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V Factor-Dependent Members of the Family *Pasteurellaceae* in the Porcine Upper Respiratory Tract

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A study was performed to obtain a better understanding of the diversity and ecology of members of the family *Pasteurellaceae* in the porcine respiratory tract. A collection of 132 V factor-dependent strains of *Pasteurellaceae* selected from porcine field isolates mainly from the respiratory tract were subjected to detailed characterization. In addition to the three hitherto recognized species *Actinobacillus pleuropneumoniae*, *Haemophilus parasuis*, and *Haemophilus* taxon "minor group," three distinct taxa were observed. Some of these taxa, which are provisionally designated taxa D, E, and F, would by traditional criteria be mistaken for *H. parasuis* but differed by several biochemical characteristics. To study the ecology of the V factor-dependent species, swabs from the nasal and oral cavities of 29 pigs were cultivated on selective and nonselective media. By studying approximately 30 isolates from each sample, the distribution and relative proportion of the individual taxa were determined. *A. pleuropneumoniae* was detected in samples from the tonsil areas of only two acutely ill animals. *H. parasuis* was isolated from the nasal cavities of four out of nine healthy pigs but from the oral cavities of only two animals. In contrast, taxon "minor group" and taxa D, E, and F were present in the oral cavities of the majority of pigs but were not detected in samples from their nasal cavities. The results indicate that all the observed V factor-dependent species of *Pasteurellaceae*, except *A. pleuropneumoniae*, are members of the resident microflora of various mucosal surfaces of the porcine upper respiratory tract.

The family *Pasteurellaceae* includes both significant pathogens and members of the commensal flora of human beings and many animal species. The three genera *Haemophilus*, *Pasteurella*, and *Actinobacillus* constitute the family, but recent taxonomic data suggest that there is a need for the establishment of several new genera within the framework of *Pasteurellaceae* (4, 10).

Until recently, the genus *Haemophilus* was delineated from the rest of the family by a requirement of its members for either one or both of the two growth factors X (hemin) and V (NAD). However, on the basis of DNA:DNA hybridization data, several V factor-dependent gram-negative rods from fowl are now included in the genus *Pasteurella* (13) and the etiological agent of porcine pleuropneumonia has become *Actinobacillus (Haemophilus) pleuropneumoniae* (16).

In addition to *A. pleuropneumoniae*, porcine V factor-dependent members of *Pasteurellaceae* include *Haemophilus parasuis*, which causes Glässer's syndrome in pigs, and the provisionally named *Haemophilus* taxon "minor group" (9) of yet undefined pathogenic significance.

Several recent studies have described indole-positive *Haemophilus* isolates from the respiratory tracts of pigs (1, 6, 17), and the phenotypic heterogeneity of strains described as *H. parasuis* suggests that several additional species occur in this location. However, a systematic examination of the variety and ecology of V factor-dependent members of the family *Pasteurellaceae* in the porcine respiratory tract has never been carried out. Such information might provide a better background for the evaluation of the clinical significance of the individual species. This study was carried out with that purpose and revealed several hitherto undetected species that form part of the commensal flora of the porcine upper respiratory tract.

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MATERIALS AND METHODS

Animals. Ten clinically sick piglets from a previous specific-pathogen-free (SPF) herd with an acute outbreak of bacteriologically verified pleuropneumonia in the farrowing section were swabbed from the tonsil region. The pigs were from 10 to 20 days old. The swabs were taken by opening the mouth and swabbing in the tonsil area with a dry cotton swab. For comparison, five weaned pigs (approximately 25 kg) and five sows from a conventional herd were swabbed in the same manner. The 10 animals were clinically healthy.

Nine pigs, all from different herds, were swabbed in the nasal cavity and on four different mucosal surfaces in the oral cavity. Prior to the sample collection, the animals were anesthetized with thiopental sodium (Leopental; Løven, Copenhagen, Denmark). The pigs weighed from 40 to 50 kg and were all apparently healthy. Seven of the pigs were from conventional herds, and two were from SPF herds. All the conventional herds were known to be endemically infected with *A. pleuropneumoniae*.

Cultivation and isolation. All swabs were transported to the laboratory in Stuart's transport medium and were processed within 3 h. After suspension of the swabs in 2.0 ml of cold brain heart infusion broth (Difco Laboratories, Detroit, Mich.), 10-fold serial dilutions were performed in cold brain heart infusion broth. Samples of 0.1 ml of appropriate dilutions were plated with a Drigalski spatula on duplicate sets of blood agar and chocolate agar containing 300 mg of bacitracin per liter. After incubation for 24 h at 37°C in jars containing atmospheric air, the plates were examined. From chocolate agar plates with 80 to 150 colonies, a random area was chosen, and 30 colonies were subcultivated for purification and identification. Relative proportions of the V factor-dependent members of the family *Pasteurellaceae* to the total flora were calculated on the basis of colony counts obtained on the selective and nonselective media.

Bacterial strains. A total of 132 strains were examined.

TABLE 1. Reference and field strains examined in this study

Type of isolate and designation according to this study (*n*)	No. of strains or strain designation[a]	Name as received	Source[b]
Culture isolates			
A. pleuropneumoniae	Shope 4074^T, and 4226	*A. pleuropneumoniae*	R. Nielsen
H. parasuis	NCTC 4557^T	*H. parasuis*	NCTC
	CAPM 5751, and CAPM 6276	*H. parasuis*	CAPM
	CCM 5747	*H. parasuis*	CCM
	HA66 (1), T143 (1), T128 (2), SW114 (3), SW124 (4), and B26 (5p)	*H. parasuis*	J. Nicolet
	HS 1, 2, 3, 4, 6, 7	*H. parasuis* (serovars)	R. Nielsen
	HPS KL-1	*H. parasuis*	H. J. Riising
	HPS 84-15995		R. F. Ross
Taxon "minor group"	HK683 (202)	Taxon "minor group"	E. L. Biberstein
Taxon C	CAPM 5113, 5015, 5066, 5080	Taxon C	CAPM
Taxon F	F6, F7	Indole-positive/urease negative *Haemophilus* sp.	M. Heidt
	PIC2NP	Indole-positive/urease negative *Haemophilus* sp.	V. Rapp Gabrielson
Field isolates			
A. pleuropneumoniae (8) *H. parasuis* (2) *Haemophilus* sp. "minor group" (5) Taxon D (10)	25 strains	*Haemophilus* sp.	J. Musser
Taxon D (2) Taxon E (2)	4 strains	*Haemophilus* sp.	R. Nielsen
A. pleuropneumoniae (4) *Haemophilus* sp. "minor group" (3) Taxon D (5)	12 strains	*Haemophilus* sp.	S. Rosendal
A. pleuropneumoniae (3) *H. parasuis* (8) *Haemophilus* sp. "minor group" (14) Taxon D (5) Taxon E (8) Taxon F (25)	63 strains	*Haemophilus* sp.	This study

[a] The figures in the parentheses indicate the serovar designation of *H. parasuis* strains.

[b] E. L. Biberstein, University of California, Davis; M. Heidt, Justus Liebig-Universität, Giessen, Federal Republic of Germany; R. Nielsen, National Veterinary Laboratory, Copenhagen, Denmark; J. Musser, University of Pennsylvania, Philadelphia; J. Nicolet, University of Berne, Bern, Switzerland; H. J. Riising, Nordisk Droge og Kemikalie A/S, Copenhagen, Denmark; V. Rapp Gabrielson, North Dakota State University, Fargo; S. Rosendal, University of Guelph, Guelph, Ontario, Canada. Abbreviations: CAPM, Collection of Animal Pathogenic Microorganisms, Brno-Type Medlánky, Czechoslovakia; CCM, Czechoslovak Collection of Cultures, Brno, Czechoslovakia; NCTC, National Collection of Type Cultures, Colindale, London, United Kingdom.

Sixty-three of these were selected from more than 500 field isolates examined and represented the taxa defined on the basis of the initial biochemical characterization. For the detailed characterization, the 63 strains were supplemented with appropriate type and reference strains and with strains from several other laboratories. The sources and designations of the strains are shown in Table 1.

Characterization of isolates. The methods used for the biochemical and cultural examinations were those used previously (8) with some minor modifications and additions. The requirement for V factor was determined by observing the satellite phenomenon on autoclaved 10% horse blood agar plates cross-streaked with a strain of *Staphylococcus aureus* and was confirmed by placing small paper disks moistened with a 10 mg/liter NAD solution on brain heart infusion agar plates (Difco) inoculated with the test strain. The requirement for X factor was determined by the porphyrin test (7).

Hemolysis and the CAMP phenomenon were examined on 10% (vol/vol) bovine blood agar. The strains were streaked at right angles to a streak of a β-toxigenic *S. aureus* strain. The plates were read after 24 and 48 h.

Hydrogen sulfide production was determined by placing a lead acetate strip (Merck, Darmstadt, Federal Republic of Germany) in the lid of an inoculated chocolate agar plate and by reading it after a 2-day incubation. Distinct blackening of the paper was recorded as a positive result.

Reduction of nitrate and nitrite was tested after 5 days of growth in brain heart infusion broth containing 10 mg of NAD per liter and 0.1% (wt/vol) KNO_3, as described by Cowan and Steel (3).

Production of acid from carbohydrates was determined as previously described (8), except that hemin was excluded from the medium. The tubes were inoculated with 2 drops of a 24-h Levinthal broth culture. Uninoculated tubes served as controls. The reaction was read after incubation for 5 days,

TABLE 2. Biochemical characteristics of V factor-dependent species of the family *Pasteurellaceae* isolated from pigs

Biochemical characteristic[a]	% of strains positive for characteristics						
	A. pleuro-pneumoniae (*n* = 17)	*Haemophilus* taxon "minor group" (*n* = 23)	*H. parasuis* (*n* = 28)	Taxon D (*n* = 22)	Taxon E (*n* = 10)	Taxon F (*n* =28)	Taxon C (*n* = 4)
V factor requirement	100	100	100	100	60	100	100
X factor requirement	0	0	0	0	0	0	0
Indole	0	0	0	0	0	100	0
Urease	100	100	0	0	0	0	0
Lysine decarboxylase	0	0	0	0	0	0	0
Ornithine decarboxylase	0	0	0	0	0	0	0
Arginine dehydrolase	0	0	0	0	0	0	0
Hemolysis	77	0	0	0	0	0	0
CAMP reaction	100	0	0	0	0	0	0
Catalase	12	13	100	0	0	100	100
Oxydase	71	78	53	59	60	82	100
β-Galactosidase (ONPG)	100	100	100	100	80	100	0
β-Glucuronidase (PGUA)	0	0	0	0	0	0	0
α-Fucosidase	0	0	96	68	80	46	0
α-Glucosidase (PNPG)	0	70	4	64	20	89	100
Alkaline phosphatase	100	100	100	91	60	100	100
Neuraminidase	0	0	86	68	60	0	0
Nitrate reduction	100	100	100	96	30	100	100
Nitrite reduction	100	48	0	0	0	0	100
H_2S	94	100	82	50	30	100	100
CO_2-improved growth	0	0	0	0	0	0	0
Fermentation of:							
L-(+)-Arabinose	0	0	0	86	0	0	100
D-(−)-Ribose	100	9	93	82	0	71	100
D-(+)-Xylose	88	30	0	50	0	25	0
D-(+)-Galactose	100	87	100	96	0	100	100
D-(+)-Glucose acid	100	100	100	100	30	100	100
Glucose gas	0	0	0	0	0	0	0
D-(+)-Mannose	100	100	100	100	10	100	100
Lactose	6	91	0	96	0	32	0
Maltose	100	100	100	100	20	100	100
D-(+)-Melibiose	0	78	0	96	0	18	0
Sucrose	100	100	100	100	10	100	100
Trehalose	0	78	0	55	0	79	0
D-(+)-Raffinose	0	100	0	100	0	93	100
Inulin	0	0	0	0	0	0	0
Soluble starch	0	83	0	86	0	61	0
Esculin	0	0	0	0	0	0	0
Salicin	0	4	0	0	0	0	0
D-(−)-Mannitol	100	0	0	100	0	0	0
D-(−)-Sorbitol	0	0	0	100	0	0	0
Inositol	0	0	4	77	0	0	100

[a] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside; PGUA, *p*-nitrophenyl-β-D-glucopyranosiduronic acid; PNPG, *p*-nitrophenyl-β-D-glucopyranoside.

and the pH was measured in tubes not showing distinct red or yellow color. Fermentation cultures with a pH drop of one pH unit or more relative to the uninoculated control were recorded as positive.

Selected glycosidases, alkaline phosphatase, and amino acid decarboxylases were demonstrated as described previously (8).

Production of neuraminidase was determined by using 4-methylumbelliferyl-α-D-*N*-acetylneuraminate as a substrate at a concentration of 0.1 mM in 0.1 M sodium cacodylate buffer (pH 6.4) (20). One loopful of bacteria from a chocolate agar culture was suspended in 0.2 ml of the substrate solution, and the preparation was incubated for 24 h at 37°C. After centrifugation, 0.1 ml of the supernatant was mixed with 1 ml of 0.1 M sodium bicarbonate buffer (pH 9.1). Release of the fluorescent product was demonstrated with a fluorescence spectrophotometer (excitation, 365 nm; emission, 450 nm).

RESULTS

Description of the individual taxa. On the basis of the results of the detailed characterization, the 63 selected strains were assigned to six taxa defined by distinct biochemical activity profiles. Three of the taxa were identical with *A. pleuropneumoniae*, *H. parasuis*, and *Haemophilus* taxon "minor group" and contained the appropriate type strains and other strains received under these labels (Table 1). Their characteristics are listed in Table 2. None of the strains assigned to *A. pleuropneumoniae* were independent of the V factor.

A fourth taxon contained four strains from the Collection of Animal Pathogenic Microorganisms (Brno-Medlánky, Czechoslovakia) and has previously been designated taxon C (8). Two of these strains are stated to have been isolated from pigs with pneumonia, and two were isolated from

TABLE 3. Detection rate and relative proportion of V factor-dependent species of *Pasturellaceae* on the tonsillar surfaces of pigs

Herd (no. in herd)[a]	No. of pigs harboring (median, range)[b]:				
	A. pleuro-pneumoniae	*H. parasuis*	Taxon "minor group"	Taxa D and E[c]	Taxon F
With outbreak (10)	2 (0, 0–47)	0	7 (2.7, 0–40)	5 (6.5, 0–51)	1 (0, 0–10)
Without outbeak (10)	0	0	8 (2.3, 0–20)	9 (0.9, 0–90)	3 (0, 0–2)

[a] Herds examined were conventional herds, one with an acute outbreak of pleuropneumonia and one without.
[b] Median and range are of percentage of total flora determined on blood agar.
[c] The identification tests used for the ecological studies did not allow separation of taxa D and E.

cocks. None of our field isolates and no strain received from other laboratories could be assigned to taxon C.

The remaining taxa, provisionally designated taxa D, E, and F, differed in several important ways from the recognized species. Taxa D and E were both urease and indole negative and nonhemolytic. Although these two taxa thus resembled *H. parasuis*, they differed from this species by several biochemical activities (Table 2).

The 22 strains assigned to taxon D grew on chocolate agar as colonies indistinguishable from those produced by strains of *H. parasuis*. They were smooth, greyish, and translucent and usually reached a diameter of 0.5 to 1 mm after incubation for 48 h. However, occasional strains showed a more feeble growth that resembled the strains assigned to taxon E. In Gram-stained smears, taxon D and *H. parasuis* showed thin, pleomorphic rods of various lengths, and taxon D often showed filamentous forms. In addition, taxon D differed from *H. parasuis* by a negative catalase reaction and by the ability to ferment several carbohydrates not fermented by *H. parasuis*, notably, mannitol, sorbitol, lactose, melibiose, and raffinose.

Growth of taxon E strains on chocolate agar was very feeble even after 48 to 72 h of incubation. The colonies were translucent and reached a diameter of about 0.2 mm. All strains showed symbiotic growth around a staphylococcus on blood agar. The requirement for NAD was confirmed for 6 of the 10 strains by showing growth only around a NAD disk on brain heart infusion agar. However, the remaining four strains did not grow at all on brain heart infusion agar, even when it was supplemented with NAD-containing disks. The poor growth of taxon E strains was also evident in fermentation and other test media and may be responsible for the negligible biochemical activities shown by most of the 10 strains. Only three strains showed detectable fermentation of glucose and reduction of nitrate. Addition of 10% horse serum to the media did not change the results. Most of the strains were positive in tests for β-galactosidase and α-fucosidase, tests which do not depend on growth of the inoculum. In addition to its poor growth and lack of strong fermentation activities, taxon E differed from *H. parasuis* by its negative catalase activity. All strains of *H. parasuis* showed strong catalase activity (Table 2). In Gram-staining smears, taxon E showed thin pleomorphic rods of various lengths with filamentous forms.

Taxon F consisted of 28 strains, including 3 strains received from a German and a North American laboratory (Table 1). They differed from all other strains of the collection by showing a positive indole reaction, which also gave agar plate cultures a characteristic pungent smell. All strains grew abundantly on chocolate agar to greyish opaque colonies reaching a diameter of 1.5 to 2 mm. They were catalase positive, although the reaction was sluggish, and differed from recognized species and the other taxa by several biochemical characteristics (Table 2).

The majority of strains of *H. parasuis* and taxa D and E showed neuraminidase activity, in contrast to strains assigned to the remaining taxa (Table 2).

Ecology of the individual taxa. To study the ecology of the demonstrated taxa, pigs from conventional and SPF herds as well as pigs from herds with acute outbreaks of pleuropneumonia were examined.

During an acute outbreak of pleuropneumonia in a Danish pig herd, 10 pigs were swabbed in the tonsil region. The samples were suspended, and the suspensions were serially diluted 10-fold and inoculated on selective and nonselective agar plates. After incubation, 30 colonies from each sample were isolated and identified and the proportions of the individual taxa were calculated. For comparison, 10 healthy animals from a conventional herd were examined in the same way. The results are shown in Table 3. In spite of the acute outbreak of *A. pleuropneumoniae* infection, the species could be detected only in the tonsillar regions of 2 of the 10 animals examined. None of the healthy animals harbored *A. pleuropneumoniae*. *H. parasuis* was not detected in any of the 20 animals, whereas taxon "minor group" and taxa D and E were present in most animals, often amounting to a considerable proportion of the flora (Table 3).

The primary habitats of the individual taxa were further studied by examining nine pigs from seven different conventional herds and two SPF herds. Samples were taken from the nose, the tonsils, and various surfaces of the oral cavity. The results, based on the examination of a mean of 30 isolates from each surface, are shown in Table 4. None of the nine pigs harbored *A. pleuropneumoniae* on the sampled surfaces. The nasal cavities of four of the nine animals, including one of the two pigs from SPF herds, harbored detectable proportions of *H. parasuis*, which otherwise was found only in the mouths of two animals. Taxon "minor group" and taxa D, E, and F were found in the tonsil regions of the majority of pigs and usually on the buccal mucosa and on the tongue. The proportions of the flora made up by the individual species are shown in Table 4.

DISCUSSION

Among the three genera *Haemophilus*, *Actinobacillus*, and *Pasteurella*, which constitute the family *Pasteurellaceae*, the genus *Haemophilus* has traditionally been defined by a requirement of its members for one or both of the two growth factors X and V. However, DNA:DNA hybridization studies published over the last 7 years have revealed that V factor dependency is not an exclusive generic feature of *Haemophilus* but may be a feature of members of all three genera of the family *Pasteurellaceae*. Thus, the former V factor-dependent "*Haemophilus avium*" has been subdivided into three species, now all members of *Pasteurella*: *Pasteurella avium*, *Pasteurella volantium*, and the yet unnamed *Pasteurella* species A (13). Likewise, the former

TABLE 4. Detection rate and relative proportion of V factor-dependent species of *Pasturellaceae* on various mucosal surfaces in nine healthy pigs

Taxa	No. of pigs harboring strain in following area (median, range)[a]:				
	Nasal cavity	Tonsil surface	Buccal mucosa	Floor of mouth[b]	Tongue dorsum
A. pleuropneumoniae	0	0	0	0	0
H. parasuis	4 (0, 0–68)	0	0	1 (0, 0–3)	1 (0, 0–4)
Taxon "minor group"	0	8 (3.9, 0–20)	9 (4.3, 0.1–17)	4 (0, 0–11)	7 (0.5, 0–12)
Taxa D and E	0	7 (1.1, 0–34)	4 (0, 0–4)	0	3 (0, 0–1)
Taxon F	0	6 (0.1, 0–4)	7 (0.9, 0–4)	5 (0, 0–10)	6 (0.7, 0–12)

[a] Total number of pigs was 9. Median and range are of percentage of total flora determined on blood agar.
[b] The area under the tongue.

"*Haemophilus pleuropneumoniae*" has been transferred to the genus *Actinobacillus* as *A. pleuropneumoniae*. The species includes both V factor-dependent (biovar 1) and V factor-independent (biovar 2) members (16), although biovar 2 still appears to require V factor-related compounds (14). These examples illustrate the present difficulties in determining the correct generic affiliation of old and new members of *Pasteurellaceae* taxa on the basis of phenotypic characteristics.

The problem is further intensified by the recognized need for several new genera within the family *Pasteurellaceae* (12). As an example, recent DNA:DNA homology data suggest that *H. parasuis* does not belong in any of the present genera (4; S. Burbach, Ph.D. thesis, University of Marburg, Marburg, Federal Republic of Germany).

Our study primarily focused on V factor-dependent members of *Pasteurellaceae* in the upper respiratory tract of pigs, but during the initial screening of field isolates special attention was paid to similar bacteria lacking this requirement. However, no strains with the characteristics of *A. pleuropneumoniae* biovar 2 were detected during the study. Biovar 2 strains have been repeatedly isolated since 1975 as the etiologic agents of enzootic porcine pleuropneumonia in Switzerland (16) but so far have not been detected in other countries.

A. pleuropneumoniae biovar 1 was not isolated from any of the healthy animals. However, it is remarkable that the use of a selective medium allowed its detection on the tonsil surfaces of only 2 of 10 pigs during an acute outbreak of pleuropneumonia. This finding is in accordance with previous observations (5, 19, 21) and emphasizes the need to identify the primary habitat of the organism in the upper respiratory tract during the initial colonization.

Our study demonstrated that the porcine upper respiratory tract harbors a wider spectrum of V factor-dependent *Pasteurellaceae* species than hitherto recognized. In addition to *A. pleuropneumoniae*, *H. parasuis*, and the yet unnamed species taxon "minor group," three distinct taxa were observed among our field strains and among strains received from other laboratories. The three taxa provisionally designated taxa D, E, and F differed distinctly from the recognized species by several biochemical properties (Table 2).

Of particular interest is taxon D. By the criteria used in most routine microbiological laboratories, strains assigned to this taxon would have been identified as *H. parasuis*. However, taxon D strains differed from *H. parasuis* in that they fermented several carbohydrates that are not attacked by *H. parasuis* and had a negative catalase reaction (Table 2). It is likely that some of the *H. parasuis* strains described by Heidt and Weiss (6) and O'Reilly and coworkers (15) as being catalase negative and mannitol positive represent strains of taxon D.

The fact that taxon D was regularly present on the tonsil surfaces of the healthy pigs examined (Tables 3 and 4) indicates that this species is a member of the resident microflora of the porcine upper respiratory tract. In contrast, *H. parasuis* was never isolated from the tonsillar region of the 29 pigs examined (Tables 3 and 4). However, four of nine apparently healthy animals examined harbored *H. parasuis* in the nasal cavity, and in one case the species constituted 68% of the microflora (Table 4).

It has previously been demonstrated that strains of *H. parasuis* may vary in their pathogenecity (11). In addition to a recognition of this fact, it is likely that a clear differentiation of taxon D from *H. parasuis* in future studies will provide additional insight into the pathogenic potential of *H. parasuis*.

Likewise, this study indicates that taxon "minor group" is a member of the resident flora of the porcine respiratory tract. It constituted up to 20% of the microflora in healthy animals and was present both on the tonsils and on buccal and tongue mucosae (Tables 3 and 4). This is compatible with the previous finding that taxon "minor group" readily colonizes but is unable to induce clinical disease in gnotobiotic piglets (2), although it may induce focal necrotic and fibrotic pulmonary lesions when inoculated into the lower airways of SPF pigs (18). This emphasizes the importance of distinguishing it from *A. pleuropneumoniae*, which has significant pathogenic potential.

Indole-positive V factor-dependent bacteria have been previously isolated from the respiratory tracts of pigs (1, 6, 17). Representative isolates from these laboratories were included in this study and were identical to the cluster provisionally labeled taxon F. Taxon F was distinct from recognized species and other taxa described in this study and undoubtedly warrants specific recognition. The isolation frequency in healthy pigs (Table 4) suggests that taxon F constitutes a part of the resident microflora of the porcine upper respiratory tract.

The 10 strains assigned to taxon E differed from the other taxa by showing very poor growth both on agar plates and in biochemical test media. Growth was not stimulated by the addition of horse serum to the media. Although the strains appear to be different from the other taxa, their feeble growth does not allow a definitive evaluation of their biochemical characteristics and relationships to other taxa.

Because of the unstable taxonomic situation within the family *Pasteurellaceae*, it is premature to make any suggestions as to the correct genus affiliation of the discovered taxa. Although traditionally they would have belonged in the genus *Haemophilus*, this is very unlikely to be their future home. Thus, taxon D resembles *Pasteurella* species A in several ways, notably by its V factor requirement and L-arabinose, trehalose, mannitol, and sucrose fermentation,

but differs by the ability to ferment lactose, sorbitol, and inositol. *Pasteurella* species A has been isolated from chickens and pigeons (13). Comprehensive nucleic acid homology studies are required to establish the final taxonomic relationship of these and other members of the family *Pasteurellaceae*.

This study demonstrates that the porcine upper respiratory tract harbors several V factor-dependent species of the family *Pasteurellaceae* that are easily mistaken for some of the pathogenic species. An evaluation of the possible significance of the taxa D, E, and F as opportunistic pathogens has to await further investigations, including isolations from lungs and inoculation experiments.

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