"Haemophilus felis": a potential pathogen for cats?

E Olsson and E Falsen

Letter to the Editor

"Haemophilus felis": a Potential Pathogen for Cats?

Inzana et al. (3) recently reported the isolation of a new Haemophilus species from a cat with chronic pulmonary disease. This and other, similar isolates were tentatively named "Haemophilus felis." According to the authors, the isolation of "H. felis" from 6 of 28 apparently normal cats indicated that "H. felis" or "H. felis"-like organisms may be relatively common inhabitants of the feline upper respiratory tract.

It is well known that many Haemophilus species, although found on the mucous membranes of healthy individuals, are capable of causing disease in animals and humans (1). It was therefore surprising to find that the authors did not even discuss whether this newly identified Haemophilus species is a potential pathogen.

The article alerted us to reinvestigate bacterial isolates obtained earlier from five young cats with respiratory tract infections and conjunctivitis. The strains had been isolated on blood agar incubated in CO2-supplemented atmosphere on which they formed satellite colonies close to a streak with a Staphylococcus species. The isolates had been preliminarily named Haemophilus species because it was impossible to identify them as any known Haemophilus species by conventional biochemical tests.

After the recognition of "H. felis," we decided to compare the cellular fatty acid methyl ester (FAME) profiles from these five strains with those of two of the reference strains of Inzana et al. (3): ATCC 49733T (CCUG 31170T) and ATCC 49734 (CCUG 31171). The FAME profiles of all seven strains were extremely similar. Dodecanoic acid which could be detected in only trace amounts in Haemophilus, Actinobacillus, and Pasteurella species was detected in "H. felis" at 9 to 12%. Less tetradecanoic and cis-delta 9-hexadecenoic acid and more cis-delta 9,12-octadecadienoic, cis-delta 9-octadecenoic, and octadecanoic acids were found in "H. felis" than in species belonging to the present Haemophilus-Pasteurella-Actinobacillus complex. Cluster analysis by the Ecorola formula (2) was quite clear-cut.

We also tested the new API NH (bioMérieux) system. All strains of "H. felis" displayed the pattern - + + + + - + + + - (profile 7170), and we found no other species with similar reactions in our data base. The API lipase was found positive; this positivity was a property shared with some Moraxella species and Kingella orale only.

Our five strains had all been isolated from cats with pronounced clinical signs; CCUG 30833, CCUG 30834, and CCUG 31243 were isolated from nose samples from cats with rhinitis, CCUG 30832 was from the eye of a cat with conjunctivitis, and CCUG 30831 was from the pharynx of a cat with rhinitis and conjunctivitis. In the three nose samples, the Haemophilus species had been isolated in pure culture; in the two other samples, the growth of Haemophilus species was abundant, although not pure. The finding of Haemophilus species was therefore in all cases judged as a significant bacterial finding.

It is obvious that further research in this field is needed in order to establish the clinical significance and phylogenetic position of "H. felis." However, on the basis of our findings, we suggest that (i) our five isolates are identical to the proposed species "H. felis" and (ii) "H. felis" is a potential pathogen causing respiratory tract infections and conjunctivitis especially in young cats.

REFERENCES


Author's Reply

In their letter, Olsson and Falsen report five isolates from respiratory tract infections and conjunctivitis in cats that they concluded were identical to "H. felis." They correctly suggest that further research is needed to establish the clinical significance and genus classification of "H. felis" but question why we did not discuss its pathogenic potential.

We agree that "H. felis" is capable of causing opportunistic infections and thank Drs. Olsson and Falsen for sharing their findings. It would be useful to know whether the five cats described had any other compromising respiratory problems, such as viral infections, stress, or a physical complication. We also agree that additional research needs to be done to determine the virulence potential of "H. felis" and the predisposing conditions necessary for it to cause infection.

In the case we reported (2), the cat clearly had a predisposing condition that resulted in mucus accumulation in the respiratory tract. This cat also had multiple previous infections with other agents. Therefore, we could not confirm whether this was a true opportunistic infection or colonization of a respiratory tract previously damaged and lacking normal defense mechanisms. Although we isolated the same or similar bacterium from a number of other cats, all of these isolates appeared as part of the normal flora, and no infection was evident. Therefore, we lacked strong evidence that "H. felis" was a potential pathogen. Our main objective in reporting this case was to raise awareness as to the existence of this agent in cats to investigators and clinical microbiologists.

In regard to the phylogenetic classification of this agent, it was tentatively assigned to the genus Haemophilus as "H. felis" because of its growth dependence on V factor (by tests with NAD). Such dependence fits the traditional definition of the genus Haemophilus (3). We stressed that this is a preliminary
affiliation, as several recent studies have revealed that (i) the growth requirement for V factor is not an exclusive characteristic of members of the genus Haemophilus (5) and (ii) there is a need for radical reorganization of the family Pasteurellaceae, which probably will include the establishment of several new genera (1, 4). The final affiliation of “H. felis,” which will most probably belong to a new genus, can only be determined after that process has been completed.

REFERENCES


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Isolation and Characterization of a Newly Identified *Haemophilus* Species from Cats: "*Haemophilus felis*"

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Received 20 February 1992/Accepted 29 April 1992

A gram-negative coccobacillus was isolated from the lower respiratory tract of a cat with chronic obstructive pulmonary disease. The isolate required CO2 and V factor for growth and was initially identified as *Haemophilus paraphrophilus* on the basis of its nutritional requirements, colony morphology, and some biochemical tests. Because of the host specificity of *Haemophilus* species and discrepancies in catalase, oxidase, and hemolytic activities, additional testing was done. Extensive biochemical testing, G+C content, and DNA reassociation studies indicated that the organism was distinct from other *Haemophilus* species. Therefore, the organism was identified as a previously unrecognized *Haemophilus* species and was tentatively named "*Haemophilus felis*." Bacteria identical to the original isolate were isolated from the nasopharynxes of 6 of 28 apparently normal cats, indicating that *H. felis* or *H. felis*-like organisms may be common members of the feline upper respiratory tract flora.

*Haemophilus* species are common commensal organisms of the mucosal membranes of humans and animals (1, 8). While most species may cause opportunistic infections only in compromised or stressed hosts, some species like *H. influenzae* type b in humans and *H. parasuis* in swine cause more serious diseases. Most animals and birds are known to carry at least one species of *Haemophilus* (1). *Haemophilus* infections in cats, however, are rare, and no particular *Haemophilus* species has been associated with cats. In this report, we describe the isolation and characterization of a previously unrecognized *Haemophilus* species that we have tentatively named "*H. felis*." The organism was originally isolated from a cat with chronic pulmonary disease. In addition, similar or identical bacteria were isolated from the nasopharynxes of 6 of 28 apparently normal cats.

CASE REPORT

A 6-year-old, spayed, domestic short-haired cat was admitted to the Virginia-Maryland Regional College of Veterinary Medicine Teaching Hospital because of a 4-year duration of raucous breathing described by the owner as audible "gurgling." Sneezing, coughing, and nasal discharge were absent. The raucous breathing was unresponsive to chlorpheniramine and various antibiotics (amoxicillin, chloramphenicol, erythromycin, cefadroxil, and cephalaxin) administered sporadically over the past 4 years. The cat was housed indoors with another healthy cat. Routine vaccinations were current, and the cat was negative for feline leukemia virus.

On examination, increased airway noise was characterized by fluidlike sounds that were most prominent over the nasal passages and trachea. Chest radiographs showed a left collapsed cranial lung lobe causing a mediastinal shift to the left. The bronchial structures in the right lung were markedly dilated, with some calcification of the bronchial wall. Under general anesthesia, the right nasal cavity had an increased density not associated with bony destruction. When the cat was extubated, a thick, yellow exudate was observed in the endotracheal tube. Cytological examination of this material indicated the presence of nondegenerate and degenerate neutrophils, macrophages, small lymphocytes, and plasma cells. A small number of bacterial rods and coccobacilli were also present. The animal was administered tetracycline for 1 month, during which time clinical symptoms resolved. Cultures could not be obtained after treatment, however, because the animal failed to return home one evening shortly after treatment.

MATERIALS AND METHODS

Bacterial strains, cultures, and media. The reference strains *H. paraphrophilus* ATCC 29241, *H. parasuis* ATCC 19417, *H. haemoglobinophilus* ATCC 19416, and *H. paracuniculus* ATCC 29986 were obtained from the American Type Culture Collection, Rockville, Md. *H. influenzae* type b strain EAg was originally obtained from Porter Anderson, University of Rochester Medical Center, Rochester, N.Y. Strains of taxon D have been described previously (7).

Strains of *H. paraphrophilus*, *H. parasuis*, *H. paracuniculus*, and the isolates from the cat were subcultured onto brain heart infusion agar or broth supplemented with 5 μg of NAD per ml. *H. influenzae* was subcultured onto the same medium to which 10 μg of solubilized hemin per ml was added. *H. haemoglobinophilus* was subcultured onto blood agar or brain heart infusion broth supplemented with 10 μg of solubilized hemin per ml. Clinical specimens were cultured on enriched chocolate agar (GC base, hemoglobin, and Gonococcus-*Haemophilus* influenzae [GCHI] supplement), blood agar, and MacConkey agar (Remel, Richmond, Va.). Nasopharyngeal cultures were obtained from 28 clinically normal cats housed at the Veterinary Microbiology Research Laboratories animal facilities. Specimens from these cats were cultured on enriched chocolate agar and enriched

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chocolate agar supplemented with 300 μg of bacitracin (Remel) per ml. Colonies morphologically similar to the isolate from the cat were subcultured onto brain heart infusion agar (Difco, Detroit, Mich.) containing 5 μg of NAD per ml or onto blood agar and were incubated in 7% CO₂ or air, respectively, at 35°C.

Biochemical studies. The requirement for V factor was determined by satellite growth around a *Staphylococcus* strain on blood agar (7), growth around commercial disks impregnated with NAD (Remel) on brain heart infusion agar, and growth on brain heart infusion agar with and without an NAD supplement in the medium. The requirement for X factor was determined by the porphyrin test (6) and by growth around commercial disks supplemented with X factor (Remel) on brain heart infusion agar. Oxidase activity was determined by the spot method with 1% tetramethyl-p-phenylenediamine dihydrochloride (Becton Dickinson and Co., Cockeysville, Md.). Reduction of nitrite and nitrate (2, 12) and production of neuraminidase (12, 16) were determined as described previously. Sugar fermentation, hydrogen sulfide production, hemolytic and CAMP activities, and production of selected enzymes were determined as described previously (7, 12). The MicroScan *Haemophilus-Neisseria* Identification Panel (HNID; American Microscan, Sacramento, Calif.) was used according to the manufacturer's instructions. Biochemical data on each *Haemophilus* species used for comparison with "*H. felis*" have been reported previously (1, 7, 9, 12).

DNA isolation and mole percent G+C content. Bacteria were grown with vigorous shaking to the mid-log phase in 500 ml of supplemented brain heart infusion broth. The bacteria were washed three times in 0.1 M phosphate-buffered saline (pH 7.4), and DNA was isolated from all species at the same time by using the isolation procedure described by Marmur (10). The final DNA preparations were dissolved in 1× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate [pH 7.0]) diluted 1:10 (0.1× SSC) and were stored at -20°C.

The mole percent G+C content of the DNA preparations was estimated from the thermal melting profile by using *Escherichia coli* DNA as a standard (5, 11).

Determination of DNA similarity values. For the reassociation of DNA samples, each DNA preparation was diluted to 0.4 mg/ml, fragmented by passage (three times) through a French pressure cell at 16,000 lb/in², denatured by heating in a boiling water bath for 5 min, and after cooling, centrifuged at 12,000 x g for 15 min to remove particulate debris. Labeled DNA was prepared by iodination of 3- to 5-μg amounts of the fragment-denatured DNA preparations (15). The specific activities of the labeled preparations ranged from 1 × 10⁵ to 3 × 10⁶ cpm/μg. The reassociation vials each contained 10 μl (0.01 to 0.03 μg) of labeled DNA, 50 μl (30 μg) of unlabeled DNA, and 50 μl of 13.2× SSC (containing 1 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid] [pH 7.0]). The vials were incubated for 3 h at 65°C. The DNA samples were then heated to 95°C for 3 min in a boiling water bath. The samples were then allowed to cool to room temperature before being centrifuged. The samples were then centrifuged at 12,000 x g for 15 min to remove particulate debris. The DNA samples were then reassociation by using the S1 nuclease procedure (5). The S1 nuclease-resistant duplexes were acid precipitated and collected on Whatman G/FF glass fiber filters (Whatman, Hillsboro, Ore.), and the radioactivity was measured with a gamma scintillation counter. The DNA from each species was radiolabeled and cross-hybridized with DNA from each of the other species. Four replicates of each hybridization were tested, and all replicate values were within 1%.

Identification of *H. felis* from cats. The reassociation with homologous DNA was normalized to 100%.

Antibiotic susceptibility studies. The in vitro antibiotic susceptibilities of each *Haemophilus* isolate obtained from cats was done by disk diffusion on *Haemophilus* Test Medium (HTM), as described by the National Committee for Clinical Laboratory Standards (13). The bacteria were suspended in unsupplemented Mueller-Hinton broth to a density equivalent to that of a 0.5 McFarland standard, and swabbed onto HTM; the disks were applied; and the inverted plates were incubated for 18 h at 35°C in 7% CO₂. Interpretation of inhibitory zone diameters was determined as recommended previously (3, 13).

RESULTS

The respiratory exudate of the cat described in the case report was cultured onto MacConkey agar in air and on blood agar and enriched chocolate agar in 7% CO₂ at 35°C. After 24 h, one quadrant of growth of small (0.5- to 1.0-mm), raised, rounded colonies was present only on chocolate agar and in pure culture; the colonies remained small for up to 48 h. Growth was absent on blood and MacConkey agars at 72 h postinoculation. The organism failed to grow when it was initially subcultured onto chocolate agar in air. After several subcultures on chocolate agar in CO₂, however, it no longer required CO₂ for growth. Growth occurred on brain heart infusion agar in the presence of a disk containing V factor but not around a disk containing X factor. The requirement for V factor was confirmed by growth of the bacterium on brain heart infusion agar supplemented with NAD (growth was absent on the same medium without NAD) and by satellite growth around a streak of *Staphylococcus* species on blood agar. After 3 days the test colonies were weakly hemolytic on the blood agar. On supplemented brain heart infusion agar, the colonies were raised, round, 0.5 to 1.0 mm in diameter, very adherent, and difficult to remove from the plate; they had a yellow pigment when they were collected on a loop. Initial tests indicated that the organism was catalase positive, reduced nitrate, and was urease and indole negative. Oxidase activity by the spot method was negative within 10 s. All sugar fermentations in unsupplemented CTA medium (Remel) were negative. The MicroScan HNID identified the organism as *H. felis*. The MicroScan HNID and American Type Culture Collection type strains of *H. paraphrophilus* and *H. parasuis* were tested in the system, the isolate from the cat had a profile identical to that of *H. parasuis* 677000, which is not in the HNID data base. The discrepancy was due to two biochemicals, *p*-nitrophenyl-α-D-glucosidase and *N*-γ-L-glutamyl-α-naphthylamide, both of which were positive for the *H. paraphrophilus* strain but negative for *H. parasuis* and the isolate from the cat. In addition, *H. parasuis* is catalase positive and oxidase negative, whereas *H. paraphrophilus* is catalase negative and oxidase positive. The G+C ratio was 37% for *H. parasuis*, 40% for *H. paraphrophilus*, and 38% for the isolate from the cat.

Since *H. paraphrophilus* is a human commensal organism and has not been associated with infections in animals, the isolate was sent to the Meningitis and Special Pathogens Section at the Centers for Disease Control in Atlanta, Ga. The Centers for Disease Control reported the isolate as *H. paraphrophilus* on the basis of characteristics similar to those of reference strains. Because of the discrepancy in catalase and oxidase activities between *H. paraphrophilus* and the isolate from the cat, however, additional biochemi-
TABLE 1. Biochemical characteristics of “H. felis” and other Haemophilus species

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>“H. felis”</th>
<th>Hpa</th>
<th>Hpe</th>
<th>Taxon D</th>
<th>Hic</th>
<th>Hhg</th>
<th>Hpc</th>
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<td>+</td>
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<td>CO2 required</td>
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<td>+</td>
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<tr>
<td>Colony morphology (on chocolate agar)</td>
<td>Y</td>
<td>Y</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>T</td>
<td>G</td>
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<tr>
<td>Catalase</td>
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<td>Oxidase</td>
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<td>V</td>
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<td>Indole</td>
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<td>V</td>
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<td>±e</td>
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<td>β-Galactosidase (ONPG)</td>
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<td>+</td>
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<td>+</td>
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<td>V</td>
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<td>V</td>
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<td>V</td>
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<td>H2S (lead acetate paper)</td>
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<td>V</td>
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<td>V</td>
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<td>-</td>
<td>+</td>
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<td>-</td>
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<td>D(+)-Xylose</td>
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<td>-</td>
<td>V</td>
<td>+</td>
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</table>

a All species were positive for nitrate reduction and acid from glucose and maltose. All species were negative for acid from esculin, inulin, and salicin.

b Abbreviations: Hpa, H. paraphrophilus; Hpe, H. parasuis; Hi, H. influenzae; Hhg, H. haemoglobinophilus; Hpc, H. paracunicular; d, delayed; V, 11 to 89% of strains are positive; ND, not determined; Y, yellow; G, gray; T, translucent; A, adherent; S, smooth.

c Data for these strains were obtained from references 1, 7, and 9.

d Biovar IV is variable.

Positive, negative, and variable reactions occur depending on biovar; see reference 9.

f ONPG, o-nitrophenyl-β-D-galactopyranoside.

g PNPG, p-nitrophenyl-β-D-glucoside.

h The pH of the uninoculated medium was 7.6, and the pH was 6.54 after incubation.

cal tests specifically for Haemophilus species were done in our laboratories (6, 9, 12). Biochemical results for H. paraphrophilus, H. parasuis, taxon D, H. haemoglobinophilus, H. paracunicular, H. influenzae, and the isolate from the cat are presented in Table 1. The biochemical profile of the isolate from the cat was unique and distinct from those of all other Haemophilus species (9). The isolate from the cat was also resistant to bacitracin in chocolate agar (300 μg/ml).

The average DNA similarity of labeled DNA from the cat isolate ranged from 6% with H. paracunicular DNA to 17% with H. paraphrophilus and H. haemoglobinophilus DNA (Table 2). However, the reassociation similarity of labeled DNA from H. haemoglobinophilus with unlabeled DNA from the isolate was only 9%, suggesting that some homologous contaminating DNA may have been present in the unlabeled H. haemoglobinophilus sample. Some cross-contamination of unlabeled DNA from the isolate from the cat and H. influenzae may also have occurred with labeled DNA from H. paraphrophilus because of the higher reassociation similarity that occurred compared with that which occurred with the labeled DNA (32 and 17% for the isolate from the cat and 35 and 9% for H. influenzae with labeled and unlabeled DNA from H. paraphrophilus, respectively). Nonetheless, on the basis of the DNA reassociation similarity, colonial morphology, and biochemical profiles, we concluded that the isolate from the cat was a distinct species in the genus Haemophilus and have tentatively given it the name “Haemophilus felis.” The type strain is TI189, which has been deposited in the American Type Culture Collection (ATCC 49733). Two isolates from normal cats, TI 7B and TI 14B, have also been deposited and have been designated ATCC 49734 and ATCC 49735, respectively.

An Haemophilus species from cats has not been reported previously. Therefore, samples from the nasopharynxes of 28 apparently normal cats housed at the Veterinary Microbiology Research Laboratories were cultured to determine whether the bacterium is common to the feline upper respiratory tract. Eleven isolates that required NAD and had colony morphologies identical to that of the original isolate were obtained from six cats. Six isolates were isolated from six cats on chocolate agar with bacitracin, and five isolates were obtained from five of the same cats on chocolate agar.
without bacitracin; all plates were incubated in CO₂. In addition to colony morphology, all of the isolates were identical to the original isolate on the basis of their catalase, oxidase, indole, nitrate, and urease test results. Two of the isolates were lost during subculture, and seven of the isolates were identical to the original isolate in their ability to ferment all carbohydrates tested (arabinose, galactose, glucose, inositol, lactose, maltose, mannitol, raffinose, ribose, salicin, sorbitol, and sucrose). In contrast to the type strain, two isolates were weakly positive for raffinose and one isolate was weakly positive for salicin, as determined by pH measurement, but they were otherwise identical to the type strain. Because the cats from which these bacteria were isolated were involved in another noninfectious disease-related project, we were not able to do more extensive examinations to determine whether any underlying respiratory pathology was present.

Disk diffusion susceptibility testing of the primary isolate obtained from the case cat and each of the isolates obtained from the normal cats was done on HTM. The original isolate was susceptible to amoxicillin-clavulanate, ceftriaxone, cefuroxime, chloramphenicol, ciprofloxacin, imipenem, tetra-cycline, and trimethoprim-sulfamethoxazole and was resistant to ampicillin. The other antibiotics approved for testing with HTM were not available (3, 13). Of the nine additional isolates obtained from healthy cats, one was resistant to ampicillin and ampicillin-clavulanate and intermediate to cefuroxime, one was resistant to ampicillin and ampicillin-clavulanate, and two were intermediate to ampicillin; these isolates were susceptible to all other drugs tested. The remaining isolates were susceptible to all drugs tested. The antibiotic susceptibility profiles of isolates obtained from the same cats were identical.

**DISCUSSION**

Chronic bronchitis in cats can be triggered by many causes, such as immotile cilia syndrome, bronchiectasis, feline asthma, and viral or parasitic infection. The resulting inflammation may cause mucosal hyperemia and edema, proliferation of bronchial glands and goblet cells, and increased secretions. In the case of the cat described in this report, chronic accumulation of mucus in the respiratory tract (similar to human cystic fibrosis) was apparently the predisposing condition causing obstructive pulmonary disease. Secondary bacterial colonization and infection can exacerbate the clinical signs and prevent normal recovery by inducing inflammation. The isolate from this cat was obtained from the specimen in pure culture. An Haemophilus species from cats has not previously been recognized as a pathogen, and therefore, the isolate described here probably colonized the respiratory tract of an already compromised animal. The isolate was initially identified as H. paraphrophilus, but H. paraphrophilus is a commensal organism of the mouths and throats of healthy humans (4) and has not previously been recovered from cats or any other animal species. Because the organism was strongly catalase positive, oxidase negative, and biochemically more similar to H. parasuis, extensive biochemical and DNA testing was done.

Besides catalase, other predominant features that distinguished the isolate from the cat from H. paraphrophilus was a delayed hemolytic reaction on sheep blood agar in the presence of Staphylococcus species; the lack of gas from glucose (although H. paraphrophilus strains were variable for gas production); failure to ferment melibiose, trehalose, and ribose; and fermentation of mannitol. In addition, the acid produced by the isolate from the cat in fermentation tests was relatively weak, reducing the pH of the medium (e.g., mannitol) from 7.6 to only 6.5, which is similar to the degree of acidification produced by H. parasuis (7). In contrast, the acid produced by H. paraphrophilus carboxylate fermentation reduces the pH of the medium to 5.4 or less (7). The isolate differed from H. parasuis in colony morphology, CO₂ requirement, α-fucosidase activity, and mannitol and ribose fermentation. However, some isolates tentatively identified as H. parasuis from pigs have demonstrated improved growth in the presence of CO₂ (7). The isolate was substantially different from taxon D in many of the biochemicals. The G+C content of 38% for the isolate from the cat was similar to the G+C content of 37% for our reference strain of H. parasuis and the mean 38.7% G+C content reported for the Haemophilus type species H. influenzae (7, 14). The G+C content of 40% for our reference strain of H. paraphrophilus was very similar to the average G+C content of 41.9% for five H. paraphrophilus strains reported previously (7).

DNA reassociation data between the isolate from the cat and five other Haemophilus species confirmed that the isolate is a distinct species. However, DNA reassociation was greatest between the isolate from the cat and H. paraphrophilus, supporting the similarity in colonial morphology, supplemental CO₂ requirement, and some biochemical tests. The next closest association was with H. parasuis, which also shared some biochemical similarities with the isolate from the cat. Therefore, the isolate was tentatively assigned to the genus Haemophilus as “H. felis.” Differentiation of “H. felis” from other Haemophilus species is most easily determined by host specificity, colony

### TABLE 2. Percent DNA reassociation similarity between “H. felis” and other *Haemophilus* species

<table>
<thead>
<tr>
<th>Unlabeled DNA source</th>
<th>Percent reassociation with labeled DNA*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>1. <em>H. paracocculitus</em> ATCC 29986</td>
<td>100</td>
</tr>
<tr>
<td>2. <em>H. parasuis</em> ATCC 19417</td>
<td>20</td>
</tr>
<tr>
<td>3. <em>H. paraphrophilus</em> ATCC 29241</td>
<td>8</td>
</tr>
<tr>
<td>4. “<em>H. felis</em>” TI189</td>
<td>9</td>
</tr>
<tr>
<td>5. <em>H. haemoglobinophilus</em> ATCC 19416</td>
<td>7</td>
</tr>
<tr>
<td>6. <em>H. influenzae</em> type b, strain EAG</td>
<td>7</td>
</tr>
</tbody>
</table>

* Percent reassociation were determined with iodinated DNA from each species, and cross-hybridization with DNA from each species was done in triplicate by the S1 nuclease procedure (5). The numbers above each column refer to the numbers of the DNA sources given in the left column.

* Percent reassociation with homologous DNA was normalized to 100%.

The proportionately higher reassociation with this DNA may have been due to minor contamination with homologous DNA, because all samples were prepared at the same time.
morphology, catalase and oxidase activities, and mannitol and ribose fermentations.

Culture samples from the nasopharynxes of 28 apparently normal cats resulted in the isolation of bacteria that required NAD and that were morphologically identical to the original isolate from six cats. For nine isolates that survived, all biochemical tests were identical to those for the original isolate from the cat except for weak raffinose fermentation by two isolates and weak salicin fermentation by one isolate, as determined by pH measurement. Therefore, "H. felis" or "H. felis"-like organisms may be relatively common inhabitants of the feline upper respiratory tract, but it has only now been identified because of its isolation in pure culture from a cat with chronic respiratory disease.

Antibiotic susceptibility testing indicated that resistance to ampicillin can occur, but the organism would be susceptible to most cephalosporins, chloramphenicol, imipenem, tetracycline, and trimethoprim-sulfamethoxazole.

ACKNOWLEDGMENTS

We thank Judy Rittenhouse and Donita Moore for isolation and initial characterization of the isolate from the cat, Juliana Toth for technical assistance, and Robert E. Weaver and Dannie G. Hollis of the Special Bacterial Pathogens Laboratory, Centers for Disease Control, for biochemical testing.

REFERENCES