in the analysis are shaded in dark gray. Numbers on the state indicates the total positive case submissions received in the period, and the number in parentheses indicates the number of positive herds included in each state over the period from July 1, 2010 to October 31, 2013.

Table 2. Profile of *Moraxella* spp. isolated from lacrimal secretion swab cultures (n = 1,042).*

Species isolated	No. of herds yielding the indicated species (1)	No. of individuals yielding the indicated species (2)
Moraxella bovis	18	193
Moraxella bovoculi	140	600
Moraxella ovis	2	4
Moraxella sp.	7	37
M. bovis and *M. bovoculi*	102	99
M. bovoculi and *Moraxella* sp.	2	1
M. bovoculi and *M. ovis*	1	1
M. bovis and *Moraxella* sp.	2	3
M. bovis, *M. ovis*, and *M. bovoculi*	1	0

* The number of herds with the corresponding species composition of isolated organisms is indicated in column (1). Column (2) indicates the number of herds with the corresponding species composition isolated from samples.

tiamulin, 99%; tilmicosin, 92%; trimethoprim–sulfamethoxazole, 87%; tulathromycin, 92%; and tylosin, ND (Table 3).

For *M. bovis*, 106 total isolates were subjected to MIC testing against 18 antimicrobials with a range of concentrations (Table 1). The MIC_{90} values were as follows: ampicillin, ≤0.25 µg/ml; ceftiofur, ≤0.25 µg/ml; chlortetracycline, 1 µg/ml; clindamycin, 2 µg/ml; danofloxacin and enrofloxacin, ≤0.12 µg/ml; florfenicol, 0.5 µg/ml; gentamicin, 1 µg/ml; neomycin, 4 µg/ml; oxytetracycline, 1 µg/ml; penicillin, ≤0.12 µg/ml; spectinomycin, 16 µg/ml; sulfadimethoxine, ≤256 µg/ml; tiamulin, ≤0.5 µg/ml; trimethoprim–sulfamethoxazole, ≤2 µg/ml; tulathromycin, 2 µg/ml; and tylosin, 8 µg/ml (Table 4). The total susceptible phenotypes observed for *M. bovis* were as follows: ampicillin, 99%; ceftiofur, 100%; chlortetracycline, 91%; clindamycin, 21%; danofloxacin, 100%; enrofloxacin, 100%; florfenicol, 100%; gentamicin, 100%; neomycin, ND; oxytetracycline, 96%; penicillin, 99%; spectinomycin, 100%; sulfadimethoxine, 98%; tiamulin, 99%; tilmicosin, 100%; trimethoprim–sulfamethoxazole, 98%; tulathromycin, 94%; and tylosin, ND (Table 4).

Discussion

While previous data indicated that *M. bovoculi* is isolated in association with outbreaks of IBK in the absence of *M. bovis*,[9,21] the current study has characterized the composition of *Moraxella* isolates associated with IBK from a large number of geographically diverse case submissions in the United States. Other authors have characterized genetic diversity using fingerprinting among *Moraxella* spp. in other countries but have not examined these on a herd or individual animal level.[27] It was observed in the current study that *M. bovoculi*

Table 3. Count of *Moraxella bovoculi* isolates with the minimum inhibitory concentration (MIC) for each antibiotic tested. Additionally, the MIC required to inhibit 50% and 90% (MIC_{50} and MIC_{90}, respectively) of each isolate as well as the total percentage of *M. bovoculi* isolates that were susceptible to each antibiotic are included (n = 213 isolates).

	MIC (µg/ml)														
Antimicrobial	0.12	0.25	0.5	1	2	4	8	16	32	64	≤256	>256	MIC_{50}	MIC_{90}	% susceptible
Ampicillin		211		2									≤0.25	≤0.25	99
Ceftiofur		207	4										≤0.25	≤0.25	100
Chlortetracycline			185	11	2	11		4*					≤0.5	≤0.5	93
Clindamycin		11	13	119	62	4	2	2*					1	2	11
Danofloxacin	207	2		4*									≤0.12	≤0.12	98
Enrofloxacin	207	2			4*								≤0.12	≤0.12	98
Florfenicol		28	164	2	2	11	4*						0.5	0.5	92
Gentamicin				204	4	2	2						1	1	98
Neomycin						200	9	2	2				4	4	†
Oxytetracycline			153	26	2	11	19*						≤0.5	4	85
Penicillin	183	21	4	2	2								≤0.12	0.25	96
Spectinomycin							45	142	9	17*			16	32	92
Sulfadimethoxine											183	30	≤256	>256	86
Tiamulin			181	26		2	2		2				≤0.5	1	99
Tilmicosin						179	17	2	11	4			≤4	8	92
Trimethoprim–sulfamethoxazole‡					185	28§							≤2	4	87
Tulathromycin				179	13		4		6	6			≤1	2	92.0
Tylosin				2	2	147	70						4	8	†

* Endpoint of > for the MIC indicated.
† No interpretive criteria available.
‡ A single concentration of 2 µg/ml trimethoprim in combination with 38 µg/ml sulfamethoxazole (2/38) was tested.
§ Indicates MIC of >2 µg/ml as additional concentrations were not evaluated.

was the only *Moraxella* sp. isolated from the majority of case submissions, when evaluated at either the herd or individual level. These findings were unexpected, given that *M. bovis* has been isolated from experimentally infected calves up to 54 days following inoculation, and thus it was thought that *M. bovis* would be the predominant organism isolated in association with IBK outbreaks.[17] However, recovery of *M. bovis* may be variable as pathogenesis studies have shown that *M. bovis* was not observed on the conjunctival surface, using light microscopy, 10-hr postinoculation when introduced in gnotobiotic calves.[23] Other studies have shown that *M. bovoculi* can be isolated from ulcers in calves experimentally inoculated with *M. bovis*.[18] Epidemiologic investigations have also failed to establish a temporal relationship between prior exposure and clinical IBK outbreaks with isolations of *M. bovoculi*.[21] Analysis of the speciation data in the current study indicates that *M. bovoculi* is present and viable in most of the submissions in higher numbers than *M. bovis*. *Moraxella bovis*, when cultured, was frequently isolated in association with *M. bovoculi*, and was the sole *Moraxella* spp. isolated in only 18 of the 282 herd submissions. The generally accepted method for sample collection for IBK culture is to place the swab in the ventral conjunctival sac to sample lacrimal secretions. Differences and variability in and among sampling methods and timing of collection may result in samples that may not be representative of all organisms present in the eye conjunctiva. Additionally, the isolation of *M. ovis* from only 5 swabs indicates that this species, at least in this set of samples, is likely not significantly contributing to IBK in cattle.

Previous studies have evaluated antimicrobial resistance patterns in *M. bovis* and *M. bovoculi* in smaller collections of isolates individually.[5,25] Additionally, a study assessing the diversity of *Moraxella* spp. in IBK cases from Uruguay evaluated antimicrobial susceptibility; however, the methods utilized disk diffusion and did not measure zone sizes for interpretation, as recommended by the CLSI, and did not use minimum inhibitory concentration testing making comparison with this data difficult.[27] The antimicrobial susceptibility data from the present study, for *M. bovoculi*, is similar to a study that examined 57 isolates from California.[5] However, there were some differences observed. *Moraxella bovoculi* isolates showed a reduced level of in vitro susceptibility to the tetracycline class drugs, where the MIC_{90} for oxytetracycline was found to be 4 µg/ml instead of 1 µg/ml. This is a four-fold increase in MIC_{90} value over the previously published data.[6] Contrastingly, the MIC_{90} for *M. bovis* against oxytetracycline was previously reported as 32 µg/ml,[25] whereas a much lower MIC_{90} of 1 µg/ml was found in the current study. The MIC_{90} of tulathromycin for both *M. bovis*

Table 4. Count of *Moraxella bovis* isolates with the minimum inhibitory concentration (MIC) for each antibiotic tested. Additionally, the MIC required to inhibit 50% and 90% (MIC_{50} and MIC_{90}, respectively) of each isolate as well as the total percentage of *M. bovis* isolates that were susceptible to each antibiotic are included (*n* = 106 isolates).

	MIC (µg/ml)														
Antimicrobial	0.12	0.25	0.5	1	2	4	8	16	32	64	≤256	>256	MIC_{50}	MIC_{90}	% susceptible
Ampicillin		105			1								≤0.25	≤0.25	99
Ceftiofur		104	2										≤0.25	≤0.25	100
Chlortetracycline			93	2	1		10*						≤0.5	1	91
Clindamycin			22	67	15	2							1	2	21
Danofloxacin	100	6											≤0.12	≤0.12	100
Enrofloxacin	103	3											≤0.12	≤0.12	100
Florfenicol		72	33	1									0.5	0.5	100
Gentamicin				106									1	1	100
Neomycin						105	1						4	4	†
Oxytetracycline			94	8		1	3						≤0.5	1	96
Penicillin	99	6	1										≤0.12	≤0.12	99
Spectinomycin							98	8					16	16	100
Sulfadimethoxine											104	2	≤256	≤256	98
Tiamulin			98	6	1	1							≤0.5	≤0.5	99
Tilmicosin						95	11						≤4	8	100
Trimethoprim–sulfamethoxazole‡					104	2§							≤2	≤2	98
Tulathromycin				104	1			1		6			≤1	2	94
Tylosin				8	21	45	29	3					4	8	†

* Endpoint of > for the MIC indicated.
† No interpretive criteria available.
‡ A single concentration of 2 µg/ml trimethoprim in combination with 38 µg/ml sulfamethoxazole (2/38) was tested.
§ Indicates MIC of >2 µg/ml as additional concentrations were not evaluated.

and *M. bovoculi* fell within the susceptible range of 2 µg/ml. However, there were 16 isolates of *M. bovoculi* with MIC values of 8 or greater for tulathromycin, with 6 of these isolates with MIC values of 64 µg/ml or greater. This is important to note, as oxytetracycline and tulathromycin are 2 antimicrobials with label claims for treatment of IBK associated with *M. bovis*.

Other antimicrobial drugs have been reported to be effective at treating IBK, including penicillin,[1,3] cloxacillin benzathine,[17] ceftiofur,[14] clindamycin,[24] florfenicol,[7] and tilmicosin.[31] A systematic review of randomized clinical trials examining IBK pharmacological studies indicated that direct comparisons between therapies were lacking, making drug comparisons challenging.[22] Evaluation of antimicrobial efficacy for therapy cannot be determined based on susceptibility patterns; however, in vitro testing of *M. bovis* and *M. bovoculi* indicate susceptibility to most of the antimicrobials, with MIC_{90} values falling into the susceptible category. The lowest percentage of susceptibility was seen with clindamycin; however, a significant number of organisms fell in the intermediate interpretive category for clindamycin (70 and 17 for *M. bovoculi* and *M. bovis*, respectively) making the number of isolates in the resistant category quite low, with a MIC_{90} of 2 µg/ml for both *Moraxella* species. While previous reports of antimicrobial susceptibility testing have shown elevated resistance to penicillin for *M. bovoculi* (12.3%),[5] data in the present study showed only a 4% level of resistance. The antimicrobial resistance patterns appear similar between geographically distinct *M. bovis* and *M. bovoculi* isolates, indicating that infections with either or both organisms show similar susceptibility patterns as previously noted.[5] However, it should be noted that there are some small differences, as the MIC_{90} of *M. bovoculi* for oxytetracycline was higher than for *M. bovis* (4 µg/ml compared with 1 µg/ml).

Differences in these susceptibility patterns may be indicative of regional preference for antimicrobial usage to treat IBK, and the emergence of these patterns may be based on selective pressure. Other factors that may influence this difference include transmission of resistance genotypes on a regional level, as all of the previously tested *M. bovoculi* isolates were from California and the current study was biased toward Midwestern U.S. states but included other states. Antimicrobial treatment prior to sampling was unable to be determined as this was not routinely included in the case histories on the submissions forms. However, prior treatment with antimicrobials may affect in vitro susceptibility data, and knowledge of a treatment history may enable more analysis of these patterns.

In conclusion, *M. bovoculi* is the most prevalent and frequent of the *Moraxella* sp. isolated in IBK cases in cattle

submitted to the Nebraska Veterinary Diagnostic Center on both individual and herd levels. Over 600 of the 1,042 isolates tested were *M. bovoculi*. In nearly half (102/282) of the herds tested, both *M. bovoculi* and *M. bovis* were isolated from 1 or more animals in those herds. The frequency of co-isolations may indicate that *M. bovoculi* may be serving as an opportunistic agent or colonizing eyes in conjunction with *M. bovis*, which has been shown experimentally to induce characteristic ulcers in gnotobiotic calves.[23] Further work should be conducted to elucidate the temporal factors and role of coinfections with *M. bovoculi* and *M. bovis* in IBK pathogenesis that would enable further interpretation of this data.

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Sources and manufacturers

a. Integrated DNA Technologies Inc., Coralville, IA.
b. *Taq* DNA polymerase, PCR master mix, and 100 mM dNTP set; Life Technologies, Grand Island, NY.
c. *Afa*I (*Rsa*I), Invitrogen, Life Technologies, Grand Island, NY.
d. QIAxcel advanced instrument, Qiagen Inc., Valencia, CA.
e. Bovine/porcine with tulathromycin MIC plate, Trek Diagnostic Systems, Thermo Fisher Scientific Inc., Waltham, MA.
f. Sensititre Microbiology System Trek Diagnostic Systems, Thermo Fisher Scientific Inc., Waltham, MA.

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