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Serological and Epidemiological Studies of Parapoxvirus Infection in Cattle in Ishikawa Prefecture

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Abstract

A survey of antibodies against parapoxvirus species bovine papular stomatitis virus (BPSV) was conducted by agar gel immunodiffusion (AGID) test on 750 serum samples collected from cattle in Ishikawa Prefecture, Japan, in 2007. On farms, 0-21.9% dairy cattle and 8.3-33.3% beef cattle were seropositive, but the difference was not significant. The positive reaction rate was significantly higher in dairy cattle reared on public pastures (33.3-82.4%) than in dairy and beef cattle reared on farms. Among dairy cattle on farms, the positive reaction rate was significantly higher in cattle reared on public pastures and in cattle introduced from other prefectures than in cattle that were neither reared on pastures nor introduced from other prefectures. The same serum samples were tested for antibodies against orf virus (ORFV) by AGID, and the positive reaction rate for ORFV was almost the same or less than that for BPSV, and the immunodiffusion line on the gel was weaker for ORFV. There was no correlation between positive reaction rates in cattle and grazing on public pastures that geographically overlap with the habitat of Japanese serows (*Capricornis crispus*), which are also hosts of parapoxvirus. These results suggest that the risk of BPSV infection is higher for dairy cattle reared on pastures than on farms, but the risk of infection due to grazing on pastures inhabited by Japanese serows seems to be low.

Discipline: Animal health
Additional key words: agar gel immunodiffusion test, bovine papular stomatitis virus, orf virus

Introduction

Parapoxviruses cause papules, nodules, and scabs in the skin around the mouth and teats of affected ruminants, cattle, sheep, and goats[8]. Parapoxviruses also infect humans and cause "milker's nodule" or "human orf". Parapoxvirus infection has a low fatality rate in animals, but the lesions around the mouth or on the teats can progress to ulcers in some severe cases, leading to economic losses for farmers. Genus *Parapoxvirus* in the family *Poxviridae* includes bovine papular stomatitis virus (BPSV), pseudocowpox virus, orf virus (ORFV), and parapoxvirus of red deer in New Zealand. All four viruses are serologically cross-reactive and cause similar histopathological lesions in the skin. Moreover, genetic analyses of these viruses using restriction endonuclease patterns showed that some ORFVs maintained mainly in sheep and goats can infect cattle[8].

A Japanese serological survey of parapoxvirus in cattle in 1998 indicated that the morbidity of parapoxvirus infection is very high, ranging from 40 to 98% in cattle in 27 prefectures[12]. However, the status of infection in cattle in Ishikawa Prefecture, as well as neighboring Toyama and Fukui prefectures, had not been investigated. Parapoxvirus infections are frequently seen in cattle and are continuously and frequently observed in free-ranging Japanese serows (*Capricornis crispus*) in Ishikawa Prefecture. The wide range and increasing population of Japanese serows in Ishikawa Prefecture[13] means that some pastures now overlap with the range of Japanese serows. Although parapoxvirus infections in Japanese serows are mainly caused by ORFVs[3], as mentioned above, ORFVs can infect cattle, and experimental infection from Japanese serow to cattle was successful[7]. On the other hand, a BPSV Ishikawa-S strain was previously isolated from a Japanese serow, not a cow, in Ishikawa Prefecture[2,14]. These findings indicate the possibility that the virus cycles between cattle and Japanese serows and that the spread of parapoxvirus infections in cattle is

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correlated with the expanding range of the Japanese serows. However, little is known about the association of infections in cattle and the range of the Japanese serows. In this study, we conducted a serological survey for antibodies against parapoxvirus in 750 cattle from Ishikawa Prefecture by agar gel immunodiffusion (AGID) test using two different parapoxvirus species, BPSV and ORFV, as antigens. The epidemiological relationships between seroprevalence in cattle, the range of the Japanese serows, and the introduction status from other prefectures were also analyzed.

Materials and methods

1. Serum samples

A total of 750 cattle serum samples were collected in Ishikawa Prefecture, Japan, in 2007, and stored at -30°C until use. Of the samples, 360 were collected from dairy cattle on 10 farms, 77 from beef cattle on four farms, and 303 from cattle reared on four public pastures in four districts: Okunoto, Nakanoto, Kuchinoto, and Kaga. Ten samples from beef cattle grazing on abandoned cultivated land around the mountains of Kaga were also used (Fig. 1, Table 1). The clinical appearance of parapoxvirus infection was not observed on any of the cattle examined.

2. AGID test

Primary fetal bovine muscle cells were infected with the BPSV Ishikawa-B strain or the ORFV IJS087 strain. The BPSV Ishikawa-B strain was isolated from vesicles on the teats of a dairy cow in Ishikawa Prefecture in 1995[5], and the ORFV IJS087 strain was isolated from papules on the lips of a Japanese serow in Ishikawa Prefecture in 2008[4]. After showing cytopathic effects (CPE), the cells were co-cultured with Madin-Darby bovine kidney cells. Cells showing 80% CPE were then collected by trypsinization, suspended in a small volume of phosphate-buffered saline containing 0.1% Triton X-100, and used as the viral antigen for the AGID test. Positive control serum, showing clear and dense immunodiffusion lines in the AGID test using the BPSV antigen, was chosen from the collected serum samples. The BPSV and ORFV antigens were serially diluted, reacted with the positive control serum, and the antigen titer was adjusted to the same clearness and denseness of the immunodiffusion lines, and then used as antigens in the AGID test. The AGID test was performed as described previously[12]. Briefly, plates were allowed to incubate for three days at room temperature in a closed humidified chamber, and precipitation lines were observed. When a precipitation line did not form but the control line curved slightly toward the inside of the test serum well, the sample was regarded as a weak positive. At least two independent AGID tests were carried out for each sample.

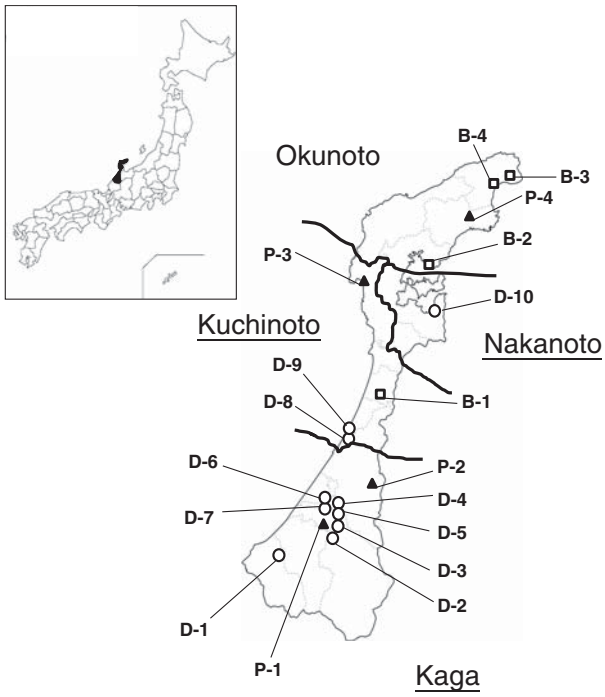

Fig. 1. Distribution of farms and pastures in Ishikawa Prefecture from which serum samples were collected from cattle

White circles represent dairy cattle breeding farms, white squares represent beef cattle breeding farms, and black triangles represent public rearing pastures. The inset shows the location of Ishikawa Prefecture, Japan. Farm and pasture IDs are the same as shown in Table 1. Regions inhabited by wild Japanese serows are underlined.

3. Statistical analysis

Significant differences in the positive reaction rate between breeding farms, regions, and type of cattle were analyzed by the χ^2 test.

Results

The positive reaction rates for BPSV varied from 0 to 21.9% in 360 dairy cattle on 10 farms, 8.3 to 33.3% in 77 beef cattle on four farms, and 33.3 to 82.4% in 303 dairy cattle grazing on four public pastures (Fig. 1, Table 1); the rates for ORFV were almost the same or less: 0-21.9%, 5.3-33.3%, and 33.3-77.8%, respectively. In 10 beef cattle grazing on abandoned cultivated land, 50.0% were seropositive against BPSV and ORFV. In general, the immunodiffusion line of ORFV on the gel was weaker than that of BPSV (Fig. 2), and a much longer time was needed for seropositive determination. In total, the positive reaction rates for BPSV were 11.9% in dairy cattle, 18.2% in beef cattle, and 65.3% in grazing dairy cattle reared on public pastures, while those for ORFV were 10.3%, 15.6%, and 61.7%, respectively. The positive reaction rate was significantly

Table 1. Positive reaction rates for BPSV and ORFV on different breeding farms

Habitat	Location	Farm ID*	No. of sera tested	Positive sera against			
				BPSV		ORFV	
				No.	%	No.	%
Serow habitat	Kaga	D- 1	30	0	0.0	0	0.0
		2	29	2	6.9	2	6.9
		3	59	10	16.9	9	15.3
		4	32	7	21.9	7	21.9
		5	38	0	0.0	0	0.0
		6	15	0	0.0	0	0.0
		7	23	4	17.4	2	8.7
		P- 1	176	145	82.4	137	77.8
		2	30	10	33.3	10	33.3
		G- 1	10	5	50.0	5	50.0
	Kuchinoto	D- 8	63	8	12.7	7	11.1
		9	46	10	21.7	10	21.7
		B- 1	24	2	8.3	2	8.3
		P- 3	49	18	36.7	17	34.7
	Nakanoto	D- 10	25	2	8.0	0	0.0
Subtotal			649	223	34.4	208	32.0
Non serow habitat	Okunoto	B- 2	19	2	10.5	1	5.3
		3	9	3	33.3	3	33.3
		4	25	7	28.0	6	24.0
		P- 4	48	25	52.1	23	47.9
Subtotal			101	37	36.6	33	32.7
Total			750	260	34.7	241	32.1

* D: dairy cattle breeding farm, B: beef cattle breeding farm, P: public rearing pasture, G: beef cattle grazing on abandoned cultivated land.

higher in grazing dairy cattle reared on public pastures than in dairy and beef cattle on farms (Table 2). Differences in positive reaction rates for different ages of cattle could not be analyzed because serum samples could not be collected from cattle over a range of ages. Of the 360 dairy cattle on farms, 121 cattle that had been reared on public pastures, and of these, 21 (17.4%) were seropositive; and 58 cattle were introduced from other prefectures, and of these, 10 (17.2%) were seropositive. These positive rates were significantly higher than for cattle that were neither reared on pastures nor introduced from other prefectures (6.6%, 12/181 cattle) (Table 3). On the other hand, for 77 beef cattle on farms, no significant difference was observed between introduced and non-introduced cattle (Table 3). Among the 57 dairy and beef cattle seropositive to BPSV cattle on farms, 14 were introduced to Ishikawa Prefecture from other prefectures: nine from Hokkaido in 2001-2006, two from Fukushima Prefecture in 2006, one from Aichi Prefecture in 2004, one from Miyazaki Prefecture in 2005, and one from Oita Prefecture in 2006. In Ishikawa Prefecture, most of the introduced cattle came from Hokkaido. However, the positive reaction rate in Hokkaido during 1997-1998 was not

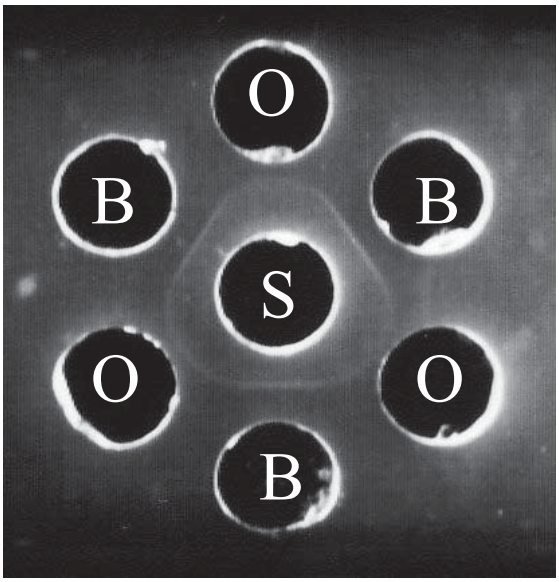

Fig. 2. Immunodiffusion lines in the AGID test using BPSV and ORFV antigens
The immunodiffusion line of the serum sample (S) was stronger for BPSV antigen (B) than for ORFV (O).

markedly higher than other parts of Japan[12], and in this study, there was no significant correlation between positive reaction rates and the prefectures of origin, including Hokkaido.

The correlation of positive reaction rates and geographical proximity to Japanese serow habitat was also assessed. Positive reaction rates were 34.4% in districts inhabited by serows (Kaga, Kuchinoto, and Nakanoto) and 36.6% in the district with no serows (Okunoto) (Table 1). A significant difference was found for the positive reaction rate between one public pasture (P-1) and the other three pastures, but no correlation was found for Japanese serow habitats among the four pastures (Table 1). No relationship between the positive reaction rate in cattle and areas overlapping serow habitat was found.

Discussion

Screening tests for antibodies against parapoxvirus in breeding dairy and beef cattle from farms in Ishikawa Prefecture showed positive reaction rates of 0-33.3%. In particular, seroprevalence was significantly higher in dairy cattle reared on public pastures (33.3-82.4%). Among the 43 BPSV seropositive dairy cattle on farms, 21 had been reared on public pastures with 15 on pasture P-1. Together with the epidemiological status results, these findings suggest that once parapoxviruses are introduced to a pasture from infected cattle, the viruses may persist subclinically in cattle grazed on the pasture, and/or the infectivity may be maintained for a long period. McKeever *et al*. reported that parapoxvirus is resistant to temperature fluctuations and ultraviolet radiation typical of several months of the British winter[6]. Resistance to environmental extremes is considered to result in a higher chance for direct contact with other cattle, enabling the parapoxvirus to persist. Consequently, disinfection is much more difficult on pastures than on farms, and the risk of parapoxvirus infection in cattle is likely to be higher on pastures than on farms. Since parapoxvirus has been isolated from the peripheral blood of cattle showing no clinical symptoms, persistent infection is indicated[1,11], and it is possible that dairy cattle infected at the pasture introduce the virus to other cattle within the home farm. Similar to our findings on dairy cattle on pastures, Saori *et al.* reported the high risk of infection in beef cattle on public pastures[10].

We found no correlation between positive reaction rates and the overlap of pasture and serow habitat. Previously, we reported that Japanese serows are mainly infected by ORFV and accidentally infected by BPSV[3]. In this study, the BPSV Ishikawa-B and ORFV IJS087 strains isolated in Ishikawa Prefecture were used as antigens in the AGID test and were adjusted to the same titer using positive control serum, but similar or lower positive reaction rates were observed and the immunodiffusion line of ORFV was weaker than that of BPSV. Therefore, although parapoxviruses cross-react serologically with each other and cattle, sheep, and goats can be experimentally infected with ORFV from Japanese serows, the positive reaction to parapoxvirus in breeding cattle in Ishikawa Prefecture is most likely not induced by ORFV infection. This is consistent with previous findings that the envelope of parapoxviruses and the plasma membrane of parapoxvirus-infected cells contain specific antigens, and that the antiserum reacts more strongly with homologous strains than with heterologous strains[9].

We found three of the 14 farms in our survey to be serologically parapoxvirus-free, as had been reported by Sentsui *et al*.[12], and we found the positive reaction rate in Ishikawa Prefecture to be lower than in other prefectures[5,12]. With the exception of one cow, all 83 cattle in the three parapoxvirus-free farms had no history of grazing on public pastures and were not introduced from other prefectures. The one exception was introduced from neighboring

Table 2. Positive reaction rates according to cattle type

Cattle type	Antigen*			
	BPSV		ORFV	
Dairy	11.9%	(43/360)[a]	10.3%	(37/360)[a]
Beef	18.2%	(14/77)[a]	15.6%	(12/77)[a]
Grazing	65.3%	(198/303)[b]	61.7%	(187/303)[b]
Total	34.5%	(255/740)	31.9%	(236/740)

*Number of positive reactions/number of sera tested.
A significant difference was found between a and b ($p<0.01$).

Table 3. Positive reaction rates in dairy and beef cattle on farms*

Cattle type	Not introduced from outside the prefecture			Introduced	
	Not reared on public pastures		Reared on public pastures		
Dairy	6.6%	(12/181)[a]	17.4%	(21/121)[b]	17.2% (10/58)[c]
Beef	25.0%	(10/40)	–		10.8% (4/37)
Total	10.0%	(22/221)	17.4%	(21/121)	14.7% (14/95)

*Number of positive reactions/number of sera tested.
Significant differences were found between a and b ($p<0.01$), and a and c ($p<0.05$).

Toyama Prefecture but had no history of grazing on public pastures. In contrast, the positive reaction rate on farm D-4 was the highest among the 10 dairy farms. Since this farm is managed by a livestock dealer, the risk of contact with parapoxvirus would be high. These results indicate that the risk of parapoxvirus infection on farms is higher when cattle graze on pastures and if cattle are introduced from outside of Ishikawa Prefecture. Therefore, the risk of parapoxvirus infection in cattle in Ishikawa Prefecture might be reduced by preventing grazing at high-risk pastures and introducing infected cattle from other prefectures, along with general hygiene control. Thus, further serological and epidemiological analyses are required to control parapoxvirus infection in cattle, and continuous serological testing for parapoxvirus infection is important for preventing the spread of infection on farms within Ishikawa Prefecture.

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References

1. Iketani, Y. et al. (2002) Persistent parapoxvirus infection in cattle. *Microbiol. Immunol.,* **46**, 285–291.
2. Inoshima, Y. et al. (2001) Genetic heterogeneity among parapoxviruses isolated from sheep, cattle and Japanese serows (*Capricornis crispus*). *J. Gen. Virol.,* **82**, 1215–1220.
3. Inoshima, Y. et al. (2002) Characterization of parapoxviruses circulating among wild Japanese serows (*Capricornis crispus*). *Microbiol. Immunol.,* **46**, 586–587.
4. Ito, M. et al. (2009) Molecular characterization of parapoxviruses from infected wild Japanese serows (*Capricornis crispus*) in Ishikawa Prefecture. *Jpn. J. Zoo Wildl. Med.,* **14**, 103–106.
5. Kuroda, Y. et al. (1999) An epidemic of parapoxvirus infection among cattle: isolation and antibody survey. *J. Vet. Med. Sci.,* **61**, 749–753.
6. McKeever, D. J. & Reid, H. W. (1986) Survival of orf virus under British winter conditions. *Vet. Rec.,* **118**, 613–614.
7. Okada, H., Matsukawa, K. & Chihaya, Y. (1986) Experimental transmission of contagious pustular dermatitis from a Japanese serow, *Capricornis crispus*, to a calf and goats. *J. Jpn. Vet. Med. Assoc.,* **39**, 578–581.
8. Robinson, A. J. & Lyttle, D. J. (1992) Parapoxviruses: their biology and potential as recombinant vaccines. *In* Recombinant poxviruses. eds. Binns, M. & Smith, G. L., CRC Press, Boca Raton, 285–327.
9. Rosenbusch, R. F. & Reed, D. E. (1983) Reaction of convalescent bovine antisera with strain-specific antigens of parapoxviruses. *Am. J. Vet. Res.,* **44**, 875–878.
10. Saori, N., Miki, T. & Noma, S. (2009) Occurrence of bovine papular stomatitis and surveillance. *In* Abstracts of the Meeting of the Japan Veterinary Medical Association, Kinki, 2009. p.35. Osaka, 2009. [In Japanese].
11. Sentsui, H. et al. (1999) Isolation of parapoxvirus from a cow treated with interferon-γ. *Vet. Microbiol.,* **70**, 143–152.
12. Sentsui, H. et al. (2000) Survey on antibody against parapoxvirus among cattle in Japan. *Microbiol. Immunol.,* **44**, 73–76.
13. Ueuma, Y. & Nozaki, E. (2003) Distribution of Japanese serow in Ishikawa Prefecture -enlargement toward the lower-lying districts and Noto region-. *Research Report of Hakusan Nature Conservation Center, Ishikawa* 30:37–41. [In Japanese].
14. Yata, S. et al. (1996) A case of parapoxvirus infection in wild Japanese serow (*Capricornis crispus*) in Ishikawa Prefecture. *Jpn. J. Zoo Wildl. Med.,* **1**, 93–97. [In Japanese with English summary].