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Bacteriological Variation Among *Bordetella bronchiseptica* Isolates from Dogs and Other Species

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Bacteriological properties of 50 isolates of *Bordetella bronchiseptica* were compared. Phase variation, which involved colonial morphology and its associated characters of hemagglutination, hemolysis, acriflavine agglutination, crystal violet staining, flagellation, and fimbriation, occurred among these isolates. Organisms representing the three observed morphotypes did not have different growth rates, nor were any differences in their bacteriological characteristics observed after repeated subculture on agar. There were also variations in antimicrobial drug susceptibility, especially to sulfonamide-trimethoprim, and in nitrate reduction. The relationships among these variable parameters were not apparent. None of the observed variations could be attributed to differences in the species of origin.

Bordetella bronchiseptica has long been known as a disease-producing organism of laboratory animals and has been implicated in several disease syndromes of domestic animals. It has been isolated from a variety of species including dogs, cats, guinea pigs, rats, mice, rabbits, pigs, horses, turkeys, monkeys, humans, and a number of wild animals (9, 10, 11, 19, 21, 30, 31, 33, 37). Despite the wide host range of *B. bronchiseptica*, taxonomic relationships among strains of this organism are generally unknown, and subspecies classification into serogroups, phage types, or biotypes has not yet been accomplished.

Although methods for the clinical identification of *B. bronchiseptica* have been well established (18, 27), there is considerable disagreement regarding some of the classical cultural and biochemical properties of the organism. Reports describing the colonial (23, 24) and ultrastructural (22, 26) morphologies of *B. bronchiseptica* have been few, and differences in these morphological properties are not well known.

It has been suggested that *B. bronchiseptica* isolates from different animal species vary in pathogenicity for swine (28); however, this observation has not been confirmed, and other bacteriological differences between *B. bronchiseptica* isolates from different species have not been reported.

To select representative *B. bronchiseptica* isolates for further pathogenicity studies in dogs, we examined the biochemical, cultural, and morphological characteristics of *B. bronchiseptica* isolates from dogs and other species. The purpose of this study was to determine the degree of bacteriological variation among *B. bronchiseptica* isolates and to observe to what extent these variations might be used to distinguish *B. bronchiseptica* isolates from different species.

MATERIALS AND METHODS

Bacterial strains. Fifty strains of *B. bronchiseptica* were studied (Table 1). The species of origin were as follows: 30 from dogs, 10 from swine, 3 from cats, 2 from guinea pigs, 2 from rats, and 1 from a monkey. The species of origin was undetermined for the 2 remaining strains of *B. bronchiseptica*. One strain each of *Bordetella pertussis*, *Bordetella parapertussis*, and *Alcaligenes faecalis*, and two strains of unidentified, nonfermentative, gram-negative bacilli isolated from the upper respiratory tract of dogs, were also studied. The strains studied represented both recent isolates and type cultures. On receipt, all cultures were checked for purity on brucella agar. Forty-eight-hour bacterial growth was lyophilized in a suspension of 50% of a stabilizing medium consisting of sucrose, phosphate buffers, glutamate, and albumin (3) and stored at 4°C until used. Subcultures were kept to a minimum prior to testing, except that strains 87, 110 H and 110 NH were subcultured at 2- to 5-day intervals for a total of over 100 passages. These strains were then tested at both high and low passage.

Media. Bacteria were maintained on brucella agar (Pfizer). Primary isolation was on Levine eosin-methylene blue agar (Difco) and blood agar. The blood agar consisted of brucella agar base plus 5% sterile sheep blood and IsoVitaleX enrichment (BBL) at a final concentration of 1:50. Bordet-Gengou agar (Difco) was prepared according to the manufacturer's instructions with 20% sheep blood. Cultures on Bordet-Gengou agar were incubated at 35°C in an atmosphere of 5% CO_2; all other cultures were

TABLE 1. *Bacterial strains studied*

Strain	Species of origin	Source[a]
Strains received as *B. bronchiseptica*		
BB Manhattan	Dog	Appel
BB 24 II 69	Dog	Appel
BB Ithaca	Dog	Appel
B. para	Dog	George
87	Dog	Bemis
110 H	Dog	Bemis
110 NH	Dog	Bemis
138 An Hus	Dog	Bemis
J696	Dog	Bemis
K704	Dog	Bemis
501	Dog	Bemis
17640 SAC	Dog	JBS
475 An Hus	Dog	Bemis
Dahlund DR64	Dog	Tischler
Hopper DS43	Dog	Tischler
18731 SAC	Dog	JBS
CD-C47	Dog	Bemis
19022 SAC	Dog	JBS
19141 SAC	Dog	JBS
1388B-3	Dog	House
BB-FTC	Dog	Lauerman
19395 ATCC	Dog	ATCC
780 ATCC	Dog	ATCC
10580 ATCC	Dog	ATCC
482-74	Dog	Bailie
695-74	Dog	Bailie
Strains received as *B. bronchiseptica*		
AMC-Lamazor	Dog	Wilkins
19705	Dog	Bemis
D-1	Dog	Harris
SS-232-75	Dog	Augst
Rat #1	Rat	Boyer
Rat #2	Rat	Boyer
BTS	Swine	Harris
S. madrid	Swine	Harris
Ct. madrid	Cat	Harris
Columbus	Cat	Kahn
SHGP-1	Guinea pig	Bemis
7-8 NADL	Swine	Croghan
495 NADL	Swine	Croghan
7-11 NADL	Swine	Croghan
2-9 NADL	Swine	Croghan
5-8 NADL	Swine	Croghan
5-4 NADL	Swine	Croghan
Phase-I Tuskegee	Swine	Jenkins
NYS #4	Unknown	Smull
NYS #10	Unknown	Smull
ILG	Monkey	House
GPA	Guinea Pig	House
B4	Swine	House
52190 SAC	Cat	JBS
Other strains studied		
8750 ATCC[b]	Unknown	ATCC
15237 ATCC[c]	Human	ATCC
9340 ATCC[d]	Human	ATCC
H 475[e]	Dog	Bemis
I 98[e]	Dog	Bemis

[a] Abbreviations: Appel, M.J.G. Appel, James A. Baker Institute for Animal Health (JABIAH), Cornell University, Ithaca, N.Y.; ATCC, American Type Culture Collection, Rockville, Md.; Augst, V. Augst, LRE, Inc., Kalamazoo, Mich.; Bailie, W. E. Bailie, College of Veternary Medicine, Kansas State University, Manhattan, Kans.; Bemis, D. A. Bemis, JABIAH, Cornell University, Ithaca, N.Y.; JBS, J. Bentinck-Smith, N.Y.S. College of Veternary Medicine, Cornell University, Ithaca, N.Y.; Boyer, C. I. Boyer, Jr., N.Y.S. College of Veternary Medicine, Cornell University, Ithaca, N.Y.; Croghan, D. Croghan, U.S.D.A., National Animal Disease Laboratory, Ames, Iowa; George, L. W. George, JABIAH, Cornell University, Ithaca, N.Y.; Harris, D. L. Harris, College of Veternary Medicine, Iowa State University, Ames, Iowa; House, J. A. House, Pitman-Moore, Inc., Washington Crossing, N.J.; Jenkins, E. M. Jenkins, School of Veternary Medicine, Tuskegee Institute, Tuskegee, Ala.; Kahn, D. E. Kahn, College of Veternary Medicine, Ohio State University, Columbus, Ohio; Lauerman, L. H. Lauerman, College of Veternary Medicine & Biomedical Science, Colorado State University, Fort Collins, Colo.; Smull, L. L. Smull, Tompkins County Hospital, Ithaca, N.Y.; Tischler, S.A. Tischler, Valley Veterinary Hospital, Walnut Creek, Calif.; Wilkins, R. J. Wilkins, Animal Medical Center, New York, N.Y.

[b] Received as *Alcaligenes faecalis*.

[c] Received as *B. parapertussis*.

[d] Received as *B. pertussis*.

[e] Unidentified nonfermentable gram-negative strains.

incubated at 37°C. Conventional test media were inoculated with colonies from 48-h brucella agar cultures. Test results were read at varying intervals, but each test was completed 7 days after inoculation.

Colonial morphology. Colonial morphologies were described after observation with direct, oblique, and transmitted lighting. Average colony size was estimated by measuring at least three well-isolated colonies. Colony type was judged by gross observation to be either smooth or rough, and the degree of dissociation was determined by the acriflavine agglutination and crystal violet-staining methods described for brucellae (1). The dissociation rate of a smooth colony-producing strain (110 H) was determined by making repeated plate counts from

brucella broth cultures that were incubated at 37°C on a rotating table (250 rpm). Brucella and blood agar cultures were observed at 48 h, except for *B. parapertussis*, which grew slowly on this medium and was therefore observed at 7 days. Growth on Bordet-Gengou agar was examined at 1 and 2 days, for most isolates, and at 7 days for the slow-growing strains.

Hemagglutination. One drop of a heavy bacterial suspension in phosphate-buffered saline (packed-bacterial-cell volume approximately 8 to 10%) was mixed with one drop of 20% washed sheep erythrocytes on a white plastic plate. This mixture was rotated and observed under direct lighting for hemagglutinating activity. Final reactions were recorded after 5 min.

Microscopic morphology. Gram-stained smears of 24-h brucella broth cultures were examined for microscopic morphology.

Ultrastructural morphology. Brucella broth cultures were incubated on a rotating table (250 rpm) at 37°C for 10 to 15 h. Negative-contrast staining was performed as follows: Formvar-coated and carbon-stabilized copper grids (200 mesh) were suspended on a drop of broth culture for 30 s to 1 min, drained, suspended on a drop of 0.1% to 0.5% aqueous potassium phosphotungstic acid, pH 7.0, for 30 s to 1 min, and drained until dry. Grids of *B. pertussis* and *B. parapertussis* were prepared from 7-day growth on Bordet-Gangou agar and required 2% aqueous potassium phosphotungstic acid for adequate staining. Grids were examined with a Philips EM 300 electron microscope (Philips Electronic Instruments, Mount Vernon, N. Y.). The overall density of organisms and the staining quality were recorded. In most cases, a minimum of 20 bacterial cells were observed, and their typical ultrastructural morphologies were described. Because the observation of pili was sometimes obscured in the more poorly stained specimens, each negative finding was repeated at least twice.

Cultural properties. Growth patterns in fluid thioglycolate and brucella broths (Pfizer) were described after 48 h of incubation. The ability of strains to grow on salmonella-shigella agar (Difco) and brucella agar containing potassium tellurite (320 mg/liter) was determined after 7 days. Growth rates of three strains, 87, 110 H, and 110 NH, were compared in brucella broth.

Antibiotic susceptibility tests. Sensitivity to antibiotics was measured according to the principles outlined by Bauer and Kirby et al. (2). The antibiotic disks used (BBL) were as follows: ampicillin (10 μg), cephalothin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), gentamicin (15 μg), kanamycin (30 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), penicillin (10 U), polymyxin B (300 U), streptomycin (10 μg), sulfamethoxazole-trimethoprim (25 μg), and tetracycline (30 μg).

Biochemical tests. Biochemical tests were performed according to standard methods (8, 36). All isolates were screened for their inability to ferment glucose on Kligler iron agar (Difco). Ability to utilize citrate as a sole carbon source was tested on Simmon citrate medium (Difco). Nitrate reduction was determined after 48 h of incubation in indole-nitrate broth (Difco), and the isolates were tested for nitrate with sulfanilic acid and alpha-naphthylamine solutions. Negative reactions were confirmed by addition of zinc dust. Urea hydrolysis was observed on Christensen urea agar (Difco) after 4 and 18 h. Oxidation of glucose was tested in an open tube containing oxidation-fermentation basal medium (Difco) and 1% glucose. This same medium was also used to observe motility. Oxidase activity was tested by flooding a 48-h brucella agar slant with a 1% solution of ρ-aminodimethylaniline oxalate (Difco). Tetrazolium reduction was tested in motility GI medium (Difco) containing 75 mg of 2,3,5-triphenyltetrazolium chloride (Eastman) per liter. Cultures were observed for formation of a red-insoluble formazan dye. Gelatin hydrolysis was determined in tubes of brucella broth containing 12% gelatin.

RESULTS

Detailed bacteriological findings and individual strain results are on record elsewhere (D. A. Bemis, Ph.D. thesis, Cornell University, Ithaca, N.Y., 1976). All organisms studied were gram-negative rods. None of the strains were able to ferment or oxidize glucose, nor were they able to hydrolyze gelatin. Each was aerobic in its growth requirements and grew only in the oxidized portion of thioglycollate broth (if able to grow in this medium). Strains received as *B. bronchiseptica* were motile within 7 days, produced oxidase, rapidly (within 4 h) hydrolyzed urea, and were capable of reducing tetrazolium to a red-insoluble formazan. None of these strains were able to grow in the presence of potassium tellurite. Forty-nine strains (98%) were capable of utilizing citrate. By using the above criteria of motility, oxidase activity, urea hydrolysis, tetrazolium reduction, utilization of citrate, and growth on potassium tellurite medium, the other related organisms studied could be clearly differentiated and excluded as *B. bronchiseptica*. With one exception, *B. bronchiseptica* grew in brucella broth as a heavy pellicle adhering to glass with turbidity throughout the tube. The following represents the main findings pertaining to variation among *B. bronchiseptica* strains.

Colonial morphology. When grown on brucella agar, three distinct morphological forms, tentatively called phase I, intermediate phase, and rough phase, were recognized among the strains of *B. bronchiseptica*. A summary of their properties is given in Table 2. The small, pearllike phase-I colonies were present in only 4 of the 50 *B. bronchiseptica* strains. These colonies were observed to dissociate to rough-phase organisms, especially when cultured in broth. The rate of dissociation was determined to be 3 to 4% after 4 days of incubation on a

TABLE 2. *Summary of morphological phase variation and related properties among B. bronchiseptica isolates*[a]

Phase	No. of strains	Size	Configuration	Texture	Appearance in transmitted light	Margin	Hemolysis on blood agar[b]	Hemagglutination[c]	Acriflavine agglutination[d]	Crystal violet staining[e]	Flagella (10- to 15-h broth	Pili
I	4	Small (≤1 mm)	Pulvinate (compact, pearllike)	Smooth	Opaque	Entire	+	+	−, ±, or +	+	−	+
Intermediate	42	Medium (1 to 2 mm)	Convex (rounded)	Smooth	Opaque-translucent	Entire	−	+ to −	± to +++	±	+	+ or −
Phase	4	Large (≥2 mm)	Umbonate (raised center, spreading perimeter)	Rough	Translucent	Undulate	−	+ or ±	+	+ or ±	+	+ or −

[a] Morphology examined on brucella agar.
[b] +, Hemolysis occurred; −, hemolysis not present.
[c] + = Strong; ± = weak; − = negative.
[d] − = Negative; ± = weak or trace agglutination; + = ca. 25% of cell suspension agglutinated (++, +++ = 50%, 75%).
[e] + = Colony completely stained; ± = colony partially strained.

rotary shaker. Dissociation to rough phase was not reversible in vitro. Colonies in phase I that had been subcultured over 100 times on agar (each time selecting for original colony type) did not lose any of their morphological properties. The large, rough-phase colonies differed from their smooth predecessors in that they were translucent, raised in the center, and had an undulating outer margin (Fig. 1 and 2). Only 4 of 50 *B. bronchiseptica* strains represented rough-phase organisms. The remaining majority of strains (42 of 50) were of smooth, intermediate colony types. Although six of these strains were observed to dissociate to rough-phase organisms, it appeared that some strains might represent stable intermediate forms. Strain 87 was subcultured over 100 times on agar and numerous times in broth without any changes in morphology occurring.

Acriflavine agglutination, hemagglutination, and crystal violet staining of these colonies were variable. Each of the four phase-I strains gave weak- (≤1+) or negative-agglutination reactions in acriflavine, had some hemagglutinating activity, and stained completely with crystal violet. The remaining two colony types gave variable results on these three tests. All of the strains that gave no hemagglutination reaction were smooth, intermediate-phase organisms, as were those that gave strong acriflavine agglutination. Crystal violet staining was found in all phase-I organisms, in 7 of 42 intermediate-phase organisms, and in only 1 of 4 rough-phase organisms.

Morphological variation was less apparent on blood agar. Only the four strains that produced phase-I colonies on brucella agar were hemolytic. Although the two properties were not well correlated, there were more pearl-like colonies (30 of 50) and more hemolytic strains (23 of 50) observed on Bordet-Gengou agar.

Microscopic morphology. Gram staining revealed that the *B. bronchiseptica* strains were thin, gram-negative rods with tapered ends, occurring mostly singly or in pairs. Some strains also possessed long filamentous forms.

Ultrastructural morphology. When observed by negative-contrast staining, *B. bronchiseptica* strains were seen to possess a highly convoluted cell wall with one to two less electron-dense spots on their surfaces (Fig. 3). The other species of bacteria examined did not possess these cell wall convolutions. Flagella were observed on all *B. bronchiseptica* strains except the four phase-I strains. When present, the flagella were arranged peritrichously. Numerous filamentous appendages (consistent with the description of pili in other bacteria [5, 7])

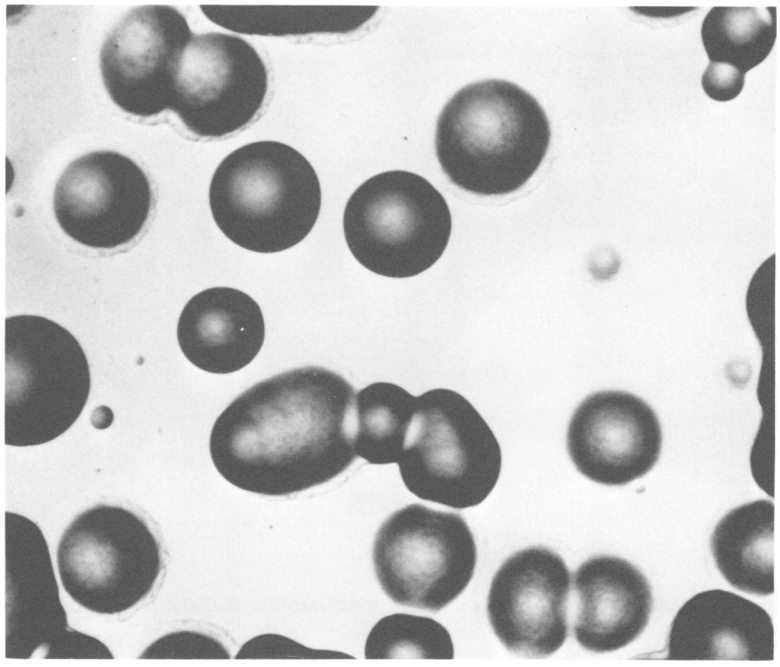

FIG. 1. *Appearance of smooth- and rough-phase colonies of B. bronchiseptica on brucella agar (25× magnification).*

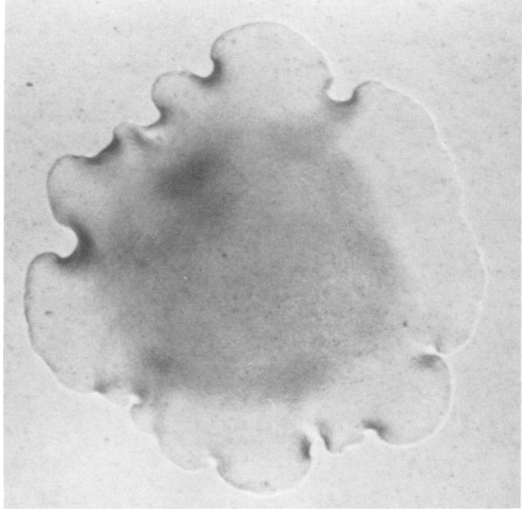

FIG. 2. *Rough-phase colony of B. bronchiseptica on brucella agar (100× magnification).*

were found on 82% of the strains tested (Fig. 4). *B. parapertussis* and *B. pertussis* were the only organisms other than *B. bronchiseptica* to possess pili. Most of the piliated strains were capable of producing smooth, phase-I type colonies on Bordet-Gengou agar.

Cultural properties. All but three strains of *B. bronchiseptica* were able to grow on salmonella-shigella agar, although some grew poorly. Growth rates of three strains (110 H, 110 NH, and 87) representing each of the observed morphological phases were not different. Near the end of the logarithmic phase of growth, cultures of 110 H autoagglutinated. Sequential Gram stains made during the growth studies showed that the incidence and length of filamentous forms increased during the later stages of log-phase growth. In strain 110 H, these filaments were arranged in palisades and formed lattice structures when the density became great enough. Macroscopically, these lattice structures were observed as granular agglutination throughout the entire broth culture.

Antibiotic susceptibility. As determined by existing clinical standards (19), *B. bronchiseptica* strains were all sensitive to polymyxin B, chloramphenicol, and tetracycline. Ninety-eight percent of the strains were susceptible to gentamicin, and 96% were susceptible to kanamycin. Nalidixic acid, cephalothin, ampicillin, and sulfonamide-trimethoprim were somewhat less effective (70, 76, and 66%, respectively, were susceptible). All strains were resistant to streptomycin and penicillin, and only one strain was partially susceptible to nitrofurantoin. The zone diameters were distributed over

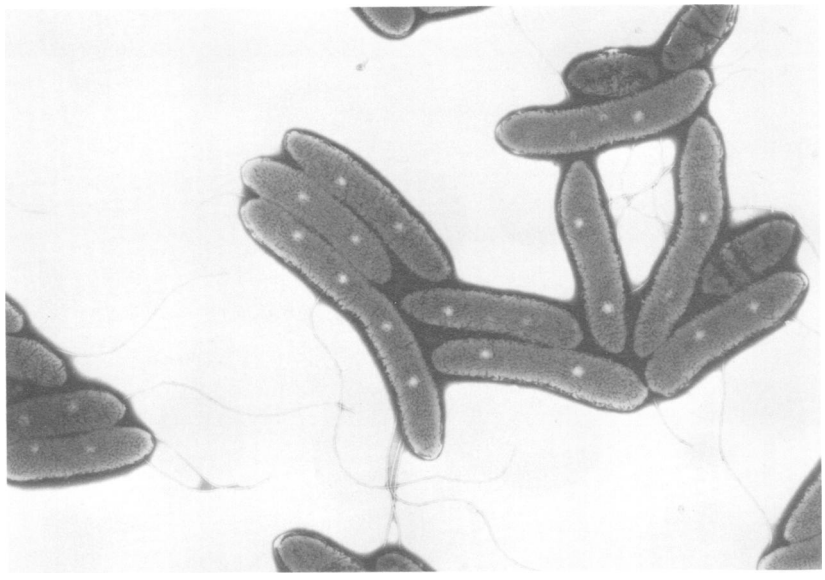

FIG. 3. *Negative-contrast staining of B. bronchiseptica (6,000× magnification).*

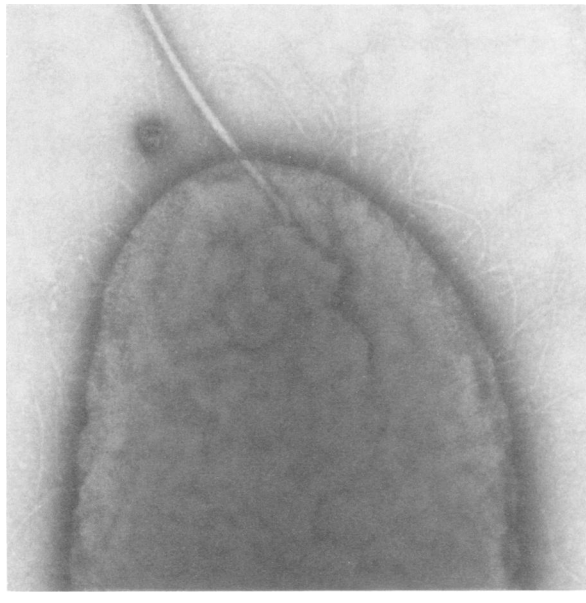

FIG. 4. *Negative-contrast staining of B. bronchiseptica (61,000× magnification).*

a narrow range for all antibiotics except sulfonamide-trimethoprim. Zone diameters for this drug ranged from 9 to 63 mm, and a bimodal type of distribution was suggested.

Biochemical tests. Only the test for nitrate reduction gave variable results. Thirty-three strains reduced nitrate to nitrite.

Effect of serial passage. Strains 87, 110 H, and 110 NH were subcultured over 100 times on brucella agar with care taken to select for the original colony type. The bacteriological characteristics of these high-passage strains were identical to those observed at low passage.

***B. bronchiseptica* variation between different host species.** Table 3 summarizes the variability of *B. bronchiseptica* isolates from differ-

TABLE 3. *Variation of B. bronchiseptica from different species*

Species	No. of strains	Phase-I colonies (brucella agar)	Rough-phase colonies (brucella agar)	Hemolysis (blood agar)	Hemolysis (Bordet-Gengou agar)	Hemagglutination (+ or ±)[a]	Acriflavine agglutination (≥++)[b]	Crystal violet staining	Pili	Growth on salmonella-shigella agar	Full susceptibility to nalidixic acid	Full susceptibility to cephalothin	Full susceptibility to ampicillan	Full susceptibility to sulfonamide-trimethoprim	Nitrate reduction
Dog	30	2[c]	3	2	8	24	9	6	24	27	22	20	24	17	20
Combined noncanine isolates	20	2	1	2	15	17	1	6	17	20	10	10	14	16	13
Noncanine isolates															
Swine	10	0	0	0	9	10	1	1	10	10	6	5	8	8	3
Cat	3	0	1	0	1	3	0	1	3	3	0	2	3	2	3
Guinea pig	2	0	0	0	1	2	0	1	2	2	1	0	1	2	2
Rat	2	2	0	2	2	2	0	2	2	2	1	1	1	2	2
Monkey	1	0	0	0	0	0	0	1	0	1	0	1	0	1	1
Unknown	2	0	0	0	2	0	0	0	0	2	2	1	1	1	2

[a] See footnote *c*, Table 2, for explanation.
[b] See footnote *d*, Table 2, for explanation.
[c] Number indicates number of strains giving positive reaction or possessing character described.

ent species. The same degree of bacteriological variation was observed in canine and noncanine isolates of *B. bronchiseptica*.

DISCUSSION

Many authors have stressed the use of "smooth" or "phase-I" strains of *Bordetella bronchiseptica* for conducting pathogenicity experiments or producing antigen. However, a comparative description of such strains and the significance of their use are not generally known. Nakase (23, 24) described four different colonial morphologies of *B. bronchiseptica*, which he called, "phase I, II, III and rough phase." Colonial morphologies similar to those observed by Nakase appeared to represent the major variables among the *B. bronchiseptica* strains included in the present study. Although most produced smooth colony types, the incidence of phase-I isolates was only 8%. Contrary to the observation of Nakase, the dissociation rate of phase-I organisms was low, and morphological characteristics could be maintained for over 100 subcultures on agar. Nakase noted that organisms producing the highly convex, entire-edged phase-I colonies possessed a few flagella and were encapsulated; he also noted that the organisms of the flat, irregular-edged rough-phase colonies were not encapsulated and did not possess flagella. These findings do not represent the usual relationship between colonial morphology, possession of capsules, and possession of flagella. In the present study, organisms producing phase-I colonies did not possess flagella, while organisms producing rough-phase colonies with spreading perimeters possessed numerous flagella. Capsules, though they may be important components of *B. bronchiseptica* pathogenicity, were not readily demonstrated in any of the strains studied.

The tendency for phase-I organisms to be stained more heavily by crystal violet stain is the converse of the reaction observed with brucella organisms. Kang et al. (14) described acid agglutination as a property of phase-I *B. bronchiseptica* organisms. Acriflavine agglutination, a property of rough brucella organisms, was positive to some degree in most strains studied. Although variation is usually unidirectional in vitro, passing from smooth to rough, rough- to smooth-phase variation has been suggested in some species (6, 29). The specificity of the above reactions for distinguishing all smooth- or rough-phase organisms must be questioned.

Hemolysis on blood agar was observed only with phase-I organisms; however, some additional strains that were not hemolytic on blood agar were hemolytic on Bordet-Gengou agar. This difference in growth media may account for the reported differences in the hemolytic character of *B. bronchiseptica*. There was also a tendency to form more phase-I type colonies on Bordet-Gengou agar; so, as suggested by Lacey (17), the composition of the medium may determine to some extent the colonial structure of an organism.

Bacterial hemagglutination has been described as a characteristic of certain bacterial surface structures such as capsules or pili (7, 12, 15, 25). Hemagglutination was observed for most strains of *B. bronchiseptica* and was not inhibited by the presence of D-mannose (0.7% concentration). This finding, at least in some species, is indicative of nonfimbrial hemagglutination (35). However, fimbrial-associated hemagglutination by *Neisseria* species has recently been shown to be unaffected by D-mannose (16, 38). Hemagglutination tended to be stronger among the phase-I isolates but was also observed for some strains producing rough- and intermediate-type colonies. Kang et al. (14) suggested that differences in hemagglutinating ability may be due to differences in origin, as his single canine isolate reacted very strongly in the hemagglutination test. No such differences could be found in the present study.

In other bacterial species, especially those organisms that colonize mucosal surfaces, the possession of pili or fimbriae has been associated with colonial morphology (4, 22, 29, 32). There has been only one published electron microscope study of *B. bronchiseptica* (26), and pili were not observed on these organisms. Morse and Morse (22) suggested that pilus-like structures similar to those found on phase-I *B. pertussis* organisms were also present on two strains of *B. bronchiseptica*. Although 82% of the *B. bronchiseptica* strains studied possessed numerous thin filamentous appendages resembling pili, 82% were hemagglutinating and 92% represented smooth-colony types; an absolute relationship between these properties could not be established.

Antibiotic susceptibility and nitrate reduction, two additional characteristics that could not be readily associated with colonial morphology, also indicated that there was no difference between *B. bronchiseptica* isolates from different species. Of those antimicrobials agents having variable effects, sulfonamide-trimethoprim was of greatest interest. Interpretation of susceptibility relies on the correlation between zone diameters and minimum inhibitory concentrations of the drugs against many different bacteria (20). Strains producing zone diameters

smaller than the smallest zone observed for bacteria considered to be susceptible are reported as resistant. Conversely, strains producing zone diameters larger than the largest zone observed for bacteria considered to be resistant are reported as susceptible. An area of overlap exists between these two ranges of zone diameters and presents difficulty in estimating the true susceptibility of some strains. Interpretation of susceptibility of *B. bronchiseptica* strains by the diffusion test may be further influenced by their somewhat slower growth rates than most bacteria used to standardize the test. Regardless of the determined susceptibility, it is of importance to note that two populations of organisms emerged with regard to zones of inhibition produced by sulfonamide-trimethoprim. There are likely to be some additional characters associated with these two populations. R-factors conferring resistance to sulfonamides and other antimicrobial agents have been found in isolates from swine (13, 34). There was no associated species distribution nor any accompanying antibiotic resistance in these sulfonamide-trimethoprim-reactive populations.

It was apparent from this study that nitrate reduction, though frequent, was not a consistent feature of *B. bronchiseptica* strains. A tenuous relationship between nitrate reduction and the previously mentioned pattern of susceptibility to sulfonamide-trimethoprim was noted. All strains that produced low-zone diameters against the sulfonamide drug were nitrate positive, and, conversely, all strains that were nitrate negative produced high-zone diameters against the sulfonamide.

The degree of bacteriological variation among strains of *B. bronchiseptica* was surprisingly small considering the parasitic nature and wide host range of this organism. Because the same degree of variation was observed in canine and noncanine isolates, there does not appear to be any promise of differentiating *B. bronchiseptica* isolates from different species. Serological studies on *B. bronchiseptica* isolates from different species are needed; however, initial studies on isolates from guinea pigs indicate that serological differences are again related to colonial phase variation (23). The significance of capsules, pili, and other properties associated with colony morphology in the pathogenicity of *B. bronchiseptica* requires further study.

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