Pasteurella avium (Hinz and Kunjara 1977) comb. nov. and *Pasteurella volantium* sp. nov.

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A deoxyribonucleic acid-deoxyribonucleic acid hybridization study (spectrophotometric method) showed that the species *Haemophilus avium* Hinz and Kunjara 1977 is composed of three deoxyribonucleic acid homology groups; these three groups are genetically closer to *Pasteurella multocida*, the type species of the genus *Pasteurella*, than to *Haemophilus influenzae*, the type species of the genus *Haemophilus*. We propose to transfer the species *Haemophilus avium* to the genus *Pasteurella* as *Pasteurella avium* comb. nov. (type strain, ATCC 29546) and to change the circumscription of this taxon to include only strains that are unable to ferment L-arabinose, maltose, and mannitol and unable to hydrolyze *o*-nitrophenyl- β -D-galactopyranoside. The name *Pasteurella volantium* sp. nov. (type strain, NCTC 3438) is proposed for strains (formerly identified as *H. avium*) that are unable to ferment L-arabinose and are able to ferment maltose and mannitol and to hydrolyze *o*-nitrophenyl- β -D-galactopyranoside. A third unnamed species consists of former *H. avium* strains that are able to ferment L-arabinose.

Hinz and Kunjara (6) classified V-factor (nicotinamide adenine dinucleotide [NAD])-dependent avian haemophili which were different from *Haemophilus paragallinarum* as *Haemophilus avium*. These organisms can be isolated from chickens and other fowl (4). Unlike *H. paragallinarum*, *H. avium* is not involved in the pathogenesis of a specific disease of chickens, coryza infectiosa gallinarum. Most strains of *H. avium* appear to be harmless mucosal commensal organisms which are spread via direct contact or airborne transmission (5), but with some of the L-arabinose-fermenting strains marked serofibrinous infraorbital sinusitis and edema of the face and, in some cases, of the wattles have been produced in chickens after inoculation into the submucosa of the infraorbital sinus (Hinz, unpublished data). The phenotypic diversity among a series of *H. avium* strains studied motivated us to determine their genetic interrelationships and their taxonomic positions in the family *Pasteurellaceae* Pohl 1981 (11, 16). Our results indicated that the species *H. avium* is genetically heterogeneous and can be separated into three groups which are phenotypically distinguishable. We propose to transfer the former species *H. avium* to the genus *Pasteurella* and to split it into the following three species: *Pasteurella avium* sensu stricto, *Pasteurella volantium* sp. nov., and a third unnamed species.

MATERIALS AND METHODS

Microorganisms. The bacterial strains investigated are listed in Table 1. They were propagated on chocolate agar (tryptic soy agar [Difco Laboratories, Detroit, Mich.] supplemented with 5% [vol/vol] defibrinated sheep blood and heated to 80°C for 15 min before pouring), purified by subculturing typical single colonies, and stored in the lyophilized state until needed.

Phenotypic characterization. Both the identification methods of Kilian and Frederiksen (8) and those of Mannheim et al. (12) were used. *Acinetobacter* sp. strain HIH ST661/60 served to supply V-factor, as described by Mannheim (9). The media used for testing the metabolism of selected amino

acids were supplemented with NAD to a final concentration of 1 μ g/ml.

Preparation of DNA. Deoxyribonucleic acid (DNA) was prepared by the method of De Ley et al. (2) from cells of the early stationary phase of growth, which were harvested from aerated cultures grown at 35°C.

DNA base composition and molecular weight of genome DNA. Thermal denaturation midpoints were used to determine the guanine-plus-cytosine (G+C) contents of the DNAs (2). *Escherichia coli* B (NCTC 10537) DNA served as a reference (G+C content, 52 mol%). The molecular weights of genome DNAs were calculated from initial renaturation rates by the method of Gillis et al. (3).

DNA-DNA hybridization. Initial renaturation rates for homologous and heterologous reassociations were determined by the method of De Ley et al. (2), as described previously (13, 15, 17).

RESULTS AND DISCUSSION

Phenotypic features. The 19 strains of *H. avium* studied fit the description of the family *Pasteurellaceae* Pohl 1981 (11). They were gram negative, nonmotile, and coccoid to rod shaped and contained demethylmenaquinones (always) and ubiquinones (usually) but no menaquinones (10). They were nonhemolytic, showed no colonial adherence, and formed smooth colonies 2.5 mm in diameter after 24 h of growth on chocolate agar. No growth occurred on MacConkey agar or in Simmons citrate medium. All strains except the ornithine decarboxylase-negative type of Bisgaard taxon 13 (E. B. Madsen, M. Bisgaard, R. Muttters, and K. B. Pedersen, Can. J. Comp. Med., in press), represented by strain K117, required V-factor. Nitrates were reduced to nitrites. Prompt positive reactions were observed for *N,N,N',N'*-tetramethyl-1,4-phenylenediamine hydrochloride oxidase, catalase, alkaline phosphatase, and acid production without gas from D-glucose, D-galactose, D-mannose, D-fructose, sucrose, and trehalose. Negative results were obtained for indole production, hydrolysis of urea and arginine, decarboxylation of lysine, liquefaction of gelatin, and acid production from L-sorbose, L-rhamnose, starch, salicin, esculin, adonitol, dulcitol, and meso-inositol.

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As shown in Table 2, the *H. avium* strains studied and some other strains could be separated phenotypically into three groups on the basis of their different capabilities for catabolizing L-arabinose, maltose, and mannitol and by the *o*-nitrophenyl- β -D-galactopyranoside test. All of the reference strains used in this study could be separated from these three groups by using an appropriate set of biochemical reactions, as described previously (12, 14).

Base compositions and molecular weights of genome DNAs. As shown in Table 1, no significant differences in the G+C contents and the genome sizes of the DNAs of the three groups of *H. avium* were found; these values ranged from 43 to 47 mol% and from 1.5×10^9 to 2.1×10^9 daltons, respectively.

DNA reassociation. On the basis of DNA binding values (Tables 3 through 5), the strains of *H. avium* studied could

be divided into three groups, which were identical to those mentioned above and should receive the rank of species as compared with the genetic relatedness in well-established *Pasteurellaceae* species.

Our experience with the renaturation method of DNA hybridization has shown that well-defined species of the *Pasteurellaceae* exhibit high degrees of DNA homology (i.e., at or above 85% binding) (13, 17). Therefore, discrimination of closely related species may be impaired by the error in the renaturation method and may require taxometric evaluation of the data. Ultimately, the recognition of species remains a matter of convention.

The strains representing the first *H. avium* group proved to be highly related mutually and exhibited DNA binding values of about 80% with two *Pasteurella* strains that are as yet unclassified on the species level (strains NCTC 11623

TABLE 1. Bacterial strains used in this study

Strain designation in this study ^a	Received as:	Other strain no. ^a	Isolated from:	Country of isolation ^b	G+C content (mol%) ^c	Genome mol wt ($\times 10^9$) ^d
NCTC 4189 ^T	<i>A. lignieresii</i>	L. Thompson 1	Cattle	USA	44.2	1.8
NCTC 5906 ^T	<i>H. aphrophilus</i>	O. Khairat 320	Human	USA	44.5	1.5
ATCC 29546 ^T	<i>H. avium</i>	K.-H. Hinz IPDH 2654	Chicken	USA	44.7	1.9
HIM 789-1	<i>H. avium</i>	K.-H. Hinz IPDH 1762	Chicken	FRG	45.9	2.1
HIM 789-2	<i>H. avium</i>	K.-H. Hinz IPDH 94	Chicken	FRG	44.0	1.8
HIM 789-3	<i>H. avium</i>	K.-H. Hinz IPDH 0003	Chicken	FRG	44.7	1.8
HIM 789-4	<i>H. avium</i>	K.-H. Hinz IPDH 0002	Chicken	FRG	44.3	1.9
HIM 789-5	<i>H. avium</i>	K.-H. Hinz IPDH 280	Chicken	FRG	45.4	1.7
HIM 789-6	<i>H. avium</i>	K.-H. Hinz IPDH 306	Chicken	FRG	44.2	1.7
HIM 859-1	<i>H. avium</i>	K.-H. Hinz IPDH 219-74	Turkey	FRG		
HIM 841-3	<i>H. avium</i>	K.-H. Hinz IPDH 207	Chicken	USA		
HIM 841-1	<i>H. avium</i>	K.-H. Hinz IPDH 167-79	Peacock	FRG		
HIM 859-3	<i>H. avium</i>	K.-H. Hinz IPDH 780,	Chicken	FRG		
HIM 859-7	<i>H. avium</i>	K.-H. Hinz IPDH 331-76	Chicken	FRG		
HIM 860-2	<i>H. avium</i>	K.-H. Hinz IPDH 712-76	Chicken	FRG		
HIM 838-1	<i>H. avium</i>	K.-H. Hinz IPDH 1254-70	Chicken	FRG		
HIM 859-4	<i>H. avium</i>	K.-H. Hinz IPDH 1103-77	Chicken	FRG		
HIM 838-4	<i>H. avium</i>	K.-H. Hinz IPDH 504-79	Pigeon	FRG		
NCTC 3438	" <i>Haemophilus gallinarum</i> "	R. Lovell 6	Fowl	UK	43.8	1.5
NCTC 8143 ^T	<i>H. influenzae</i>	A. B. Rosher 680	Human	USA	38.8	1.2
ATCC 29545 ^T	<i>H. paragallinarum</i>	K.-H. Hinz IPDH 2403	Chicken	FRG	42.3	1.7
NCTC 7857 ^T	<i>H. parainfluenzae</i>	A. B. Rosher	Human	UK	39.5	1.4
HIM 793-8	<i>H. parainfluenzae</i> biotype 1	M. Kilian HK128	Human	Denmark	41.7	1.4
CCM 5869 ^T	<i>Actinobacillus pleuropneumoniae</i>	R. E. Shope 4074	Swine		43.2	1.5
HIM 734-5	<i>Haemophilus somnus</i>	L. Corboz 719	Bovine	Switzerland	37.5	1.4
NCTC 4101	<i>Haemophilus</i> sp.	A. Fleming 1	Human	UK	44.6	1.9
HIM 716-7	<i>Haemophilus</i> sp.	K.-H. Hinz IPDH 2176	Kestrel	FRG	43.8	1.5
HIM 716-4	<i>Haemophilus</i> sp.	K.-H. Hinz IPDH 195	Budgerigar	FRG	38.1	1.7
HIM 700-8	<i>Haemophilus</i> sp.	K.-H. Hinz IPDH 312	Pigeon	FRG	37.0	1.7
ATCC 13361 ^T	<i>P. gallinarum</i>	K. L. Heddleston USDA P-913	Chicken	USA	43.6	1.6
ATCC 13360	<i>P. gallinarum</i>	K. L. Heddleston USDA P-892	Chicken	USA	43.7	1.6
NCTC 10322 ^T	<i>P. multocida</i>	G. R. Carter W-9217	Bovine	Canada	41.6	1.6
SSI P614	<i>P. multocida</i>	S. Namioka TS 8	Chicken	Vietnam	40.9	1.6
NCTC 11623	<i>Pasteurella</i> sp.	J. E. Smith 166	Dog	UK	42.2	1.5
NCTC 11624	<i>Pasteurella</i> sp.	W. Mannheim PE 4	Cat	FRG	40.4	1.5
HIM 844-2	<i>Pasteurella</i> sp.	M. Bisgaard K117, taxon 13	Calf	Denmark	42.8	1.9
NCTC 11411	<i>Pasteurella</i> sp.	M. Bisgaard F73, taxon 4	Chicken	Denmark	43.9	1.7
HIM 745-4	<i>Pasteurella</i> sp.	M. Bisgaard F279, taxon 1	Duck	Denmark	42.3	1.8

^a ATCC, American Type Culture Collection, Rockville, Md.; CCM, Czechoslovak Collection of Microorganisms, Brno, Czechoslovakia; HIM, Zentrum für Hygiene und Medizinische Mikrobiologie, Marburg, Federal Republic of Germany; IPDH, Institute for Poultry Disease, Hannover, Federal Republic of Germany; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England; SSI, Statens Seruminstitut, Copenhagen, Denmark.

^b USA, United States of America; FRG, Federal Republic of Germany; UK, United Kingdom.

^c Average of nine assays.

^d Average of 6 to 12 assays.

TABLE 2. Biochemical characteristics that distinguish the three species considered

Strain ^a	Test results ^b								
	Acid produced from:						ONPG hydrolyzed ^c	Ornithine decarboxylated	NAD required
	L-Arabinose	Maltose	Mannitol	Sorbitol	D-Xylose	Lactose			
<i>P. avium</i> sensu stricto									
ATCC 29546 ^T	—	—	—	—	—	—	—	—	+
HIM 789-4	—	—	—	—	+	—	—	—	+
HIM 844-2	—	—	—	—	+	—	—	—	—
<i>P. volantium</i> sp. nov.									
HIM 789-3	—	+	+	+	+	w	+	—	+
HIM 859-1 ^d	—	+	+	+	—	w	+	—	+
NCTC 3438 ^T	—	+	+	—	+	—	+	—	+
HIM 841-3 ^d	—	+	+	—	+	w	+	—	+
HIM 841-1 ^d	—	+	+	—	+	w	+	—	+
HIM 859-3 ^d	—	+	+	—	+	w	+	—	+
HIM 859-7 ^d	—	+	+	—	—	—	+	—	+
HIM 860-2 ^d	—	+	+	—	—	—	+	—	+
NCTC 4101	—	+	+	+	—	—	+	+	+
HIM 838-1 ^d	—	+	+	+	—	—	+	+	+
<i>Pasteurella</i> species A									
HIM 789-5	+	—	+	—	—	—	—	—	+
HIM 859-4 ^d	+	—	+	—	—	—	—	—	+
HIM 789-2	+	—	+	—	+	w	+	—	+
HIM 838-4 ^d	+	+	—	—	+	—	—	—	+
HIM 789-1	(+)	+	—	—	—	—	+	—	+
Other <i>Pasteurella</i> spp.									
HIM 789-6	+	+	—	—	—	—	+	—	+
NCTC 11623	—	—	—	—	—	—	—	—	—
NCTC 11624	—	—	—	—	—	—	—	—	—

^a Species assignments based on DNA-DNA hybridization results.^b +, Positive; (+), positive within 48 to 72 h; w, only weak reaction observed; —, negative.^c ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.^d Strain not tested for DNA relatedness.

and NCTC 11624) (Table 3). These strains were related to the genus *Pasteurella* via *Pasteurella multocida* biotype 6, with DNA binding levels of approximately 55%, and via the *Pasteurella pneumotropica* type Henriksen-*Pasteurella*

TABLE 3. Levels of DNA relatedness of *P. avium* strain ATCC 29546^T with selected members of the *Pasteurellaceae*

Species	Strain	% Homology with strain ATCC 29546 ^T (unless otherwise stated)
<i>P. avium</i>	ATCC 29546 ^T	100
<i>P. avium</i>	HIM 789-4	100 (2.9) ^a
<i>P. avium</i>	HIM 844-2	88 (1.7)
<i>Pasteurella</i> sp.	NCTC 11624	78 (7.9)
<i>Pasteurella</i> sp.	NCTC 11623	74 (5.5) ^b
<i>P. gallinarum</i>	ATCC 13360	61 (1.2)
<i>P. volantium</i>	NCTC 3438	60 (3.0)
<i>P. gallinarum</i>	ATCC 13361 ^T	52 (6.2)
<i>Pasteurella</i> species A	HIM 789-1	38 (4.0)
<i>Pasteurella</i> species A	HIM 789-5	36 (7.6)
<i>Pasteurella</i> species A	HIM 789-2	35 (4.0) ^c
<i>Haemophilus</i> sp.	HIM 700-8	31 (1.5)
<i>P. multocida</i>	NCTC 10322 ^T	22 (8.4)
<i>Pasteurella</i> sp.	HIM 789-6	20 (2.3)
<i>H. influenzae</i>	NCTC 8143 ^T	9 (2.8)
<i>Haemophilus</i> sp.	HIM 716-7	9 (6.7)
<i>Haemophilus</i> sp.	HIM 716-4	7 (2.5)
<i>A. lignieresii</i>	NCTC 4189 ^T	7 (4.2)
<i>Pasteurella</i> sp.	NCTC 11411	2 (3.1)

^a The values in parentheses are standard deviations.^b Tested with *P. avium* strain HIM 789-4 instead of strain ATCC 29546^T. When *P. avium* strain HIM 844-2 was tested instead of strain HIM 789-4, the level of DNA homology was 81% (standard deviation, 4.2%).^c Tested with *P. avium* strain HIM 789-4 instead of strain ATCC 29546^T.

“gas” group, with binding levels of 59 and 57% (Mutters, unpublished data), which is related to *P. multocida* at the 60% DNA homology level (Mutters, unpublished data). Furthermore, this group is linked to the genus *Pasteurella* via *Pasteurella volantium* (see below). A direct comparison of *H. avium* ATCC 29546^T (T = type strain) and *P. multocida* NCTC 10322^T yielded only 22% DNA binding, the level of DNA binding with the type species of *Haemophilus* (*Haemophilus influenzae* NCTC 8143^T) was 9%, and the level of DNA binding with the type species of *Actinobacillus* (*Actinobacillus lignieresii* NCTC 4189^T) was 7% (i.e., below the reliability of the renaturation method) (2). On the basis of these DNA relationships, the group containing the type strain of *H. avium* clearly belongs to the genus *Pasteurella* and must be reclassified as *Pasteurella avium*. A determination of the relationships of *Pasteurella* sp. strains NCTC 11623 and NCTC 11624 to *P. avium* will require more detailed investigations with more strains. Phenotypically, these strains can be separated from *P. avium* only by their ability to produce indole and their somewhat lower G+C contents (40 to 43 mol%).

The second group studied, for which we propose the name *Pasteurella volantium*, proved to be related to *P. avium* at the 60% DNA binding level. On the other hand, it was linked to *Pasteurella gallinarum* by DNA binding values of about 70% and was linked by DNA relatedness values of 57 and 49% to another new *Pasteurella* species from chickens, namely, Bisgaard taxon 4 (1), represented by strain NCTC 11411 (Table 4). A DNA binding level of 59% was determined with *P. multocida* NCTC 10322^T. DNA binding values below 40% have been observed for *P. volantium* with *Haemophilus aegyptius* ATCC 11116^T, *Haemophilus aphrophilus* NCTC 5886^T, *Haemophilus haemoglobin-*

philus NCTC 1659^T, *Haemophilus influenzae* NCTC 4560, *Haemophilus paragallinarum* ATCC 29545^T, *Haemophilus parahaemolyticus* ATCC 10014^T, *Haemophilus parainfluenzae* NCTC 7857^T, and ATCC 7901, *Haemophilus parasuis* NCTC 4557^T, *Actinobacillus seminis* ATCC 15768^T, *Actinobacillus* sp. strain ATCC 27072, *Pasteurella haemolytica* NCTC 9380^T and NCTC 11610, *Pasteurella pneumotropica* type Jawetz strain NCTC 8284, *Pasteurella pneumotropica* type Heyl strain ATCC 12555, *Pasteurella ureae* NCTC 10219^T, *Pasteurella* sp. ("Pasteurella bettii") strain NCTC 10535, and *Pasteurella* sp. strain NCTC 10699 (S. Pohl, Ph.D. thesis, Philipps-Universität, Federal Republic of Germany, Marburg, 1979; S. Pohl, unpublished data).

The third group of former *H. avium* strains consists of L-arabinose-fermenting strains. This group was shown to be remote from both of the other groups, with DNA binding values of less than 40%, but was also included in the genus *Pasteurella* on the basis of DNA relatedness values with *P. gallinarum* ranging from 63 to 67% (Table 5). We suggest that this species remain unnamed until its exact taxonomic position within the genus *Pasteurella* can be determined by studying more strains.

Our data contain strong evidence that the three groups of *H. avium* should be classified as distinct species of the genus *Pasteurella*.

The incorporation of V-factor-dependent strains in the genus *Pasteurella*, which was first suggested by Pohl (15), is not surprising since close genetic relationships between V-factor-dependent and V-factor-independent taxa have been described for the *Actinobacillus lignieresii*-*Haemophilus pleuropneumoniae* group (15, 17) and for the *Haemophilus aphrophilus*-*Haemophilus paraphrophilus* group (15). These observations indicate that V-factor dependency is not

TABLE 5. Levels of DNA relatedness of *Pasteurella* species A strain HIM 789-5 with selected members of the *Pasteurellaceae*

Species	Strain	% Homology with strain HIM 789-5 (unless otherwise stated)
<i>Pasteurella</i> species A	HIM 789-5	100
<i>Pasteurella</i> species A	HIM 789-2	91 (7.0) ^a
<i>Pasteurella</i> species A	HIM 789-1	83 (4.4)
<i>Pasteurella</i> sp.	HIM 789-6	66 (7.8) ^b
<i>P. gallinarum</i>	ATCC 13361 ^T	63 (5.2) ^c
<i>H. paragallinarum</i>	ATCC 29545 ^T	36 (7.6)
<i>Pasteurella</i> sp.	NCTC 11411	26 (1.4) ^d
<i>Pasteurella</i> sp.	NCTC 11623	24 (9.1)
<i>P. multocida</i>	NCTC 10322 ^T	19 (11.7)
<i>P. multocida</i>	SSI P614	3 (0.7)
<i>Haemophilus</i> sp.	HIM 716-7	1 (3.5)
<i>H. parainfluenzae</i>	HIM 793-8	0 (1.4)
<i>Haemophilus somnus</i>	HIM 734-5	0 (4.2)
<i>Haemophilus</i> sp.	HIM 716-4	0 (6.4)
<i>Haemophilus</i> sp.	HIM 700-8	0 (8.0)
<i>H. aphrophilus</i>	NCTC 5906 ^T	0 (7.1)
<i>Actinobacillus pleuropneumoniae</i>	CCM 5869 ^T	0 (7.8)
<i>H. influenzae</i>	NCTC 8143 ^T	0 (11.5)

^a The values in parentheses are standard deviations.

^b When *Pasteurella* species A strain HIM 789-1 was tested instead of strain HIM 789-5, the level of DNA homology was 58% (standard deviation, 7.8%).

^c When *Pasteurella* species A strain HIM 789-1 was tested instead of strain HIM 789-5, the level of DNA homology was 67% (standard deviation, 6.6%); when *Pasteurella* species A strain HIM 789-2 was used, the level of DNA homology was 63% (standard deviation, 9.5%).

^d Tested with *Pasteurella* species A strain HIM 789-1 instead of strain HIM 789-5. When *Pasteurella* species A strain HIM 789-2 was tested instead of strain HIM 789-1, the level of DNA homology was 18% (standard deviation, 4.7%).

TABLE 4. Levels of DNA relatedness of *P. volantium* strain NCTC 3438^T with selected members of the *Pasteurellaceae*

Species	Strain	% Homology with strain NCTC 3438 ^T (unless otherwise stated)
<i>P. volantium</i>	NCTC 3438 ^T	100
<i>P. volantium</i>	HIM 789-3	90 (5.7) ^a
<i>P. volantium</i>	NCTC 4101	81 (7.4) ^b
<i>P. gallinarum</i>	ATCC 13361 ^T	72 (7.9)
<i>P. gallinarum</i>	ATCC 13360	70 (8.1)
<i>P. avium</i>	ATCC 29546 ^T	60 (3.0) ^c
<i>P. multocida</i>	NCTC 10322 ^T	59 (4.9)
<i>Pasteurella</i> sp.	NCTC 11411	49 (5.2) ^d
<i>Pasteurella</i> sp.	NCTC 11624	42 (11.4)
<i>Pasteurella</i> species A	HIM 789-5	35 (10.6) ^e
<i>Pasteurella</i> species A	HIM 789-1	35 (7.8)
<i>H. paragallinarum</i>	ATCC 29545 ^T	35 (16.8)
<i>Pasteurella</i> sp.	HIM 745-4	33 (5.8)
<i>Pasteurella</i> sp.	HIM 789-6	28 (8.4) ^f
<i>H. parainfluenzae</i>	NCTC 7857 ^T	18 (3.6) ^g

^a The values in parentheses are standard deviations.

^b When *Pasteurella volantium* strain HIM 789-3 was tested instead of strain NCTC 3438^T, the level of DNA homology was 88% (standard deviation, 3.1%).

^c When *P. volantium* strain HIM 789-3 was tested instead of strain NCTC 3438^T, the level of DNA homology was 44% (standard deviation, 9.3%).

^d When *P. volantium* strain HIM 789-3 was tested instead of strain NCTC 3438^T, the level of DNA homology was 57% (standard deviation, 4.6%).

^e When *P. volantium* strain HIM 789-3 was tested instead of strain NCTC 3438^T, the level of DNA homology was 35% (standard deviation, 4.7%).

^f Tested with *P. volantium* strain HIM 789-3 instead of strain NCTC 3438^T.

^g Tested with *P. volantium* strain NCTC 4101 instead of strain NCTC 3438^T.

a generic feature of *Haemophilus* but is a common feature among various members of the family *Pasteurellaceae*. Considering the foregoing, two new *Pasteurella* species are proposed, as described below.

***Pasteurella avium* (Hinz and Kunjara) comb. nov.** *P. avium* contains V-factor-dependent strains hitherto classified as *Haemophilus avium* and V-factor-independent strains classified as the ornithine decarboxylase-negative type of Bisgaard taxon 13. The cells are gram-negative, coccoid, nonsporeforming rods which occur singly or in short chains. They are mesophilic, chemoorganotrophic, facultatively aerobic, and fermentative. Ubiquinones and demethylmenaquinones are produced. Oxidase, catalase, and alkaline phosphatase reactions are positive. Nitrates are reduced to nitrites. No growth occurs on MacConkey agar and in Simmons citrate medium. The surface colonies on chocolate agar are smooth, convex, and grayish white. On sheep blood agar supplemented with NAD no hemolysis occurs. Acid is produced from D-glucose without gas formation and from D-galactose, D-mannose, D-fructose, sucrose, and trehalose. No acid is produced from L-sorbose, L-arabinose, L-rhamnose, starch, raffinose, maltose, mannitol, sorbitol, m-inositol, adonitol, or dulcitol. Some strains produce acid from D-xylose. Negative reactions are obtained for indole production, hydrolysis of urea and arginine, decarboxylation of lysine and ornithine, and liquefaction of gelatin. Found in the hearts and infraorbital sinuses of chickens and in the lungs of calves suffering from pneumonia. The G+C content of the DNA is 43 to 45 mol%. The genome molecular weight is 1.9×10^9 . Strain IPDH 2654 (= ATCC 29546) is the type strain.

Description of the type strain. The type strain has all of the characteristics given above for the species. It does not

produce acid from D-xylose and was isolated from a chicken (6).

Pasteurella volantium sp. nov. *Pasteurella volantium* (vo.lan'ti.um. L. fem. gen. pl. n. *volantium* from fowl) cells are gram-negative, immotile, coccoid, nonsporeforming rods which occur singly or in short chains. They are mesophilic, chemoorganotrophic, facultatively aerobic, fermentative, and V-factor dependent. Oxidase, catalase, and alkaline phosphatase reactions are positive. Ubiquinones and demethylmenaquinones are produced. Nitrates are reduced to nitrites. No growth occurs on MacConkey agar or on Simmons citrate medium. On chocolate agar the surface colonies are smooth and convex and may produce yellowish pigments. On sheep blood agar supplemented with NAD no hemolysis occurs. The phenotypic features of *P. volantium* are similar to those of *P. avium*, but *P. volantium* differs in being maltose and mannitol positive and often sorbitol positive. Ornithine is decarboxylated by some strains. The G+C content is 44 to 45 mol%. The genome molecular weight is 1.5×10^9 to 1.9×10^9 . Found in the wattles of domestic fowl. One strain has been isolated from a human tongue (7). The type strain is strain Lovell 6 (= NCTC 3438 = ATCC 14385).

Description of the type strain. The type strain has all of the characteristics of the species. In addition, it produces acid from D-xylose but not from sorbitol or lactose. Ornithine is not decarboxylated. This strain was isolated from a chicken.

Although a third group of former *H. avium* strains was included in the genus *Pasteurella* on the basis of DNA homology, it appeared to be heterogeneous (Table 2) and should be described when more strains have been studied. We suggest strain IPDH 280 (HIM 789-5) as a reference strain for this species, provisionally named *Pasteurella* species A.

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