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REVIEW

Epidemiological Findings on Yersiniosis in Nonhuman Primates in Zoological Gardens in Japan

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Abstract

Yersiniosis, which is caused by pathogenic *Yersinia enterocolitica* or *Yersinia pseudotuberculosis*, poses a serious problem for zoological gardens engaged in breeding nonhuman primates. In Japan, *Y. pseudotuberculosis* in particular frequently causes fatal infection, and affected nonhuman primates may die unexpectedly or after a very short illness. Our epidemiological study in 17 zoological gardens in Japan suggested that *Yersinia pseudotuberculosis*-derived mitogen (YPM), which is a kind of superantigenic toxin, might be the cause of, or at least the most important factor in, the high mortality of breeding nonhuman primates infected by *Y. pseudotuberculosis* in Japan. Furthermore, seroepidemiological study proved that pathogenic *Yersinia* is highly prevalent among breeding squirrel monkeys in Japan. It is likely that the monkeys that are pathogenic *Yersinia* positive have been inapparently or mildly infected by low pathogenic strains of *Yersinia*, not highly pathogenic strains of *Yersinia*, such as YPM-producing *Y. pseudotuberculosis*. In this review, we will describe the epidemiology of yersiniosis in breeding nonhuman primates in Japan.

Discipline: Animal health
Additional key words: epidemiology, virulence factor, *Yersinia enterocolitica*, *Y. pseudotuberculosis*, zoological garden

Introduction

The genus *Yersinia*, a member of the family *Enterobacteriaceae*, consists of 14 species of gram-negative bacilli[27,40,41,43]. Three of those species are pathogenic species, *Y. pestis*, the causative agent of plague, and the enteric food- and water-borne pathogens *Y. enterocolitica* and *Y. pseudotuberculosis*. In Japan, *Y. enterocolitica* and *Y. pseudotuberculosis* are distributed, not *Y. pestis*, and the important causal agents of zoonosis, yersiniosis. *Y. enterocolitica* is classified into 51 serovars, based on O-antigen, and includes both non-pathogenic and pathogenic groups. *Y. enterocolitica* serovars O:3, O:5,27, O:8, and O:9 are known to be representative pathogenic serovars, which are frequently isolated from humans and animals[1,21,37]. On the other hand, *Y. pseudotuberculosis* is classified into 15 serovars, and serovars 1 to 6, 10, and 15 have been isolated from clinical samples[8,30,31].

Pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* have a wide distribution in wild animals, livestock, and the environment. Both pathogens generally contaminate food and are responsible for foodborne disease in humans. The usual clinical symptom is gastrointestinal disease, but highly pathogenic strains of *Yersinia*, for example, *Y. pseudotuberculosis* and *Y. enterocolitica* serovar O:8, sometimes cause septicemia[2]. On the other hand, many animal species carry the agents as an inapparent infection, with some exceptions. Pigs are known to be an important reservoir of pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis*[6,9,25]. Pathogenic *Yersinia* is frequently found on the tonsils and in the intestinal content of clinically healthy pigs at slaughterhouses around the world, so pork is suspected to be one of the major sources of this food poisoning. In the wild, wild rodents are a major reservoir of pathogenic *Yersinia*, and in Japan, *Y. pseudotuberculosis* is frequently isolated from raccoon dogs[7,44]. Wild birds are also representative reservoirs of pathogenic *Yersinia*, and in Europe, it has been reported

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that wild birds are a major reservoir of *Y. pseudotuberculosis* serovar 1a[8,24]. It is thought that these wild animals contaminate water with their feces, which contains pathogenic *Yersinia*, and the contaminated water causes yersiniosis in humans.

In contrast, nonhuman primate species are very sensitive to pathogenic *Yersinia*, and fatal cases of yersiniosis have been reported in various nonhuman primate species throughout the world[23,39,45]. In Japan, *Y. pseudotuberculosis* outbreaks frequently occur during cold seasons[14,20,26,29,35,46]. The nonhuman primates affected with *Y. pseudotuberculosis* may die unexpectedly or after a very short illness, and following death, they show typical pathological findings, such as severe enteritis, swelling of a Peyer's patch, and multiple abscesses in the spleen and liver. Therefore, it is difficult to provide medical treatment based on confirmed diagnosis before death. Many nonhuman primate species kept at zoological gardens are formally recognized as "threatened" by the International Union for Conservation in Nature (IUCN), and their deaths pose a serious loss to the zoological gardens. There are many clinical and/or pathological reports of fatal infection with pathogenic *Yersinia* in breeding nonhuman primates. However, there has not been any detailed information on the epidemiology of yersiniosis in breeding nonhuman primates. This paper reviews the epidemiology of yersiniosis in breeding nonhuman primates in Japan.

1. Characteristics of pathogenic *Yersinia* isolated from breeding nonhuman primates in Japan

It has been reported that the pathogenicity of pathogenic *Yersinia* is associated with several virulence factors. Pathogenic *Yersinia* harbors 70-kb virulence plasmid (pYV), which encodes a number of important virulence and virulence-associated proteins[5,42]. Of those, *Yersinia* outer membrane proteins (Yops) are known to be an important factor in virulence, and their production is controlled by the *virF* gene[5]. As the chromosomal genes, the *inv* and *ail* genes are involved in bacterial attachment to and invasion of epithelial cells in vitro, and are specifically harbored by *Y. pseudotuberculosis* and *Y. enterocolitica*, respectively. Additionally, high-pathogenicity islands (HPIs), which carry the genes involved in siderophore synthesis and the acquisition of iron during mammalian infection, for example, the *irp2* gene[38], and *Y. pseudotuberculosis*-derived mitogen (YPM), which is a superantigenic toxin, are known to play an important role in causing severe systemic infection[3,16]. However, it remains unclear which virulence factor is connected with the high mortality of nonhuman primates in pathogenic *Yersinia* infection. As a result, an investigation of the characteristics of pathogenic *Yersinia* isolated from dead breeding nonhuman primates was performed in Japan.

The 74 breeding nonhuman primates of nine species that died at 17 zoological gardens in Japan were examined for the presence of pathogenic *Yersinia* between 2001 and 2007. Pathogenic *Yersinia* was isolated from 35 breeding nonhuman primates of eight species that died in 13 of the 17 zoological gardens, characterizing 23 yersiniosis outbreaks (Table 1). *Yersinia* was isolated in almost all the nonhuman primates, which showed the typical pathological findings of yersiniosis. A total of 19 *Y. pseudotuberculosis* strains and four pathogenic *Y. enterocolitica* strains, i.e., one strain per outbreak isolated from the dead nonhuman primates, were examined for serovars and for virulence genes (Table 2).

The predominant serovars of *Y. pseudotuberculosis* isolated from dead nonhuman primates were serovar 1b (36.8%) and 4b (42.1%) (Table 1). These serovars were also predominant serovars isolated from clinical samples, e.g., of human patients. The majority of the strains of these serovars are highly pathogenic with *ypmA*[8]. It is known that the presence of *ypmA* is limited to the Far East (Japan, Korea, and Far-Eastern Russia), and also that it exacerbates the toxicity of *Y. pseudotuberculosis* in systemic infection in mice[8]. Moreover, it has been reported that the clinical signs of *Y. pseudotuberculosis* infection found in the Far East include not only fever, gastroenteric symptoms, and mesenteric lymphadenitis, which are the main symptoms in Europe, but also a variety of systemic manifestations, such as rash, desquamation, erythema nodosum, and arthritis[36]. A variety of monkeys that are native to South America, Southeast Asia or Africa are bred in zoological gardens in Japan, as listed in Table 1, as well as Japanese macaques. It has been noted that nonhuman primates from those regions, where the presence of *Y. pseudotuberculosis* with the *ypm* gene has not been identified, frequently die when infections with this pathogen occur, while there has been little mortality among Japanese macaques due to *Y. pseudotuberculosis* infection[20]. Because of the persistent exposure of Japanese macaques to *Y. pseudotuberculosis* with the *ypm* gene from ancient times, they may have acquired resistance to that pathogen, unlike imported nonhuman primates. Thus, YPM seems to be the main cause of the high mortality of the monkeys imported from abroad.

The strain of serovar 7 has never been isolated from a clinical case and has been considered a nonpathogenic serovar, i.e., the first reported isolation of *Y. pseudotuberculosis* serovar 7 from a clinical sample anywhere in the world. This serovar has been isolated from dogs, raccoon dogs, moles, wild mice, and water[8]. However, there have been no reports about *Y. pseudotuberculosis* serovar 7 isolated from samples of primate origin. Pathological analysis of the squirrel monkey, from which serovar 7 was isolated, showed the typical pathological findings of yersiniosis, and PCR analysis demonstrated that the strain of serovar 7 also harbored pYV and *ypmA* genes (Table 2). These results suggest that serovar 7 has the same degree of pathogenicity as other pathogenic serovars. Therefore, we should pay

attention to the possibility of humans and other animal species being infected by serovar 7.

Of the four *Y. enterocolitica* strains isolated in our study, two were of serovar O:8, and there was one each of

Table 1. Cases of pathogenic *Yersinia* fatal infection in breeding nonhuman primates in Japan

No.	Species of isolated bacteria	Institution	Region	Incident Month-Year	Animal species (species and number of other monkeys dead in the same outbreak)
1	*Y. pseudotuberculosis*	A	Kanto	Apr-02	Squirrel monkey
2		B	Kanto	Nov-03	Orangutan
3		C	Kanto	Nov-03	Squirrel monkey (1 Squirrel monkey)
4		C	Kanto	Jan-05	Squirrel monkey
5		D	Kanto	Apr-07	Dusky leaf monkey
6		E	Kanto	May-07	Squirrel monkey
7		F	Kinki	Dec-03	Squirrel monkey (2 Squirrel monkeys)
8		G	Chugoku	Mar-04	Squirrel monkey
9		H	Sikoku	Apr-01	Squirrel monkey
10		H	Sikoku	Apr-03	Squirrel monkey
11		H	Sikoku	Jan-05	Squirrel monkey
12		H	Sikoku	Dec-05	Squirrel monkey
13		I	Kyusyu	May-03	Squirrel monkey
14		I	Kyusyu	Jun-03	Squirrel monkey
15		I	Kyusyu	Feb-07	Squirrel monkey
16		J	Kyusyu	Jul-03	Squirrel monkey (1 Squirrel monkey)
17		K	Kyusyu	Feb-05	Hamadryas baboon (1 Hamadryas baboon and 1 Dark-handed gibbon)
18		L	Kyusyu	Mar-05	Squirrel monkey (1 Squirrel monkey)
19		M	Kyusyu	Mar-05	White-faced saki (1 White-faced saki, 1 Ruffed lemur and 1 Ring-tailed lemur)
20	*Y. enterocolitica*	E	Kanto	Dec-02	Squirrel monkey (2 Squirrel monkey)
21		E	Kanto	Apr-03	Dark-handed gibbon
22		I	Kyusyu	May-05	Squirrel monkey
23		I	Kyusyu	May-07	Squirrel monkey

Table 2. Characteristics of pathogenic *Yersinia* isolated from dead breeding nonhuman primates

No.	Species	Virulence genes[a]							Serovar
		virF	*ail*	*inv*	*ypm*			*irp2*	
					ypmA	*ypmB*	*ypmC*		
1	*Y. pseudotuberculosis*	+	–	+	+	–	–	–	4b
2		+	–	+	+	–	–	–	4b
3		+	–	+	+	–	–	–	4b
4		+	–	+	+	–	–	–	4b
5		+	–	+	+	–	–	–	1b
6		+	–	+	+	–	–	–	4b
7		+	–	+	+	–	–	–	4b
8		+	–	+	+	–	–	–	4b
9		+	–	+	+	–	–	–	1b
10		+	–	+	+	–	–	–	6
11		+	–	+	+	–	–	–	1b
12		+	–	+	+	–	–	–	2b
13		+	–	+	–	–	–	–	4b
14		+	–	+	+	–	–	–	7
15		+	–	+	+	–	–	–	1b
16		+	–	+	+	–	–	–	1b
17		+	–	+	–	–	–	–	3
18		+	–	+	+	–	–	–	1b
19		+	–	+	+	–	–	–	1b
20	*Y. enterocolitica*	+	+	–	–	–	–	+	O:8
21		+	+	–	–	–	–	+	O:8
22		+	+	–	–	–	–	–	O:3
23		+	+	–	–	–	–	–	O:5,27

[a]+: positive, –: negative

serovars O:3 and O:5,27. *Y. enterocolitica* serovar O:8 is often related to human infection; however, there have been no reports about natural infection in animals caused by this serovar. Thus, this is also the first report of a fatal case of *Y. enterocolitica* serovar O:8 infection in animals anywhere in the world. *Yersinia* outbreaks in breeding nonhuman primates have been reported in Japan, but all except one reported outbreak were of *Y. pseudotuberculosis*[28]. The outbreaks of *Y. enterocolitica* were confirmed in breeding nonhuman primates, not only low pathogenic serovars O:3 and O:5,27 but also the high pathogenic serovar O:8. These results indicate the need for more attention to the possibility of *Y. enterocolitica* outbreaks in breeding nonhuman primates in Japan.

2. Epidemiological study of *Y. enterocolitica* serovar O:8 infection in breeding nonhuman primates in Japan

Y. enterocolitica serovar O:8 has unique features. This serovar, which harbors HPIs, is known to be the most pathogenic serovar of *Y. enterocolitica*[32]. Its geographic distribution is very limited. Human patients infected with serovar O:8 have been sporadically reported in the northern Tohoku region[11,34]. We will introduce here an outbreak of *Y. enterocolitica* serovar O:8 in breeding nonhuman primates at a zoological garden in the Kanto region of Japan (Table 1), which we observed in the process of investigating occurrences of serovar O:8 infection in breeding nonhuman primates. From December 2002 to January 2003, five of 50 squirrel monkeys housed in a zoological garden in the Kanto region of Japan died following a few days of diarrhea. After this outbreak in the squirrel monkeys had ended, one of two dark-handed gibbons died in April 2003, showing similar clinical signs. All of the dead nonhuman primates showed the typical pathological findings of yersiniosis. The organs of three of the dead squirrel monkeys and of the dark-handed gibbon were examined, and *Y. enterocolitica* serovar O:8 was isolated.

In order to determine the source and the transmission route of infection, 98 fecal samples (45 from squirrel monkeys, 20 from other nonhuman primates of 18 different species, and 33 from black rats captured around the nonhuman primate houses) and seven water samples were collected in the zoological garden, and were examined for the prevalence of *Y. enterocolitica* serovar O:8. Serovar O:8 was isolated from 21 of the 65 nonhuman primates (32.3%) and five of the 33 (15.2%) black rats (Table 3). Furthermore, the 30 isolates, consisting of 26 isolates from fecal samples and four isolates from the organs of the dead nonhuman primates (one from each nonhuman primate), were examined by molecular typing, pulsed field gel electrophoresis (PFGE), ribotyping using a RiboPrinter system, and restriction endnuclease analysis of virulence plasmid DNA (REAP). Molecular genetic analysis of the 30 isolates from the analyzed samples showed that all but one had the same molecular genotype, suggesting that these isolates originated from a common source. Moreover, the order in which the infection occurred in the different nonhuman primate species in the zoological garden, with the initial outbreak among the squirrel monkeys (December 2002 to January 2003), followed by the dark-handed gibbon (April 2003), together with the results of the molecular genetic analysis, suggests that O:8 infection occurred first in the colony of squirrel monkeys, and was then transmitted to the dark-handed gibbon. Since the isolates from the black rats had the same molecular genotypes of the two nonhuman primate species, these rats might have been the vector between the two species. Moreover, given that the prevalence of *Y. enterocolitica* serovar O:8 in the black rats captured in this area was relatively high, and considering the time lag between the infection of the two colonies of the nonhuman primates, the black rats might have played an important role as a vector of strains of this serovar in the zoological garden.

Hayashidani et al.[12] have classified Japanese O:8 isolates into seven genotypes based on a combination of the results of PFGE and ribotyping. A comparison of the molecular genotypes of the 30 isolates from our study with other Japanese isolates analyzed by Hayashidani et al.[12] showed that the molecular genotype of 29 isolates was very similar to that of the strains isolated from wild rodents captured in Niigata Prefecture, which borders the Kanto region in the northwest. It also showed that the genotype of the one isolate that differed from the other 29 showed a molecular genotype similar to that of an isolate from a wild rodent in Yamagata Prefecture in northeastern Japan (Table 4). It is tempting to speculate that the strains isolated from the nonhuman primates might have originated in wild rodents.

3. Seroepidemiological study of pathogenic *Yersinia* in breeding squirrel monkeys in Japan

As described above, many fatal cases of yersiniosis occurred in breeding nonhuman primates in Japan. Zoological gardens in Japan keep a variety of nonhuman primate species that originated in semitropical and tropical region. The highest number of dead nonhuman primates by *Y. pseudotuberculosis* infection in Japan occurred among squirrel monkeys (Table 1). The habitat of the squirrel monkey is South and Central America, but many zoological gardens in Japan have been breeding monkeys imported from those regions. To determine the prevalence of pathogenic *Yersinia* infection in breeding nonhuman primates, a seroepidemiological study of squirrel monkeys in Japan by ELISA using semi-purification Yops as an antigen was carried out.

The serum samples of 252 squirrel monkeys from nine zoological gardens in Japan and 91 squirrel monkeys

Table 3. Isolation of *Yersinia enterocolitica* serovar O:8 from breeding nonhuman primates and environmental materials in the zoological garden

	Source	Number of animals examined	Number of serovar O:8 isolates (%)
Breeding nonhuman primates	Common squirrel monkey (*Saimiri sciureus*)	45	17 (37.8%)
	Common chimpanzee (*Pan troglodytes*)	2	1 (50.0%)
	Crab-eating macaque (*Macaca fascicularis*)	1	1 (100.0%)
	De Brazza's monkey (*Cercopithecus neglectus*)	1	1 (100.0%)
	Vervet monkey (*Cercopithecus aethiops*)	1	1 (100.0%)
	Others (14species)	15	0 (0.0%)
	Subtotal	65	21 (32.3%)
Environmental materials	Black rat (*Rattus rattus*)	33	5 (15.2%)
	Water	7	0 (0.0%)
	Subtotal	40	5 (12.5%)
	Total	105	26 (24.8%)

Table 4. Typing results for *Yersinia enterocolitica* serovar O:8 isolated in Japan using PFGE and ribotyping

Geno-type[a]	PFGE pattern	Ribo-pattern	REAP pattern	Strain	Region	Source	References
I	A	R1	P1	YE93009	Aomori	Patient	12
II	A	R4	P1	YE89023	Aomori	Patient	12
III	B	R1	P2	APCC Y9314	Aomori	Patient	12
IV	B	R3	P2	YE87069	Aomori	Patient	12
V	C	R1	P1	NY9504002	Aomori	Wild rodent	12
VI	D	R1	P3	NY891001	Niigata	Wild rodent	12
VII	D	R2	P3	YE9809001	Yamagata	Wild rodent	12
VI'	D'	R1	P3	NY0212001	Saitama	Squirrel monkey	17
VII'	D'	R2	P3	NY0304008	Saitama	Black rat	17

[a] Genotype was produced by combining the results obtaining using PFGE with Not I and ribotyping. The prime (') denotes a closely related type or pattern.

imported from Suriname were tested, and the results were interpreted by measuring optical density (OD). The OD of 91 monkeys from Suriname, where no presence of pathogenic *Yersinia* has been reported[10], was measured. As these monkeys showed low OD, they were considered to be a negative control. The cutoff value was calculated as the mean OD of the negative sera plus 3 standard deviations (SD); therefore, the cutoff value was 0.113. Among the 252 squirrel monkeys tested, 164 (65.1%) showed an OD higher than the cutoff value and were therefore considered positive (Fig. 1).

It was revealed that pathogenic *Yersinia* is highly prevalent among breeding squirrel monkeys in Japan. Yops used as an antigen of ELISA is encoded in pYV, which is harbored in pathogenic strains of *Yersinia*. Regardless of the species and serovars of *Yersinia*, it is known that antibodies to Yops significantly rise after humans and animals are infected with pathogenic *Yersinia*[4,13]. Therefore, the

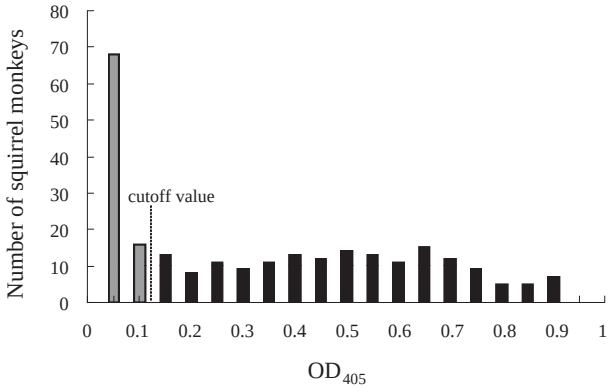

Fig. 1. Analysis of sera obtained from 252 breeding squirrel monkeys in Japan
The vertical dashed line represents the cutoff value, which was calculated as 0.113.
: negative samples, : positive samples.

squirrel monkeys that were considered Yops positive must have been infected by pathogenic *Yersinia* in the past. Squirrel monkeys that do not have any immunity to yersiniosis, such as infant squirrel monkeys, seem to die at a high rate when infected with highly pathogenic strains of *Yersinia*. On the other hand, low pathogenic *Yersinia* strains, for example, non-YPM-producing *Y. pseudotuberculosis* and *Y. enterocolitica* serovars O:3, O:5,27, and O:9, are also widely distributed in wild animals and livestock[8], while only a few fatal cases of breeding nonhuman primates with these strains have been reported in Japan[28]. These results suggested that squirrel monkeys will either show clinical signs or die when infected with highly pathogenic strains of *Yersinia*, for example, YPM-producing *Y. pseudotuberculosis*. The monkeys showing antibodies to Yops have been inapparently or mildly infected with low pathogenic strains of *Yersinia*, not highly pathogenic strains of *Yersinia.*

These positive monkeys belonged to eight of nine zoological gardens, and the percentage of seropositive monkeys ranged from 22.2 to 89.4% (Table 5). The zoological gardens that we investigated did not collect sufficient information on each individual, so it was difficult to explain the reason for the differences in the positive rate among zoological gardens. However, it seems to depend on the breeding system and the age of monkeys. Kihara et al.[22] reported that the frequency of isolation from fecal samples of outdoor-kept nonhuman primates was markedly higher than that of indoor-kept nonhuman primates. In our study, Institution I and L, which keep squirrel monkeys in outdoor surroundings, also showed high positive rates of 73.8 and 89.4%, respectively. Furthermore, Institute I has individually labeled all theirs monkeys with electronic microchips, so the prevalence of the serum antibody to Yops was arranged by age in Institute I. The positive rate of monkeys that were over one year old (95.7%) was significantly higher than that of monkeys under one year old (23.3%). It is likely that inapparent infections of low pathogenic *Yersinia* frequently occur in breeding squirrel monkeys in Japan.

Table 5. Prevalence of serum antibody to Yops in squirrel monkeys from 9 institutions in Japan

Region	Institution[a]	No. of positive samples / No. of samples tested (%)
Kanto	E	9 / 23 (39.1)
	N	12 / 15 (80.0)
	O	0 / 10 (0.0)
	P	2 / 9 (22.2)
Kinki	F	6 / 8 (75.0)
	Q	6 / 23 (26.1)
Shikoku	H	11 / 14 (78.6)
Kyusyu	I	76 / 103 (73.8)
	L	42 / 47 (89.4)
Total		164 / 252 (65.1)

[a] Institution E, F, H, I, and L represent the same institution listed in Table 1, respectively.

Future Prospects

Yersiniosis is the most severe problem in breeding nonhuman primates, so it is important to develop methods to prevent pathogenic *Yersinia* infection in nonhuman primates as soon as possible. However, at the present time, it is difficult to prevent pathogenic *Yersinia* infection in breeding nonhuman primates, even with proper attention to facility maintenance and sanitation, as well as feed hygiene. Most breeding monkeys at zoological gardens are kept in outdoor cages or enclosures for exhibition. These conditions lead to the exposure of the nonhuman primates to animals living in the wild, such as birds and rodents, and as pathogenic *Yersinia* is widely distributed in wild animals, the probability of transmission of this pathogen from those animals is very high. Moreover, it is almost impossible to completely prevent wild animals from invading the cages of nonhuman primates, and the food and water provided for nonhuman primates are easily contaminated. Therefore, development of an effective vaccine is important for preventing pathogenic *Yersinia* infection in breeding nonhuman primates.

Studies on vaccine development against pathogenic *Yersinia* have long been conducted mainly for *Y. pestis*, because *Y. pestis* has caused plague with high mortality among humans, and it can be used as a weapon[33]. Recently, a number of approaches are underway to improve the efficacy of the combination vaccine that includes *Y. pestis*-specific capsular antigen F1 and low calcium response antigen V (LcrV), although it has yet to be put to practical use[15,33,47]. On the other hand, pathogenic *Y. enterocolitica* and *Y. pseudotuberculosis* are foodborne pathogens, and in almost all cases, the infections are initiated by consumption of contaminated food or water leading to limited gastrointestinal symptoms that are not lethal to humans. Therefore, vaccine development against yersiniosis has not been considered as important. In the future, we will need to advance research into the development of an inexpensive and easily used vaccine for breeding nonhuman primates.

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