Table 2. Serovar and antibiotic resistance of *Salmonella* isolated from raccoons and masked palm civets

Origin	Species	Subspecies	Serovar	Antibiotic resistance	No. isolates
Raccoon	*Salmonella enterica*	*enterica*	*S.* Mbandaka	–*	5
			S. Infantis	ABPC, NA, OTC	1
				NA, OTC	1
				OTC	1
				–	1
			S. Typhimurium	ABPC, NA, OTC	1
				ABPC, KM, OTC	1
				–	2
			S. Nagoya	–	2
			S. Berta	–	1
			S. Manhattan	OTC	1
			S. Nigeria	OTC	1
			S. Rubislaw	–	1
			S. Thompson	–	1
		diarizonae	UT	–	6
Masked palm civet	*Salmonella enterica*	*enterica*	*S.* Enteritidis	–	1
			S. Nagoya	–	1
			4,12:i:-	–	1

UT, untypable; ABPC, ampicillin; NA, nalidixic acid; OTC, oxytetracycline; KM, kanamycin.
*Susceptible to all antimicrobial agents used in this study.

Salmonella

Salmonella enterica was isolated from the faecal samples of 26 of 459 raccoons (5.7%, 95% CI 7.8–3.5%) and three of 153 masked palm civets (2.0%, 95% CI 4.2–0%). Table 2 shows the serovars and antimicrobial resistance patterns of the isolates. Nine and three serovars were identified in the isolates from raccoons and masked palm civets, respectively. Those serovars included common serovars in gastroenteritis patients and domestic animals (e.g. *S.* Infantis, *S.* Typhimurium, *S.* Thompson, and *S.* Enteritidis) (Esaki et al., 2004; Ishihara et al., 2009; National Institute of Infectious Disease, 2009). Seven of 26 isolates (26.9%) from raccoons showed resistance to at least one antimicrobial agent used in this study, whereas all isolates from masked palm civets were susceptible to all of the antimicrobial agents.

Yersinia

Yersinia spp. were isolated from the faecal samples of 177 of 459 raccoons (38.6%, 95% CI 43.0–34.1%) and 16 of 153 masked palm civets (10.5%, 95% CI 15.3–5.6%). Among the isolates, seven strains from raccoons and six strains from masked palm civets showed positive reactions in autoagglutination tests and were subsequently identified as *Y. pseudotuberculosis*. Therefore, the prevalence of *Y. pseudotuberculosis* in raccoons and masked palm civets was 1.5% (95% CI 2.6–0.4%) and 3.9% (95% CI 7.0–0.8%), respectively. Four, one, and two *Y. pseudotuberculosis* isolates from raccoons belonged to serotypes 1b, 3, and 4b, respectively. Three, two, and one *Y. pseudotuberculosis* isolates from masked palm civets belonged to serotypes 1b, 3, and 4b, respectively. All *Y. pseudotuberculosis* isolates were susceptible to all of the antimicrobial agents used in this study. In all of the *Y. pseudotuberculosis* isolates, the gene of YPMa was detected and the gene of HPI was not detected by PCR assay.

Campylobacter

Campylobacter spp. were isolated from the faecal samples of six of 459 raccoons (1.3%, 95% CI 2.3–0.3%) and 11 of 153 masked palm civets (7.2%, 95% CI 11.3–3.1%). Three isolates from raccoons were identified as *C. jejuni* by multiplex PCR assay. The other *Campylobacter* isolates from raccoons and masked palm civets exhibited three different phenotypic patterns (Table 3). All *the Campylobacter* isolates were susceptible to all of the antimicrobial agents used in this study.

Table 3. Biochemical characteristics of *Campylobacter* spp. isolated from raccoons and masked palm civets

Origin	Species	No. isolates	Oxidase	Catalase	Growth at	
					25°C	42°C
Raccoon	*Campylobacter jejuni*	3	+	+	–	+
	Campylobacter spp.	2	+	+	–	–
		1	+	+	–	+
Masked palm civet	*Campylobacter* spp.	9	+	+	–	+
		1	+	+	+	+
		1	+	+	–	–

Discussion

Salmonella

In this study, the prevalence of *S. enterica* in raccoons (5.7%) was almost concordant with the prevalence in Western Pennsylvania, USA (7.4%) (Compton et al., 2008). Investigations in other wild mammals also have revealed similar prevalence rates in UK (6.5%) and Spain (7.2%) (Euden, 1990; Millan et al., 2004). However, Morse et al. (1983) reported that *S. enterica* was isolated from 31.1% of feral raccoons. This finding may suggest that raccoons have the potential to harbor *S. enterica* at a high rate. The prevalence of *S. enterica* in masked palm civets was somewhat lower than that in raccoons. Differences in their behaviour could be responsible for the difference in the prevalence. Further analysis of their food habits and habitat choice may verify this hypothesis.

Some of the isolates were indicated to have originated from human activities, because many of the serovars isolated have been common in human gastroenteritis and in domestic animals, and the isolates from raccoons showed a relatively high resistance rate (26.9%). Interestingly, six strains isolated from raccoons were identified as *S. enterica* subsp. *diarizonae*. Because this *Salmonella* subspecies is not common in warm-blooded animals but is common in cold-blooded animals and the environment, it may be associated with their omnivorous behaviour and proclivity for wet habitats (Zeveloff, 2002; Bopp et al., 2003; Haley et al., 2009).

Yersinia

Yersinia pseudotuberculosis isolates belonged to serotypes 1b, 3, and 4b, which are predominant serotypes in human patients and wild animals in Japan (Hamasaki et al., 1989; Fukushima and Gomyoda, 1991; Hayashidani et al., 2002). The prevalence of *Y. pseudotuberculosis* in raccoons and masked palm civets was comparable with that of those studies. It is difficult to compare the prevalence with that in its place of origin, because there are few reports on isolation of pathogenic *Yersinia* from raccoons and masked palm civets (Hacking and Sileo, 1974). All of the *Y. pseudotuberculosis* strains showed the same genotypic pattern with the YPMa^{+} HPI^{-} Far Eastern systemic-pathogenicity type. Fukushima et al. (2001) reported that most of the strains isolated in Far East Asia showed such a pattern and differed from the strains isolated in European countries. In addition to the information regarding geographical origin, this virulence characteristic has a clinical implication, because *ypmA* encodes YPMa, which contributes to the virulence of *Y. pseudotuberculosis* in systemic infection (Carnoy et al., 2000).

These results lead to the conclusion that raccoons and masked palm civets probably have acquired their infections in Japan and play a similar role to other indigenous animals on the ecology of *Y. pseudotuberculosis*. In previous studies, natural reservoirs of *Y. pseudotuberculosis* in Japan have been suggested to be wild mammals and birds, especially rodents and raccoon dogs (*Nyctereutes procyonoides*) (Hamasaki et al., 1989; Fukushima and Gomyoda, 1991; Hayashidani et al., 2002). The major transmission routes of the pathogen in wildlife are suggested as preying upon infected animals or ingesting environmental substances contaminated with *Y. pseudotuberculosis* rather than contact with human activities (Fukushima and Gomyoda, 1991). It is likely that raccoons and masked palm civets acquired their infection, as a result of sharing habitats with other reservoirs.

Campylobacter

The principal reservoirs of *Campylobacter* in the environment are wild mammals and birds (Mörner, 2001), with the occurrence of enteric *Campylobacter* higher in birds than in wild mammals. This tendency is consistent with our results. The prevalence rates in raccoons and masked palm civets were 1.3% and 7.2%, respectively, whereas the rate is often more than 10% in wild birds (Kapperud and Rosef, 1983; Matsusaki et al., 1986; Ito et al., 1988). However, wild mammals, especially species that have direct or indirect contact with human activities, are still important reservoirs of the pathogen. Workman et al. (2005) demonstrated that dogs were one of the most likely sources of human campylobacteriosis. Domestic animals, including dogs, are more likely to have opportunities to acquire infections from excretory substances or from environments contaminated by raccoons and masked palm civets.

Among the isolates, three strains were identified as *C. jejuni*. However, the other 14 isolates from both raccoons and masked palm civets could not be identified by the multiplex PCR assay. Additional biochemical tests suggested that these strains belonged to uncommon species of *Campylobacter* (Table 3). As such species (e.g. *C. hyointestinalis*, *C. larienae* or *C. rectus*) are isolated from healthy animals and enteritis patients, their pathogenicity and epidemiology have not been well known (Vandamme et al., 2005). Because there is little information about the carriage of uncommon *Campylobacter* species in wild mammals, further investigations are required to elucidate the real impact of these species in wild animals on public and animal health.

From these results, we concluded that raccoons and masked palm civets could be potential reservoirs of enteropathogenic *Campylobacter* (*C. jejuni*) and are more likely to possess uncommon *Campylobacter* species. To assess the risk and potential source, wild birds in the same habitat and the environment where the animals live should be investigated.

Conclusion

In the present study, we investigated the prevalence of three important enteric pathogens, including *Salmonella*, *Yersinia*, and *Campylobacter* spp. in feral raccoons and masked palm civets. These results lead to the conclusion that these animals are potential reservoirs of the pathogens. The characteristics of the isolates showed that these animals probably acquired the pathogens from human activities, other wild animals, and the environment. The presence of human-associated serovars and the antimicrobial resistance of the *Salmonella* isolates revealed the effect of human activities on these animals. This represents a typical spill-over of pathogens from human activities to wildlife (Daszak et al., 2000). Meanwhile, the carrying of pathogens which are usually isolated from wildlife or from the environment (e.g. *S. enterica* subsp. *diarizonae* and *Y. pseudotuberculosis*) indicated that these animals could play an important role in the life cycles of those bacteria in their habitats. These findings are in concordance with their omnivore behaviour and their wide range of habitats from forests to urban areas (Zeveloff, 2002; Abe, 2005).

Our results revealed that raccoons and masked palm civets play an important role on the spreading of human-related pathogens. Moreover, carriage of wildlife- and environment-related pathogens in these animals showed the possibility of the transmission of these pathogens to humans and domestic animals. Raccoons and masked palm civets live near areas of human habitation and often nest in attics or in the feed stores of livestock. The enteric pathogens that we investigated can be transmitted to humans and domestic animals via feces, contaminated water and soil (Fukushima et al., 1988; Humphrey and Bygrave, 1988; Handeland et al., 2002). Thus, not only the ecological threats but also the public and animal health risks presented by these animals should be assessed in detail.

Acknowledgements

This work was partially supported by Health Sciences Grant for Research on Emerging and Re-emerging Infectious Disease from the Ministry of Health, Labour, and Welfare of Japan. We are grateful to local government offices, M. Kaneda, Cacodaemon network and S. Kato, Strain Ltd. for providing fecal samples in this study.

References

Abe, E. 2005: A guide to the mammals of Japan, pp. 71–106. Tokai University Press, Hatano, Japan.

Aulisio, C. C. G., I. J. Mehlman, and A. C. Sanders, 1980: Alkali method for rapid recovery of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from foods. *Appl. Environ. Microbiol.* 39, 135–140.

Berrada, Z. L., H. K. Goethert, and S. R. Telford, 2006: Raccoons and skunks as sentinels for enzootic tularemia. *Emerg. Infect. Dis.* 12, 1019–1021.

Bigler, W. J., J. H. Jenkins, P. M. Cumbie, G. L. Hoff, and E. C. Prather, 1975: Wildlife and environmental health: raccoons as indicators of zoonoses and pollutants in southeastern United States. *J. Am. Vet. Med. Assoc.* 167, 592–597.

Bopp, C. A., F. W. Brenner, P. I. Fields, J. G. Wells, and N. A. Strockbine, 2003: *Escherichia*, *Shigella*, and *Salmonella*. In: Murray, P. R., E. J. Baro, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (eds), Manual of Clinical Microbiology. ASM Press, Washington, DC.

Carnoy, C., C. Mullet, H. Muller-Alouf, E. Leteurtre, and M. Simonet, 2000: Superantigen YPMa exacerbates the virulence of *Yersinia pseudotuberculosis* in mice. *Infect. Immun.* 68, 2553–2559.

Compton, J. A., J. A. Baney, S. C. Donaldson, B. A. Houser, G. J. San Julian, R. H. Yahner, W. Chmielecki, S. Reynolds, and B. M. Jayarao, 2008: *Salmonella* infections in the common raccoon (*Procyon lotor*) in Western Pennsylvania. *J. Clin. Microbiol.* 46, 3084–3086.

Daszak, P., A. A. Cunningham, and A. D. Hyatt, 2000: Wildlife ecology – emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science* 287, 443–449.

Esaki, H., A. Morioka, K. Ishihara, A. Kojima, S. Shiroki, Y. Tamura, and T. Takahashi, 2004: Antimicrobial susceptibility of *Salmonella* isolated from cattle, swine and poultry (2001–2002): report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. *J. Antimicrob. Chemother.* 53, 266–270.

Euden, P. R., 1990: *Salmonella* isolates from wild animals in Cornwall. *Br. Vet. J.* 146, 228–232.

Finnegan, C. J., S. M. Brookes, N. Johnson, J. Smith, K. L. Mansfield, V. L. Keene, L. M. McElhinney, and A. R. Fooks, 2002: Rabies in North America and Europe. *J. R. Soc. Med.* 95, 9–13.

Fukushima, H., 1987: New selective agar medium for isolation of virulent *Yersinia enterocolitica*. *J. Clin. Microbiol.* 25, 1068–1073.

Fukushima, H., and M. Gomyoda, 1991: Intestinal carriage of *Yersinia pseudotuberculosis* by wild birds and mammals in Japan. *Appl. Environ. Microbiol.* 57, 1152–1155.

Fukushima, H., M. Gomyoda, K. Shiozawa, S. Kaneko, and M. Tsubokura, 1988: *Yersinia pseudotuberculosis* infection contracted through water contaminated by a wild animal. *J. Clin. Microbiol.* 26, 584–585.

Fukushima, H., Y. Matsuda, R. Seki, M. Tsubokura, N. Takeda, F. N. Shubin, I. K. Paik, and X. B. Zheng, 2001: Geographical heterogeneity between Far Eastern and Western countries in prevalence of the virulence plasmid, the superantigen *Yersinia pseudotuberculosis*-derived mitogen, and the high-pathogenicity island among *Yersinia pseudotuberculosis* strains. *J. Clin. Microbiol.* 39, 3541–3547.

Gavin, P. J., K. R. Kazacos, and S. T. Shulman, 2005: Baylisascariasis. *Clin. Microbiol. Rev.* 18, 703–718.

Hacking, M. A., and L. Sileo, 1974: *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* from wildlife in Ontario. *J. Wildl. Dis.* 10, 452–457.

Haley, B. J., D. J. Cole, and E. K. Lipp, 2009: Distribution, diversity, and seasonality of waterborne salmonellae in a rural watershed. *Appl. Environ. Microbiol.* 75, 1248–1255.

Hamasaki, S., H. Hayashidani, K. Kaneko, M. Ogawa, and Y. Shigeta, 1989: A survey for *Yersinia pseudotuberculosis* in migratory birds in coastal Japan. *J. Wildl. Dis.* 25, 401–403.

Hamir, A. N., C. A. Hanlon, M. Niezgoda, and C. E. Rupprecht, 2001: The prevalence of interstitial nephritis and leptospirosis in 283 raccoons (*Procyon lotor*) from 5 different sites in the United States. *Can. Vet. J.* 42, 869–871.

Handeland, K., T. Refsum, B. S. Johansen, G. Holstad, G. Knutsen, I. Solberg, J. Schulze, and G. Kapperud, 2002: Prevalence of *Salmonella* Typhimurium infection in Norwegian hedgehog populations associated with two human disease outbreaks. *Epidemiol. Infect.* 128, 523–527.

Hayashidani, H., N. Kanzaki, Y. Kaneko, A. T. Okatani, T. Taniguchi, K. Kaneko, and M. Ogawa, 2002: Occurrence of Yersiniosis and Listeriosis in wild boars in Japan. *J. Wildl. Dis.* 38, 202–205.

Humphrey, T. J., and A. Bygrave, 1988: Abortion in a cow associated with salmonella infection in badgers. *Vet. Rec.* 123, 160.

Ikeda, T., M. Asano, Y. Matoba, and G. Abe, 2004: Present status of invasive alien raccoon and its impact in Japan. *Glob. Environ. Res.* 8, 125–131.

Inokuma, H., T. Makino, H. Kabeya, S. Nogami, H. Fujita, M. Asano, S. Inoue, and S. Maruyama, 2007: Serological survey of *Ehrlichia* and *Anaplasma* infection of feral raccoons (*Procyon lotor*) in Kanagawa Prefecture, Japan. *Vet. Parasitol.* 145, 186–189.

Ishihara, K., T. Takahashi, A. Morioka, A. Kojima, M. Kijima, T. Asai, and Y. Tamura, 2009: National surveillance of *Salmonella enterica* in food-producing animals in Japan. *Acta Vet. Scand.* 51, 35. Doi:10.1186/1751-0147-51-35.

Ito, K., Y. Kubokura, K. Kaneko, Y. Totake, and M. Ogawa, 1988: Occurrence of *Campylobacter jejuni* in free-living wild birds from Japan. *J. Wildl. Dis.* 24, 467–470.

Japan Veterinay Medical Association, 2007: Guidelines for the Management of Invasive Alien Species, The wildlife Committee Report. Division of small animal medicine in JVMA.

Junge, R., and D. F. Hoffmeister, 1980: Age determination in raccoons from cranial suture obliteration. *J. Wildl. Manage.* 44, 725–729.

Kapperud, G., and O. Rosef, 1983: Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway. *Appl. Environ. Microbiol.* 45, 375–380.

Kawabuchi, T., M. Tsuji, A. Sado, Y. Matoba, M. Asakawa, and C. Ishihara, 2005: *Babesia microti*-like parasites detected in feral raccoons (*Procyon lotor*) captured in Hokkaido, Japan. *J. Vet. Med. Sci.* 67, 825–827.

Kobayashi, T., Y. Kanai, Y. Ono, Y. Matoba, K. Suzuki, M. Okamoto, H. Taniyama, K. Yagi, Y. Oku, K. Katakura, and M. Asakawa, 2007: Epidemiology, histpathology, and muscle distribution of *Trichinella* T9 in feral raccoons (*Procyon lotor*) and wildlife of Japan. *Parasitol. Res.* 100, 1287–1291.

Laird, W. J., and D. C. Cavanaugh, 1980: Correlation of autoagglutination and virulence of yersiniae. *J. Clin. Microbiol.* 11, 430–432.

Lee, K., T. Iwata, M. Shimizu, T. Taniguchi, A. Nakadai, Y. Hirota, and H. Hayashidani, 2009: A novel multiplex PCR assay for *Salmonella* subspecies identification. *J. Appl. Microbiol.* 107, 805–811.

Luangtongkum, T., T. Y. Morishita, A. B. El-Tayeb, A. J. Ison, and Q. J. Zhang, 2007: Comparison of antimicrobial susceptibility testing of *Campylobacter* spp. by the agar dilution and the agar disk diffusion methods. *J. Clin. Microbiol.* 45, 590–594.

Machida, N., N. Izumisawa, T. Nakamura, and K. Kiryu, 1992: Canine distemper virus infection in a masked palm civet (*Paguma larvata*). *J. Comp. Pathol.* 107, 439–443.

Matsusaki, S., A. Katayama, K. Itagaki, H. Yamagata, K. Tanaka, T. Yamami, and W. Uchida, 1986: Prevalence of *Campylobacter jejuni* and *Campylobacter coli* among wild and domestic animals in Yamaguchi Prefecture. *Microbiol. Immunol.* 30, 1317–1322.

Millan, J., G. Aduriz, B. Moreno, R. A. Juste, and M. Barral, 2004: *Salmonella* isolates from wild birds and mammals in the Basque Country (Spain). *Rev. Sci. Technol.* 23, 905–911.

Montgomery, G. G., 1964: Tooth eruption in preweaned raccoons. *J. Wildl. Manage.* 28, 582–584.

Mörner, T., 2001: *Campylobacter* infection. In: Williams, E. S., and I. K. Barker (eds), Infectious Diseases of Wild Mammals, pp. 488–489. Iowa State University Press, Ames, IA.

Morse, E. V., D. A. Midla, and K. R. Kazacos, 1983: Raccoons (*Procyon lotor*) as carriers of *Salmonella*. *J. Environ. Sci. Health. A* 18, 541–560.

National Committee for Clinical Laboratory Standards, 2002: Performance Standards for Antimicrobial Disk Susceptibility Tests M100-S12. NCCLS, Villanova, PA.

National Institute of Infectious Disease, 2009: Salmonellosis in Japan as of June 2009. *Infect. Agent Surveill. Rep.* 30, 203–204.

Popoff, M. Y., and L. Le Minor, 2001: Antigenic formulas of the *Salmonella* serovars. WHO Collaboratin Centre for Reference and Research on *Salmonella*. Institute Pasteur, Paris, France.

Popoff, M. Y., and L. E. Le Minor, 2005: *Genus XXXIII.* Salmonella. In: Brenner, D. J., N. R. Krieg, and J. T. Staley (eds), Bergey's Manual of Systematic Bacteriology: The

Proteobacteria, The Gammaproteobacteria, pp. 764–798. Springer-Verlag, Berlin, Germany.
Sato, H., and K. Suzuki, 2006: Gastrointestinal helminths of feral raccoons (*Procyon lotor*) in Wakayama Prefecture, Japan. *J. Vet. Med. Sci.* 68, 311–318.
Schiemann, D. A., 1979: Synthesis of a selective agar medium for *Yersinia enterocolitica*. *Can. J. Microbiol.* 25, 1298–1304.
Tu, C., G. Crameri, X. Kong, J. Chen, Y. Sun, M. Yu, H. Xiang, X. Xia, S. Liu, T. Ren, Y. Yu, B. T. Eaton, H. Xuan, and L. F. Wang, 2004: Antibodies to SARS coronavirus in civets. *Emerg. Infect. Dis.* 10, 2244–2248.
Vandamme, P., F. E. Dewhirst, B. J. Paster, and S. L. W. On, 2005: *Genus I.* Campylobacter. In: Garrity, G. (ed), Bergey's Manual of Systematic Bacteriology: The Proteobacteria; The Alpha-, Beta-, Delta-, and Epsilonproteobacteria, pp. 1147–1160. Springer-Verlag, Berlin, Germany.
Wang, G., C. G. Clark, T. M. Taylor, C. Pucknell, C. Barton, L. Price, D. L. Woodward, and F. G. Rodgers, 2002: Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, and *C. fetus* subsp. *fetus*. *J. Clin. Microbiol.* 40, 4744–4747.
Wauters, G., M. Janssens, A. G. Steigerwalt, and D. J. Brenner, 1988: *Yersinia mollaretii* sp. nov and *Yersinia bercovieri* sp. nov, formerly called *Yersinia enterocolitica* biogroups 3A and 3B. *Int. J. Syst. Bacteriol.* 38, 424–429.
Workman, S. N., G. E. Mathison, and M. C. Lavoie, 2005: Pet dogs and chicken meat as reservoirs of *Campylobacter* spp. in Barbados. *J. Clin. Microbiol.* 43, 2642–2650.
Zeveloff, S. I., 2002: Raccoons: A Natural History. Smithsonian Institution Press, Washington, DC.