APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Mar. 1988, p. 818–821
0099-2240/88/030818-04$02.00/0

Vol. 54, No. 3

Virulence of *Yersinia pseudotuberculosis* Isolated from Pork and from the Throats of Swine

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Received 21 September 1987/Accepted 21 December 1987

***Yersinia pseudotuberculosis* was isolated from retail pork and from healthy swine throats. These wild-type strains and their representative cured isogenic strains were tested for the presence of plasmids and several virulence factors, and these characteristics were compared with those of virulent strains from humans. Two pork isolates (serotype IVB) and four swine isolates (serotypes IIB, IIC, III, and IVB) harbored a 42- to 48-megadalton plasmid which had similar fragmentation patterns resulting from digestion with restriction endonuclease. These six strains were lethal for mice via oral challenge and were positive in autoagglutination and calcium dependency tests. They also invaded HeLa cells and induced cytotoxicity. Histopathological examination and indirect fluorescent-antibody staining provided definite evidence of the pathogenicity of these strains when tissue sections from orally infected mice were used. The virulence factors of wild-type pork and swine isolates with the 42- to 48-megadalton plasmid were identical to those of two human isolates (serotypes IVB and VB). Hence, these pork and swine isolates should be considered potentially pathogenic for humans. The finding suggests that retail pork and swine may play an important role in the epidemiology of human infections caused by *Y. pseudotuberculosis*.**

Yersinia pseudotuberculosis is associated with a variety of human diseases, such as mesenteric lymphadenitis, terminal ileitis, erythema nodosum, arthritis, and septicemia (15). Since 1981, several large outbreaks of *Y. pseudotuberculosis* infection have been reported in Japan (7, 25). These reports implicated foods as the vehicle of transmission; however, the foods responsible were not bacteriologically identified.

Y. pseudotuberculosis is also a well-recognized animal pathogen which causes zoonotic infections (14, 18, 24). Studies done in Europe, Canada, and Japan have shown that swine are an important reservoir of *Y. pseudotuberculosis* (16, 29, 30, 35). The presence of the organism in swine suggests that pork meat may be contaminated. Although an association between clinical human infections and pork ingestion has been implied (32), the organism has never been found in retail pork samples (16). Consequently, the link between a swine reservoir and transmission to humans remains unclear.

Our recent survey, however, of retail meat sold in Japan revealed that 2% of pork tongue and 0.8% of ground pork samples were contaminated with *Y. pseudotuberculosis* (3, 27).

The virulence of *Y. pseudotuberculosis* is associated with a 40- to 50-megadalton (MDa) plasmid (5, 8, 21). The virulent plasmid is associated with several kinds of virulence factors, i.e., calcium dependency (5), expression of VW antigens (20), autoagglutination (12), production of specific outer membrane proteins (1, 21), cytotoxicity in tissue culture cells (17, 23), and lethality in mice (1, 12). However, no report is available on the virulence and presence of plasmids in *Y. pseudotuberculosis* strains isolated from pork or swine.

In this study, we investigated several virulence factors and plasmid profiles of *Y. pseudotuberculosis* isolated from pork or swine and compared the results with those for human isolates in terms of their significance in public health.

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MATERIALS AND METHODS

Bacterial strains. The 12 strains of *Y. pseudotuberculosis* used in this study are shown in Table 1. Strain MCP49 (serotype IVB) was isolated from pork tongue at our laboratory in Shizuoka, Japan (27). Strain M364 (serotype IVB) was isolated from ground pork in Shimane, Japan (3). The strains with the PT prefix (serotypes IIB, IIC, III, and IVB) were isolated from healthy swine throats. Two strains from humans were used as virulent control cultures. Strain CYP86-1 (serotype IVB) was isolated from the stool of a patient involved in a mass outbreak by K. Sanbe, Public Health Laboratory of Chiba Prefecture, Chiba, Japan (25). Strain K1 (serotype VB) was isolated from the stool of a patient representing a sporadic case by M. Sogawa, Kagawa Prefectural Institute of Public Health, Kagawa, Japan. Four plasmid-containing strains were cured by selection for colonies growing on a magnesium oxalate agar plate at 37°C. Isogenic cured mutants are designated by the C suffix. Stock cultures were kept as cell suspensions at −20°C in 30% glycerol–1% peptone.

Assays for virulence factors. Autoagglutination was determined by the method of Laird and Cavanaugh (12). Magnesium oxalate agar (6) was used for the determination of calcium dependency. The invasion of HeLa cell monolayers was determined as described by Vesikari et al. (33). Cytotoxicity (17) was determined by the morphological changes in HeLa cells, i.e., the appearance of round and densely stained cells with a few extended pseudopods. The production of heat-stable enterotoxin (ST) was determined by the suckling mouse assay (19). Oral infection of ddY mice (Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan) with *Y. pseudotuberculosis* was carried out as described by Bolin et al. (1). Groups of five mice weighing 20 to 22 g were deprived of water for 18 h and then allowed to drink freely from a 50-ml water suspension of each strain (~10^9 bacteria per ml) grown at 25°C. In addition, a histological examination and an indirect

TABLE 1. Results of the virulence tests for 12 *Y. pseudotuberculosis* strains

Strain[a]	Source	Serotype	Plasmid size (MDa)	Autoagglutination	Calcium dependency	Virulence in mice (oral)[b]	LD_{50} in mice (i.p.)[c]	HeLa cell		ST production
								Invasion	Cytotoxicity	
MCP49	Pork tongue	IVB	2.2, 38, 42	+	+	Lethal	5.4×10^4	+	+	−
MCP49-C			2.2, 38	−	−	—	$>10^7$	+	−	−
M364	Ground pork	IVB	2.2, 42	+	+	Lethal	1.8×10^4	+	+	−
M364-C			2.2	−	−	—	$>10^7$	+	−	−
PT593	Swine throat	IVB	36, 42	+	+	Lethal	L	+	+	−
PT593-C			36	−	−	—	NL	+	−	−
PT94	Swine throat	IIB	44	+	+	Lethal	L	+	+	−
PT508	Swine throat	IIC	46	+	+	Lethal	L	+	+	−
PT595	Swine throat	III	47	+	+	Lethal	L	+	+	−
CYP86-1	Human	IVB	42	+	+	Lethal	7.8×10^4	+	+	−
CYP86-1-C			None	−	−	—	$>10^7$	+	−	−
K1	Human	VB	48	+	+	Lethal	L	+	+	−

[a] The C suffix indicates that isogenic mutants of the organisms were selected on magnesium oxalate agar.
[b] —, No illness.
[c] LD_{50}, 50% Lethal dose. L, Lethal when 3×10^6 cells were injected i.p.; NL, nonlethal when 3×10^6 cells were injected i.p.

fluorescent-antibody examination of tissue sections from orally infected mice were performed to demonstrate the evidence for pathogenicity. The 50% lethal dose for ddY mice was determined by intraperitoneal (i.p.) injection of graded doses of a suspension in saline of each strain grown at 25°C and by the calculation method of Reed and Muench (22). The virulence after i.p. injection of six strains (PT593, PT593-C, PT94, PT508, PT595, and K1) was assessed by inoculating i.p. a single dose (3×10^6 cells) of bacterial suspension into five mice. Mortality was recorded daily for 21 days.

Plasmid detection and restriction digests. Overnight cultures grown at 25°C in brain heart infusion broth were lysed by a modification of the method of Kado and Liu (9). Lysates were incubated for 30 min at 55°C and extracted twice with a phenol-chloroform (1:1) mixture. Plasmid DNA was subjected to electrophoresis on an 0.8% agarose gel previously soaked in Tris-borate buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA [pH 8.0]). Plasmid sizes were estimated by comparison with uncut control plasmids of known sizes (S-a, 23 MDa; RP4, 36 MDa; R1, 62 MDa) and by summation of the restriction fragments treated with three restriction endonucleases (*Bam*HI, *Hin*dIII, and *Eco*RI). Restriction endonucleases were used under the conditions recommended by the supplier (Nippon Gene Co., Toyama, Japan). Restricted DNA was subjected to electrophoresis on a horizontal 0.7% agarose gel in Tris-acetate buffer (0.04 M Tris, 0.02 M sodium acetate, 0.002 M EDTA [pH 8.0]).

RESULTS

Assay for virulence factors. The results obtained in virulence tests with 12 strains of *Y. pseudotuberculosis* are given in Table 1. All the strains were serotyped in our laboratory. Wild-type strains (MCP49, M364, and CYP86-1) of serotype IVB from pork or humans were positive in autoagglutination and calcium dependency tests. The same strains were lethal for mice within 3 to 7 days after oral ingestion. Extraintestinal translocation of these strains could be easily demonstrated by recovery of the challenge organisms from the liver and spleen at necropsy. The 50% lethal doses of the three strains determined after i.p. injection were 1.8×10^4 to 7.8×10^4 cells. These strains also induced cytotoxicity in HeLa cells but did not produce ST. Histopathological changes in dead mice infected orally were necrotic lesions, mainly in the intestine, liver, and spleen, caused by all of the strains. Organisms in the colonies in the necrotic lesions were detected by the fluorescent-antibody technique. The other wild-type strains (PT593, PT94, PT508, PT595, and K1) from swine or humans were positive in six kinds of virulence tests, including the lethality test by oral ingestion or a single i.p. injection of 3×10^6 cells in mice. The virulence of the isolates from pork or swine was equal to that of the human isolates.

Isolation and characterization of plasmids. To assess the relationship between virulence factors and plasmids in the strains, we examined the plasmid contents. Eight wild-type strains carried plasmids with molecular sizes ranging from 2.2 to 48 MDa (Fig. 1). We also found that a plasmid ranging in size from 42 to 48 MDa was present in these strains, regardless of their sources and serotypes. Four isogenic calcium-independent mutant strains (MCP49-C, M364-C, PT593-C, and CYP86-1-C) lost the 42-MDa plasmid but retained the other plasmid(s). The same strains also lost three kinds of virulence factors: autoagglutination, cytotoxicity in HeLa cells, and lethal effects in mice after oral feeding. The 50% lethal dose of three cured isogenic strains (MCP49-C, M364-C, and CYP86-1-C) after i.p. injection

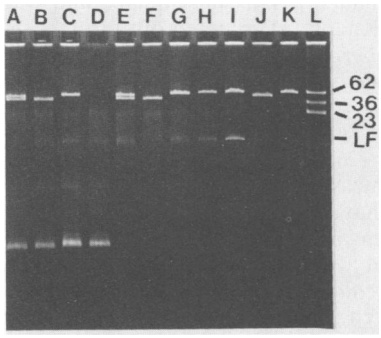

FIG. 1. Agarose gel electrophoresis of plasmid DNAs from *Y. pseudotuberculosis* MCP49 (lane A), MCP49-C (lane B), M364 (lane C), M364-C (lane D), PT593 (lane E), PT593-C (lane F), PT94 (lane G), PT508 (lane H), PT595 (lane I), CYP86-1 (lane J), and K1 (lane K). Lane L, Molecular mass standards (S-a, 23 MDa; RP4, 36 MDa; R1, 62 MDa). LF, Linear DNA fragments.

exceeded 10^7 cells, and one strain (PT593-C) was nonlethal for mice even though 3×10^6 cells were injected by the i.p. route (Table 1). These data indicate that the presence of a 42- to 48-MDa plasmid may be required for the expression of virulence factors and pathogenicity in mice. The invasiveness of cured isogenic strains in HeLa cells was identical to that of uncured wild-type strains. However, the cured strains no longer caused cytotoxicity in HeLa cells (Table 1). This fact is in accordance with observations by Okamoto et al. (17) and Rosqvist and Wolf-Watz (23). These data imply that the virulence plasmid is not essential for the invasion of HeLa cells by *Y. pseudotuberculosis* but that it apparently encodes a genetic factor responsible for cytotoxicity.

Furthermore, we investigated the DNA similarities of 42- to 48-MDa plasmids from *Y. pseudotuberculosis* by comparing fragmentation patterns resulting from digestion by restriction endonucleases. The representative patterns of DNAs digested with *Bam*HI are shown in Fig. 2. The plasmids from eight wild-type strains shared many fragments of identical size (Fig. 2, lanes B, D, F, H, I, J, K, and L), regardless of the serotypes or sources. When these plasmid DNAs were digested with *Eco*RI or *Hin*dIII, the same results were obtained (data not shown). Furthermore, all plasmids from the wild-type strains shared three common *Bam*HI restriction fragments (arrows in Fig. 2) which were equal in size to the calcium dependence region of plasmid pIB1 of *Y. pseudotuberculosis* YPIII, identified previously by Portnoy et al. (21). The sizes of the three fragments were 4.0, 4.4, and 5.3 kilobases. The similarity in the fragmentation patterns of the 42- to 48-MDa plasmids suggests a possible association between a closely related family of plasmids and the pathogenicity of our isolates from pork, swine, and humans.

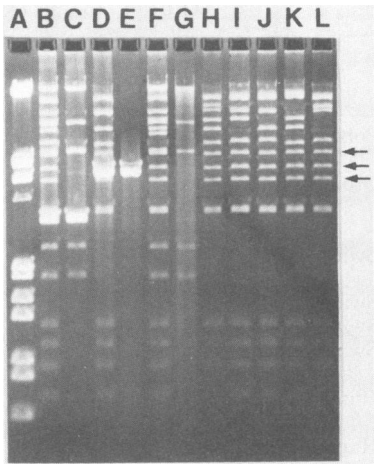

FIG. 2. *Bam*HI-digested plasmid DNAs from *Y. pseudotuberculosis* strains. Lanes: A, Molecular weight marker (lambda DNA plus *Eco*RI plus *Hin*dIII); B, MCP49; C, MCP49-C; D, M364; E, M364-C; F, PT593; G, PT593-C; H, PT94; I, PT508; J, PT595; K, CYP86-1; L, K1. Arrows indicate three common *Bam*HI restriction fragments which were equal in size to the calcium dependence region of plasmid pIB1 of *Y. pseudotuberculosis* YPIII, identified previously by Portnoy et al. (21).

DISCUSSION

Y. pseudotuberculosis has been classified into six serological types designated O types I to VI. Subtypes A and B have been designated in O types I, II, IV, and V (28). Furthermore, new serotypes IIC, VII, and VIII have been proposed by Tsubokura et al. (31). We used five serotypes of *Y. pseudotuberculosis* strains isolated from pork, swine, or humans in this study (Table 1). Since the most common types of *Y. pseudotuberculosis* isolated from human materials in Japan are serotypes IVB and VB (32), human strains with these serotypes were used as virulent control cultures.

Y. pseudotuberculosis has been frequently isolated from domestic animals, pets, wild animals, and water (4, 10, 26, 30, 34, 35). Knapp (11) pointed out three possible modes of *Y. pseudotuberculosis* infection: (i) direct contact with infected animals or their excreta, (ii) consumption of infected meat or other contaminated foodstuffs, and (iii) drinking of contaminated water or milk. Transmission from animals (2, 13) or water (26) to humans has been established by actual cases. In contrast, possible modes of transmission from pork to humans have not yet been documented. However, in Japan Shiozawa et al. (27) and Fukushima (3) recently reported that retail pork meat was contaminated with *Y. pseudotuberculosis* serotype IVB. The results of this study show that such isolates are highly virulent for mice and that they possess a 42-Mda plasmid. This class of plasmid is associated with virulence factors, such as lethality in mice, autoagglutination, calcium dependency, and cytotoxicity in HeLa cells. There were no differences in these virulence factors among pork, swine, and human clinical isolates of *Y. pseudotuberculosis*. Thus, pork and swine isolates should be considered potentially pathogenic for humans. There appears to be a close relationship between the presence of virulent *Y. pseudotuberculosis* in swine and contamination with virulent *Y. pseudotuberculosis* in pork. If pork is contaminated with virulent *Y. pseudotuberculosis* and improperly cooked or allowed to cross-contaminate other foods, its consumption may result in the development of a *Y. pseudotuberculosis* infection. Therefore, we presume that pork may play an important role in the epidemiology of human infections caused by *Y. pseudotuberculosis*.

We used six tests for virulence factors: autoagglutination, calcium dependency, lethality in mice, HeLa cell invasion, HeLa cell cytotoxicity, and ST production. The results indicate that these tests, except for HeLa cell invasion and ST production, are useful in obtaining reproducible results for assessing virulence potential. These four virulence factors seem to be closely related. Nevertheless, it seems desirable to run more than two virulence tests.

Plasmids of 42 to 48 MDa which were associated with the above-mentioned virulence factors produced similar results in the DNA restriction analysis. This fact suggests that restriction endonuclease fingerprinting may be a reliable indicator of the virulence of *Y. pseudotuberculosis*. We also believe that detection of the three common *Bam*HI restriction fragments (Fig. 2) of the calcium dependence region is useful for selecting potentially virulent strains. Further studies with additional strains of *Y. pseudotuberculosis* are needed to establish the usefulness of these fragments as indicators of the virulence of *Y. pseudotuberculosis*.

ACKNOWLEDGMENTS

We thank H. Miyamoto, Shimada Public Health Center, Shizuoka, Japan, and M. Matsumoto, Oregon State University, Corvallis, for their help with the manuscript.

LITERATURE CITED

1. **Bolin, I., L. Norlander, and H. Wolf-Watz.** 1982. Temperature-inducible outer membrane protein of *Yersinia pseudotubercu-*

losis and *Yersinia enterocolitica* is associated with the virulence plasmid. Infect. Immun. **37**:506–512.
2. **Daniels, J. J. H. M.** 1961. Enteral infection with *Pasteurella pseudotuberculosis*. Br. Med. J. **2**:997.
3. **Fukushima, H.** 1985. Direct isolation of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from meat. Appl. Environ. Microbiol. **50**:710–712.
4. **Fukushima, H., K. Hoshina, R. Nakamura, Y. Ito, and M. Gomyoda.** 1987. Epidemiological study of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in Shimane Prefecture, Japan. Contrib. Microbiol. Immunol. **9**:103–110.
5. **Gemski, P., J. R. Lazere, T. Casey, and J. A. Wohlhieter.** 1980. Presence of a virulence-associated plasmid in *Yersinia pseudotuberculosis*. Infect. Immun. **28**:1044–1047.
6. **Higuchi, K., L. L. Kupferberg, and J. L. Smith.** 1959. Studies on the nutrition and physiology of *Pasturella pestis*. III. Effects of calcium ions on the growth of virulent and avirulent strains of *Pasteurella pestis*. J. Bacteriol. **77**:317–321.
7. **Inoue, M., H. Nakashima, O. Ueba, T. Ishida, H. Date, S. Kobashi, K. Takagi, T. Nishu, and M. Tsubokura.** 1984. Community outbreak of *Yersinia pseudotuberculosis*. Microbiol. Immunol. **28**:883–891.
8. **Ishiguro, N., Y. Nakaoka, G. Sato, and M. Tsubokura.** 1985. Plasmid DNA relatedness among different serogroups of *Yersinia pseudotuberculosis*. J. Clin. Microbiol. **21**:662–665.
9. **Kado, C. I., and S.-T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145**: 1365–1373.
10. **Kaneko, K., and N. Hashimoto.** 1981. Occurrence of *Yersinia enterocolitica* in wild animals. Appl. Environ. Microbiol. **41**: 635–638.
11. **Knapp, W.** 1958. Mesenteric adenitis due to *Pasteurella pseudotuberculosis* in young people. N. Engl. J. Med. **259**:776–778.
12. **Laird, W. J., and D. A. Cavanaugh.** 1980. Correlation of autoagglutination and virulence of yersiniae. J. Clin. Microbiol. **11**:430–432.
13. **Macaulay, J. D., J. A. C. Wilson, J. D. Abbott, and N. S. Mair.** 1967. Fatal case of *Pasteurella pseudotuberculosis* infection associated with hepatic cirrhosis. Br. Med. J. **2**:553–554.
14. **Mair, N. S.** 1975. Yersiniosis, p. 174–185. *In* W. T. Hubbert, W. F. McCulloch, and P. R. Schnurrenberger (ed.), Diseases transmitted from animals to man, 6th ed. Charles C. Thomas, Publisher, Springfield, Ill.
15. **Mollaret, H. H.** 1965. Les formes cliniques de l'infection humaine a bacille de malassez et vignal. Pathol. Biol. **13**:554–566.
16. **Narucka, U., and J. F. Westendoorp.** 1977. Studies for the presence of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in clinically normal pigs. Tijdschr. Diergeneeskd. **102**:299–303.
17. **Okamoto, K., T. Kobayashi, S. Shinoda, T. Inoue, J. Yukitake, K. Shimizu, Y. Kawamoto, T. Moriyama, and A. Miyama.** 1984. Cytotoxicity and calcium-dependent antigen of *Yersinia*. Microbiol. Immunol. **28**:33–49.
18. **Paff, J. R., D. A. Triplett, and T. N. Saari.** 1976. Clinical and laboratory aspects of *Yersinia pseudotuberculosis* infections, with a report of two cases. Am. J. Clin. Pathol. **66**:101–110.
19. **Pai, C. H., V. Mors, and S. Toma.** 1978. Prevalence of enterotoxigenicity in human and nonhuman isolates of *Yersinia enterocolitica*. Infect. Immun. **22**:334–338.
20. **Perry, R. D., and R. R. Brubaker.** 1983. Vwa^+ phenotype of *Yersinia enterocolitica*. Infect. Immun. **40**:166–171.
21. **Portnoy, D. A., H. Wolf-Watz, I. Bolin, A. B. Beeder, and S. Falkow.** 1984. Characterization of common virulence plasmids in *Yersinia* species and their role in the expression of outer membrane proteins. Infect. Immun. **43**:108–114.
22. **Reed, L. J., and H. Muench.** 1938. A simple method of estimating fifty percent endpoints. Am. J. Hyg. **27**:493–497.
23. **Rosqvist, R., and H. Wolf-Watz.** 1986. Virulence plasmid-associated HeLa cell induced cytotoxicity of *Yersinia pseudotuberculosis*. Microb. Pathogen. **1**:229–240.
24. **Saari, T. N., and D. A. Triplett.** 1974. *Yersinia pseudotuberculosis* mesenteric adenitis. J. Pediatr. **85**:656–659.
25. **Sanbe, K., M. Uchiyama, K. Koiwai, K. Takagi, H. Yazaki, Y. Nanayama, and M. Ohtawara.** 1987. Community outbreak of *Yersinia pseudotuberculosis* occurred among primary school children. J. Jpn. Assoc. Infect. Dis. **61**:763–771.
26. **Sato, K.** 1987. *Yersinia pseudotuberculosis* infection in children. Contrib. Microbiol. Immunol. **9**:111–116.
27. **Shiozawa, K., M. Akiyama, K. Sahara, M. Hayashi, T. Nishina, M. Murakami, and Y. Asakawa.** 1987. Pathogenicity of *Yersinia enterocolitica* biotype 3B and 4, serotype O:3 isolates from pork samples and humans. Contrib. Microbiol. Immunol. **9**:30–40.
28. **Thal, E.** 1973. Observations on immunity in *Yersinia pseudotuberculosis*. Contrib. Microbiol. Immunol. **2**:190–195.
29. **Toma, S., and V. R. Deidrick.** 1975. Isolation of *Yersinia enterocolitica* from swine. J. Clin. Microbiol. **2**:478–481.
30. **Tsubokura, M., K. Otsuki, T. Fukuda, M. Kubota, M. Imamura, K. Itagaki, K. Yamaoka, and M. Wakatsuki.** 1976. Studies on *Yersinia pseudotuberculosis*. IV. Isolation of *Y. pseudotuberculosis* from healthy swine. Jpn. J. Vet. Sci. **38**:549–552.
31. **Tsubokura, M., K. Otsuki, Y. Kawaoka, H. Fukushima, K. Ikemura, and Y. Kanazawa.** 1984. Addition of new serogroups and improvement of the antigenic designs of *Yersinia pseudotuberculosis*. Curr. Microbiol. **11**:89–92.
32. **Tsubokura, M., K. Otsuki, Y. Kawaoka, and T. Maruyama.** 1982. Isolation and serotyping of *Yersinia pseudotuberculosis* in humans in Japan. Eur. J. Clin. Microbiol. **1**:396–397.
33. **Vesikari, T., J. Bromirska, and M. Maki.** 1982. Enhancement of invasiveness of *Yersinia enterocolitica* and *Escherichia coli* in HEp-2 cells by centrifugation. Infect. Immun. **36**:834–836.
34. **Yanagawa, Y., T. Maruyama, and S. Sakai.** 1978. Isolation of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from apparently healthy dogs and cats. Microbiol. Immunol. **22**: 643–646.
35. **Zen-Yoji, H., S. Sakai, T. Maruyama, and Y. Yanagawa.** 1974. Isolation of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from swine, cattle and rats at an abattoir. Jpn. J. Microbiol. **18**:103–105.