Moraxella boevrei sp. nov., a New *Moraxella* Species Found in Goats

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Six *Moraxella*-like strains that formed a phenotypically homogeneous group were isolated from the nasal flora of healthy goats. Total genomic DNA-DNA hybridization, rRNA gene restriction pattern, DNA base composition, and genetic transformation studies were performed to determine the relationships of these bacteria to species belonging to the genus *Moraxella* and other fastidious gram-negative species. The new group of isolates was very homogeneous, as shown by rRNA gene restriction fragment length patterns (ribotyping), and these organisms displayed high relative binding ratios (RBRs) to each other in DNA-DNA hybridization experiments (RBRs, $\geq 58\%$) but distinctly lower levels of DNA homology with all other species investigated. However, the RBRs obtained with species of the genus *Moraxella* were higher than the RBRs obtained with all other gram-negative strains examined. Although the new strains had most of the *Moraxella bovis* phenotypic characteristics except nitrate reduction, quantitative and qualitative genetic transformation data led to the conclusion that they belong to a distinct new cluster in the genus *Moraxella*. The results of this study, combined with the general morphological and phenotypic profiles of the new strains, are consistent with the creation of a new *Moraxella* species, for which the name *Moraxella boevrei* is proposed. Strain 88365 (= ATCC 700022 = CCUG 35435 = NCTC 12925 = CIP 104716) is the type strain of *M. boevrei*.

Moraxella bovis is the bacterial agent involved in acute and chronic cases of infectious bovine keratoconjunctivitis or pink-eye, a contagious disease of cattle (5). Investigators have reported that the number of *M. bovis* isolations from bovine upper respiratory tracts increases during summer months (10), and recovering the bacteria from cattle during cold seasons (i. e., autumn and winter) has proven to be difficult (2). These observations suggested that there are carriers of *M. bovis* in other animals (3). Other investigators have reported the isolation of bacteria resembling *M. bovis* from various animals, including horses (8, 13–15), sheep (20–22, 33), and goats (23, 25).

It has now been demonstrated that the characteristics of the equine *Moraxella* isolates (formerly *Moraxella equi*) are compatible with the description of a nonhemolytic variant of *M. bovis*, a member of the *Moraxella lacunata* group or the classical moraxellae. This group includes *M. lacunata*, *M. bovis*, *Moraxella nonliquefaciens*, and *Moraxella caprae* (*M. caprae* is a new species recently isolated from goats [23]). Many other strains isolated from sheep or goats or from other mammals have not been precisely identified because of a lack of adequate characteristics for distinction. Identification of these organisms to the species level by cultural and conventional biochemical techniques in clinical laboratories has proven to be difficult. Serum liquefaction, nitrate reduction, and hemolysis on blood agar are the main traits that can be used to differentiate the species of the classical moraxellae (27, 31). However, it has been demonstrated that most *Moraxella* strains can be identified by genetic transformation with nutritional auxotrophic mutants (17–19) or by genetic transformation of the high-level streptomycin resistance marker (4, 5). Ratios of interstrain transformation to intrastrain transformation close to 1.0 for members of individual species have been demonstrated in this genus (7, 12, 29, 31). The transformation ratio usually ranges from 10^{-1} to 10^{-3} from one species to another within

the classical moraxellae (4, 5). The species may also be identified by total genomic DNA-DNA hybridization experiments, in which members of one species usually exhibit relative binding ratios (RBRs) close to 70% or higher (29, 31).

Recently, we isolated from the upper respiratory tracts of healthy goats from the same herd six hemolytic *Moraxella* strains resembling *M. bovis* (21, 22). The strains had all of the conventional biochemical and enzymatic features of collection strains of *M. bovis* except one; they were all capable of nitrate reduction. These properties suggested that these isolates belong to *M. bovis*, but the sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of whole-cell protein profiles placed them in a group different from the group containing the classical *M. bovis* reference strains (22). These strains also transformed *M. bovis* only weakly in qualitative genetic transformation assays (21).

In the present study, the relationships of the six hemolytic goat strains to all members of the genus *Moraxella* were investigated by performing quantitative genetic transformation, total DNA-DNA hybridization, rRNA gene restriction pattern (ribotyping), and DNA base composition analyses.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains investigated, including the strains of various *Moraxella* species and other fastidious gram-negative bacteria used for comparison, are listed in Table 1. Strains were grown on 5% human blood agar plates (Difco) and chocolate agar plates incubated at 35°C in the presence of 5% CO₂.

Quantitative and semiquantitative genetic transformation assays. The media and reagents used have been described previously (4, 5). Spontaneous mutants for high-level streptomycin resistance were selected. DNAs were extracted from the mutants, and the DNA concentrations were adjusted to 200 µg/ml. The quantitative transformation assay was performed by exposing 0.5 ml of a recipient suspension to 0.05 ml of each DNA for 20 min. After DNase treatment, 0.1-ml aliquots of each final suspension and appropriate dilutions were inoculated onto blood agar plates. The DNase treatment step was omitted from semiquantitative assays. The plates were preincubated for 7 h before they were placed on top of an agar layer, and the final concentration of streptomycin was 50 µg/ml after diffusion. The plates were incubated for an additional 3 to 5 days before colonies were counted and ratios of interstrain transformation to intrastrain (autologous) transformation were calculated (4, 5).

DNA-DNA hybridization. (i) **DNA dot blot.** DNA was extracted and blotted for use in dot blot hybridization experiments essentially as previously described (23,

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TABLE 1. Bacterial strains used in this study

Species	Strain(s) ^a	Other designation(s) ^a	Source (year)	Reference(s) or sender ^a
<i>Moraxella boevei</i> sp. nov.	88365 ^T	ATCC 700022 ^T , CCUG 35435 ^T , NCTC 12925 ^T	Goat nasal swabs (1988)	23
	88363A, 88363B, 88364, 88365, 88369		Goat nasal swabs (1988)	23
<i>M. caprae</i>	NCTC 12877 ^T	CCUG 33296 ^T , ATCC 700019 ^T	Goat nasal swabs (1988)	23
	88100, 88103, 88104, 88105		Goat nasal swabs (1988)	23
<i>M. bovis</i>	ATCC 10900 ^T		Cattle pinkeye	4, 29
	ATCC 17948			
	CIP 649	ATCC 17947	Cattle pinkeye	CIP
	9		Cattle	29
	NCTC 11012 ^T (also called <i>M. equi</i>)	ATCC 25576 ^T	Eye of horse	29
	PG 500		Cattle IBK ^b	Field isolate
	PG 501		Cattle IBK	Field isolate
<i>M. lacunata</i>	ATCC 17967 ^T	NCTC 11011 ^T	Human conjunctivitis	ATCC
	A947		Human conjunctivitis	4, 29
	50136		Human conjunctivitis	29
<i>M. nonliquefaciens</i>	ATCC 19975 ^T		Human nasal swab	1, 4, 29
<i>M. osloensis</i>	CIP 6835 ^T	ATCC 20776 ^T	Human spinal fluid	CIP
	5873			29
<i>M. atlantae</i>	CIP 8225 ^T	ATCC 29525 ^T	Human blood	CIP
<i>M. phenylpyruvica</i>	CIP 8227 ^T	ATCC 23333 ^T	Human blood	CIP
<i>M. lincolnii</i>	CCUG 9405 ^T		Human nasopharynx	34
<i>Moraxella (Branhamella) catarrhalis</i>	Ne11 ^T	ATCC 23238 ^T	Unknown	31
<i>M. (B.) ovis</i>	199/55 ^T	ATCC 33078 ^T	Ovine keratconjunctivitis	31
<i>M. (B.) caviae</i>	ATCC 14659 ^T	CIP 7319 ^T	Guinea pig pharynx	23
<i>M. (B.) cuniculi</i>	ATCC 14688 ^T	CIP 7317 ^T	Rabbit oral mucosa	23
<i>Kingella kingae</i>	4177/66 ^T	ATCC 23330 ^T	Human nose	31
<i>K. denitrificans</i>	NCTC 10995 ^T			31
<i>Neisseria elongata</i>	6171/75			31
<i>N. weaveri</i> (formerly CDC group M5)	B5522			1
<i>Eikenella corrodens</i>	ATCC 23834 ^T	NCTC 10596 ^T	Sputum	31
<i>Psychrobacter immobilis</i>	NCMB 98	CCUG 9702	Gills of lemon sole	23
<i>Oligella urethralis</i>	ATCC 17960 ^T		Human ear	29
<i>Haemophilus aphrophilus</i>	9574/86		Sinusitis	30
<i>H. paraphrophilus</i>	NCTC 10557 ^T	ATCC 29241 ^T	Paronychia	30
<i>Pasteurella multocida</i>	646		Pig	30
<i>P. haemolytica</i>	NVI (serotype 1)			30
<i>Escherichia coli</i>	K-12			31

^a ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection Institut Pasteur, Paris, France; CCUG, Culture Collection, University of Göteborg, Göteborg, Sweden; NCTC, National Collection of Type Cultures, London, United Kingdom; NCMB, National Collection of Marine Bacteria, Aberdeen, Scotland.

^b IBK, infectious bovine keratoconjunctivitis.

31). Briefly, DNA extracted by a modification of the method of Marmur (24) was heat denatured at 95°C for 10 min, cooled on ice, and mixed with an equal volume of cold 2 M ammonium acetate. DNA was applied to nitrocellulose filters by using a Hybri-Dot system (Bethesda Research Laboratories). Eight parallel dots (1 µg each) and a Tris-EDTA buffer control were blotted for each strain. The

filters were washed in 6× SSC buffer (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and then soaked in 2× Denhardt's solution for 1 h before they were baked in a vacuum oven at 80°C for 2 h. Filters were stored dry until they were used.

(ii) **Labelling of probes.** Mechanically fragmented genomic DNA probes were labelled with [³²P]-dCTP to a specific activity of approximately 1 × 10⁷ cpm/µg

TABLE 2. DNA base compositions and affinities of *M. boevei* strains to other *Moraxella* species as determined by genetic transformation

DNA donor	G+C content (mol%) ^a	Ratios of interstrain transformation to intrastrain transformation with the following recipients:				
		<i>M. boevei</i> 88365 ^T	<i>M. bovis</i> ATCC 10900 ^T	<i>M. lacunata</i> A947	<i>M. atlantae</i> A1922	<i>M. (B.) catarrhalis</i> Ne11 ^T
<i>M. boevei</i> 88365 ^T	41	1	<10 ⁻⁵	— ^b	—	6.2 × 10 ⁻⁵
<i>M. bovis</i> ATCC 10900 ^T	41–44.5	<10 ⁻⁴	1			1.2 × 10 ⁻⁴
<i>M. lacunata</i> A947	40–44	—		1		
<i>M. atlantae</i> A1922	46.5–47.5	—			1	
<i>M. (B.) catarrhalis</i> Ne11 ^T	40–43	<10 ⁻⁴				1
<i>N. elongata</i> 6171/75	53–54.7	—				1.2 × 10 ⁻⁵

^a The data for strain 88365^T are from this study; the data for other strains are from references 6, 7, 12, and 28.

^b —, transformants were not detected in semiquantitative transformation experiments.

of DNA by using a Rediprime random priming labelling kit (Amersham International, Inc., Buckinghamshire, England) according to the manufacturer's recommendations.

(iii) **Hybridization.** Hybridization was performed by using a previously described protocol (23, 31). The sodium salt concentration of the prehybridization and hybridization fluids was 0.1 M. The hybridization results of the dot blot procedure were determined by overnight autoradiography (Hyperfilm MP; Amersham) and scintillation counting (Packard Instruments Co., Inc., Meriden, Conn.) of standard-cut pieces of the nitrocellulose filter obtained with a self-designed vacuum cutting device.

(iv) **Quantitation of the dot blot hybridization reaction mixture.** The mean of the counts per minute for the eight parallel determinations minus the control counts per minute obtained with salmon sperm DNA for each of the strains was determined. The mean counts per minute for the autologous strain minus the mean counts per minute for salmon sperm DNA was defined as a DNA homology ratio or RBR of 100%. The mean RBR of each strain was calculated as follows: [(mean counts per minute for the strain - mean counts per minute for salmon sperm DNA)/mean counts per minute for the autologous reaction] × 100. The 95% confidence interval of the sample mean of the RBRs was estimated for each experiment (data not shown).

rRNA gene restriction analysis (ribotyping). (i) **DNA endonuclease digestion.** Total DNA (5 to 7 µg) was cleaved with 15 U of *Hind*III at 37°C for 4 h. Digestion was performed with one-half of the enzyme for 2 h, and then the other half of the enzyme was added and the preparation was incubated for an additional 2 h. *Hind*III was selected on the basis of the results of preliminary experiments in which this enzyme gave the best results in terms of complete digestion, as well as number and/or distribution of rRNA gene restriction patterns. The reaction was stopped by heating the reaction mixture at 65°C for 10 min before DNA restriction fragments were separated on a 0.8% (wt/vol) agarose (type II medium EEO; Sigma Chemical Co., St. Louis, Mo.) gel by electrophoresis in 1× TAE buffer (40 mM Tris, 1 mM EDTA [pH 8, adjusted with glacial acetic acid]) for 16 h at 35 V.

(ii) **Southern blots.** DNA fragments were transferred to a neutral nylon membrane (Appligene, Illkirch, France) with a vacuum blotter apparatus (Trans-Vac TE 80; Hoefer Scientific Instruments, San Francisco, Calif.) at 5×10^3 Pa over a period of approximately 45 min, using 20× SSC as the transfer solution. The transferred DNA fragments were immobilized on the membrane by UV cross-linking at 0.120 J/cm².

(iii) **Prehybridization and hybridization.** Prehybridization was carried out in 10 ml of prehybridization buffer (2× SSC, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, 0.1% glycine) containing 1 mg of heat-denatured salmon sperm DNA per ml at 65°C for 2 h, and this was followed by 16 h of hybridization at 65°C with 500 ng of heat-denatured acetylaminofluorene-labelled *Escherichia coli* 16 + 23S rRNA (purchased from Eurogentec, Seraing, Belgium) per ml in 10 ml of hybridization fluid (2× SSC, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% glycine, 25 mM KH₂PO₄ [pH 8.0], 2 mM EDTA, 0.5% SDS, 10% polyethylene glycol 6000, 100 µg of heat-denatured salmon sperm DNA per ml). Washing and detection of hybrids were performed as recommended by the manufacturer (Eurogentec).

(iv) **DNA fragment size determination.** The sizes of the rRNA gene restriction fragments were calculated from migration distances by using the computer program Taxotron, purchased from Institut Pasteur, Paris, France. The program also included a module for normalized schematic representation of rRNA gene restriction fragments.

Determination of the G+C content of DNA. The overall base compositions of the DNAs of the new *Moraxella* strains were determined by high-performance liquid chromatography as described by Peyret et al. (26). DNA of *M. bovis* type strain ATCC 10900 was included in the study as a control. The DNA base composition of each strain was expressed as the moles percent of (G+C) in the total bases, as determined by the following relationship: [(guanine + cytosine) × 100]/[(guanine + cytosine + adenine + thymine)].

RESULTS

Genetic transformation. The results of the genetic transformation experiments are presented in Table 2. DNA from a streptomycin-resistant mutant of strain 88365^T (T = type strain) was used as the donor DNA representing the putative new *Moraxella* species.

Quantitative and semiquantitative genetic transformation assays performed with *M. bovis* ATCC 10900^T, *M. lacunata* A947, *Moraxella atlantae* A1922, and *Moraxella (Branhamella) catarrhalis* Ne11^T as the recipients and strain 88365^T DNA as the donor DNA gave ratios of interstrain transformation to intrastrain transformation of less than 5×10^{-5} to 6.5×10^{-5} (Table 2). Similar results were obtained in semiquantitative transformation experiments in which strain 88365^T was the

TABLE 3. RBRs in DNA-DNA hybridization experiments

Filter DNA source	Mean RBR with ³² P-labelled DNA from:	
	<i>M. boevrei</i> 88365 ^T	<i>M. bovis</i> ATCC 10900 ^T
<i>Moraxella boevrei</i> sp. nov. strains		
88365 ^T	100	10
88363A	95	12
88363B	78	ND ^a
88364A	58	
88366	99	
88369	>100	
<i>M. caprae</i> strains		
NCTC 12877 ^T (= CCUG 33297 ^T)	11	42
88100	7	
88103	7	
88104	7	
88105	5	
<i>M. bovis</i> strains		
ATCC 10900 ^T	10–14 ^b	100
9	23	
ATCC 17947	17	
NCTC 11012 ^T (<i>M. equi</i>)	18	47
<i>M. lacunata</i> strains		
ATCC 17967 ^T	11	
A947	4	
50136	7	
<i>M. nonliquefaciens</i> ATCC 19975 ^T	12–23 ^b	
<i>M. osloensis</i> 5873	6	
<i>M. atlantae</i> CIP 8225 ^T	4	
<i>M. phenylpyruvica</i> CIP 8227 ^T	5	
<i>M. lincolnii</i> CCUG 9505 ^T	1	
<i>Moraxella (Branhamella) ovis</i> 199/55 ^T	14	
<i>M. (B.) catarrhalis</i> Ne11 ^T	3	ND
Other gram-negative species ^c		
<i>Kingella kingae</i>	<1	
<i>K. denitrificans</i>	<1	
<i>Neisseria elongata</i>	<1	
<i>N. weaveri</i>	<1	
<i>Oligella urethralis</i>	<1	
<i>Eikenella corrodens</i>	<1	
<i>Psychrobacter immobilis</i>	3	
<i>Haemophilus aphrophilus</i>	3	
<i>H. paraphrophilus</i>	7	
<i>Pasteurella multocida</i>	<1	
<i>P. haemolytica</i>	8	
<i>Escherichia coli</i>	<1	

^a ND, not determined.

^b Range of values from three sets of experiments.

^c See Table 1 for the designations of the strains used.

recipient. When *M. (B.) catarrhalis* Ne11^T was used as the recipient, the ratio of interspecies transformation to intrastrain transformation with strain 88365^T as the donor was significantly higher than ratio obtained with *Neisseria elongata* 6171/75 (6.5×10^{-5} , compared with 1.2×10^{-5}).

DNA-DNA hybridization. The results of the DNA-DNA hybridization experiments are shown in Table 3. DNA-DNA dot blot hybridization analysis showed that DNAs from the six new *Moraxella* strains displayed mean RBRs ranging from 58 to 100% (most values were more than 78%) when DNA from putative new *Moraxella* sp. strain 88365^T was used as the probe. DNA from strain 88365^T was chosen as a total genomic probe that was representative of the new group to test the affinity of this group to different *Moraxella* species and other fastidious gram-negative bacterial species included in the study (Table 3). The new strains were clearly distinguished from previously

FIG. 1. Normalized schematic representation of the rRNA gene restriction fragments obtained after digestion of total DNA with *Hind*III and hybridization with acetylaminofluorene-labelled 16 + 23S RNA from *E. coli*. The arrows indicate common bands found in most classical moraxellae.

described *Moraxella* species and other gram-negative bacterial species included in the study by their RBRs in hybridization experiments (Table 3). However, higher RBRs were obtained with DNAs from species belonging to the genus *Moraxella* than with DNAs from other gram-negative species; in particular, high RBRs were obtained with DNAs from strains belonging to *M. bovis*, *M. nonliquefaciens*, and *Moraxella ovis*, with which RBRs as high as 14 and 23% were obtained when DNA of strain 88365^T was used as the probe and *M. ovis* 199/55^T and *M. bovis* 9 or *M. nonliquefaciens* ATCC 19975^T DNAs were used as the filterbound DNAs, respectively.

rRNA gene restriction patterns. The results of the rRNA gene restriction pattern analysis showed that the new goat strains were similar to each other and different from all other *Moraxella* species included in the study. The *Hind*III rRNA gene restriction fragments included an approximately 3.0-kb band found in most species belonging to the classical moraxellae, including the two clinical isolates, *M. bovis* PG 500 and PG 501. This DNA fragment was not found in the recently described organism *M. caprae* (Fig. 1). An additional band at approximately 1.3 kb was sometimes found in the same species (including *M. caprae*), as well as *M. bovis*, *Moraxella cuniculi*, and *Moraxella caviae*. *Moraxella osloensis*, *M. atlantae*, and *Moraxella phenylpyruvica* each displayed a unique pattern.

DNA base composition. The results of the determination of the G+C contents of DNAs are presented in Table 2. The G+C contents of the DNAs of the new goat strains were similar, ranging from 41 to 41.5 mol%. The G+C content of the DNA of *M. bovis* ATCC 10900^T included in the study was 40 mol%.

DISCUSSION

The six new bacterial strains were assigned to the genus *Moraxella* on the basis of their Gram reaction, cellular morphology, absence of motility, positive oxidase reaction, and other reactions in a panel of conventional biochemical and hydrolytic enzymatic tests and were referred as to group 1 in

previous studies (21, 22). Because they had most of the properties of *M. bovis* except nitrate reduction, we originally considered these goat isolates members of *M. bovis*. However, results obtained from SDS-polyacrylamide gel electrophoresis of whole-cell proteins showed that the group of goat isolates was very homogeneous and produced a protein profile different from the profiles produced by typical strains of *M. bovis*, *M. lacunata*, and *M. nonliquefaciens*. Nevertheless, *M. bovis* was their closest neighbor (22). Therefore, it was important to define the genetic affinities of these isolates (formerly group 1) to all members of the genus *Moraxella*, including taxa belonging to the classical moraxellae. Genetic transformation in which the high-level streptomycin or spectinomycin resistance marker or nutritional mutants have been used as a specific tool in *Moraxella* species differentiation has been exploited extensively by Bøvre (4), Bøvre and Hagen (7), Henriksen and Bøvre (11), Juni et al. (18), and other workers (12, 32). The results of experiments in which DNA-DNA dot blot hybridization was used with total genomic and pilin-specific PCR probes, as well as the results of multilocus enzyme electrophoresis, correlate well with the results of genetic transformation analyses (29, 31). The results of the present study, in addition to the results of previous protein and qualitative genetic transformation analyses (21, 22), clearly indicate that the new isolates constitute a new cluster of strains that has affinity for the genus *Moraxella*, but is different from *M. bovis* in cattle and *M. nonliquefaciens* in humans. DNAs from the new goat strains displayed high RBRs with each other ($\geq 58\%$) but distinctly lower RBRs with DNAs of all other species investigated. However, the RBRs were somewhat higher with members of the genus *Moraxella* than with other gram-negative bacteria included in the present study. The ratio of interstrain transformation to intrastrain transformation of DNA from streptomycin-resistant strain 88365^T when *M. catarrhalis* Ne11^T was the recipient is also in the range found for the other *Moraxella* species. The base compositions of the DNAs of the new strains match the base compositions determined for the

TABLE 4. Phenotypic characteristics of *M. boevrei* and other *Moraxella* species^a

Characteristic	<i>M. boevrei</i>	<i>M. bovis</i>	<i>M. caprae</i>	<i>M. lacunata</i>	<i>M. nonlique- faciens</i>	<i>M. osloensis</i>	<i>M. lincolnii</i>	<i>M. atlantae</i>	<i>M. phenyl- pyruvica</i>	<i>M. (B.) ovis</i>	<i>M. (B.) caviae</i>	<i>M. (B.) cuniculi</i>	<i>M. canis</i>	<i>M. catar- rhalis</i>
Morphology ^b	SR	R	R	R	R	R	C, R	R	R	C	C	C	C	C
Motility	— ^c	—	—	—	—	—	—	—	—	—	—	—	—	—
Catalase activity	+	(+)	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase activity	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth on MacConkey agar	—	—	—	—	—	v	NT	+	NT	—	—	—	—	—
Acids produced from glucose	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Growth on minimal medium ^d	—	—	—	—	—	+	NT	—	—	—	—	—	(+)	—
Hemolysis	+	+ ^e	+	—	—	—	—	—	—	(+)	w	—	+	—
Nitrate reduction	+	(—)	+	+	+	(—)	—	—	(+)	+	+	—	+	(+)
Liquefaction of gelatin	+	+	—	+	—	—	—	—	—	—	—	—	—	—
DNase activity	—	—	—	—	—	—	—	—	—	(—)	(—)	—	(+)	+
Proteolysis on Löffler slants	+	+	—	+	—	—	—	—	—	—	—	—	—	—
Indole	—	—	—	—	—	—	NT	—	—	—	—	—	—	—
Phenylalanine deaminase activity	—	—	—	—	—	—	—	NT	+	—	—	—	—	—
Hydrolysis of Tween 80	+	+	+	+	—	—	—	—	NT	—	—	—	—	—
Alkaline phosphatase activity	—	—	—	+	—	+	—	+	NT	+	+	+	+	+
Esterase activity	+	+	—	+	+	+	+	+	NT	+	+	+	+	+
Acid phosphatase activity	—	w	—	w	—	+	—	+	NT	—	—	w	—	—
G+C content (mol%)	41–41.5	41–44.5	40–41.5	40–44.5	40–44	43–46	44	46.5–47.5	42.5–43.5	44.5–46.5	44.5–47.5	44.5	45.5–49.6	40–43

^a Data from this study and references 6, 7, 16, 22, 23, and 34.^b C, coccus; R, rod; SR, short rod.^c —, negative reaction; +, positive reaction; w, weak reaction; (+), most strains are positive; (—), most strains are negative; NT, not tested.^d Minimal medium containing ammonium and acetate.^e *M. equi* strains which belong to the species *M. bovis* do not exhibit hemolysis.

genus *Moraxella* (6, 7, 9, 16, 28, 34). The rRNA gene restriction patterns also indicated that the new cluster should be separated from the other *Moraxella* species investigated. The presence of at least two common bands in the species included in the classical moraxellae could be useful for *Moraxella* differentiation, particularly for distinguishing the classical moraxellae from other *Moraxella* species. However, the number of strains investigated in this study is not sufficient to create a general scheme for distinguishing the members of the genus *Moraxella*.

Based on the results presented above, we propose that the goat strains belong to a new species in the genus *Moraxella*, *Moraxella boevrei* sp. nov. The number of *Moraxella* species has increased in recent years with the proposals of several new *Moraxella* species, including *Moraxella lincolnii* (34), *Moraxella canis*, (16), and *M. caprae* (23), which shows how widespread strains of the genus *Moraxella* are in mammals. The usual keys used to identify these organisms in clinical laboratories are not sufficient for differentiating most *Moraxella* species and should therefore be combined with other methods, such as genetic transformation or rapid total DNA-DNA hybridization assays. For example, although genetic analysis reveals a clear-cut distinction between *M. bovis* and *M. boevrei*, these two species have the same features (except nitrate reduction) on the basis of the phenotypic criteria listed in Table 4.

Description of *Moraxella boevrei* sp. nov. *Moraxella boevrei* (boev're.i, L. gen. n. *boevrei*, of Bøvre, in honor of Kjell Bøvre, a Norwegian microbiologist, in recognition of his many contributions to the field of *Moraxella* and *Neisseria* identification and taxonomy [4, 7, 23, 29, 32]). Cells are gram-negative thin rods to coccus-like structures and have a tendency to occur as diplobacilli or in short chains. They are nonmotile, aerobic, and mesophilic. Colonies are grey-white, rough, and small (diameter after 48 h, 0.5 mm) and are surrounded by a wide area of hemolysis. Growth occurs in an aerobic atmosphere (preferably containing 5% CO₂) on 5% sheep or human blood agar plates or chocolate agar plates. Growth does not occur or occurs weakly on nutrient agar. The oxidase and catalase reactions are strongly positive. All strains hydrolyze Tween 80 and reduce nitrate. No acid is produced from carbohydrates. All strains are proteolytic (Loeffler slants and gelatin positive). Indole and urease reactions are negative. The only conventional characteristic that distinguishes *M. boevrei* from *M. bovis* is the reduction of nitrate. Otherwise all other conventional and hydrolytic enzymes of *M. boevrei* and *M. bovis* are exactly the same. The G+C content ranges from 41 to 41.5 mol% (Table 4). The clinical significance is uncertain. Strain 88365 (= ATCC 700022 = CCUG 35435 = NCTC 12925 = CIP 104716), which was isolated in 1988 from the nasal cavity of a healthy goat, is the type strain.

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