

Prevalence of antibodies against Parainfluenza virus type 3, Respiratory syncytial virus and bovine Herpesvirus type 1 in sheep from Northern Prefectures of Japan

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Keywords

Infectious bovine rhinotracheitis, Japan, Parainfluenza virus type 3, Respiratory syncytial virus, Sheep.

Summary

Ovine sera collected in the Prefectures of Hokkaido, Aomori and Iwate in the Northern Japan were examined for the presence of antibodies against Respiratory syncytial virus (RSV), bovine Herpesvirus type 1 (infectious bovine rhinotracheitis: IBR) and Parainfluenza virus type 3 (PIV3) using serum neutralisation (SN) and enzyme-linked immunosorbent assay (ELISA) tests. Twenty-three animals (11.73%) out of the 196 tested were sero-positive to PIV3. Sixteen animals (8.69%) out of the 184 tested reacted to RSV. No animals were positive to IBR antigen. Sero-conversions to PIV3 were detected in Hokkaido and Iwate (14.92% and 8.82%, respectively). Antibodies against RSV were detected in Hokkaido (9.23%) and Aomori (14.28%). Although no diagnostic measures were in place, the infections did not appear to be related to any reduction in sheep productivity.

Prevalenza di anticorpi contro il virus Parainfluenzale di tipo 3, il virus Respiratorio sinciziale e l'Herpesvirus bovino di tipo 1 in pecore nelle Prefetture settentrionali del Giappone

Parole chiave

Giappone, Pecore, Rinotracheite infettiva bovina, Virus Parainfluenzale di tipo 3, Virus Respiratorio sinciziale.

Riassunto

Sieri ovini, raccolti nelle prefetture settentrionali del Giappone (Hokkaido, Aomori e Iwate), sono stati esaminati per la presenza di anticorpi contro il virus Respiratorio sinciziale (RSV), l'Herpesvirus bovino di tipo 1 (rinotracheite infettiva bovina: IBR) e il virus Parainfluenzale di tipo 3 (PIV3) applicando i test di siero neutralizzazione (SN) ed enzyme-linked immunosorbent assay (ELISA). Ventitré animali (11,73%) su 196 campioni testati sono risultati sieropositivi per PIV3. Sedici animali (8,69%) su 184 campioni testati hanno reagito per RSV. Nessun animale è risultato positivo per antigene IBR. Seroconversioni verso PIV3 sono state identificate nelle prefetture di Hokkaido e Iwate (rispettivamente 14,92% e 8,82%). Anticorpi contro RSV sono stati riscontrati nella prefettura di Hokkaido (9,23%) e in quella di Aomori (14,28%). Sebbene non siano state applicate misure diagnostiche, le infezioni non hanno mostrato nessuna relazione con una riduzione di produttività nelle pecore.

Respiratory disorders are among the most important problems associated with small ruminant health, causing morbidity and mortality. Respiratory syncytial virus (RSV) and Parainfluenza virus type 3 (PIV3) are among the most well known diseases that affect the respiratory system of sheep and goats (1, 2). Sheep are susceptible to bovine Herpesvirus type 1 (BoHV1), agent of infectious bovine rhinotracheitis (IBR). This is a pathogen of worldwide importance, which primarily affects cattle. So far, the studies conducted on respiratory viral infections in Japan have been mainly focused on cattle (7, 8, 9), hence only scarce information is available on epidemiology of virus pathogens in sheep. No previous epidemiological surveys on RSV, PIV3 or IBR have been undertaken on small ruminants in Japan. Furthermore, no clinical cases due to these infections have been reported among sheep flocks.

To explore the presence of the RSV, PIV3 and IBR and to obtain a preliminary picture of their epidemiology, a serological survey was carried out from September 2007 to January 2008 in the Prefectures of Hokkaido, Aomori and Iwate in the Northern Japan, where the majority of the Japanese sheep, a total of 4,775 sheep (43%), are bred. Details of the sampling methodology and descriptions of the flocks have been reported (5).

The presence of antibodies against PIV3 and RSV was determined by using serum neutralisation (SN) test. In a 96-well plate, inactivated serum samples were diluted from an initial dilution of 1:2 by doubling and placed in contact with 100TCID₅₀ of previously titrated PIV3 SF-4 or RSV RB-94 strains. After incubation for 1h at 37°C with 5% CO₂ to enable viral neutralisation, 5×10⁵/ml of Madin-Darby bovine kidney (MDBK) cells - suspended in minimum essentials medium (MEM) (Eurobio, Cortaboeuf, France) and containing penicillin 100IU/ml, streptomycin 100µg/ml, gentamicin 5µg/ml, nystatin 50 IU/ml and 10% foetal calf serum (FCS) (Sigma, Hamburg, Germany) - were added to each well. After 5 days, the cytopathic effect (CPE) in the wells was evaluated and the antibody titre was defined as the highest serum dilution able to inhibit at least 75% of the virus' CPE. Positive and negative reference sera, cell and virus controls (Istituto Zooprofilattico

Sperimentale dell'Abruzzo e del Molise 'G. Caporale', Teramo, Italy) were included in each plate.

Serological testing for antibodies against BoHV1 glycoprotein B was performed by enzyme linked immunosorbent assay (ELISA), using a commercial kit (IDEXX IBR gB, IDEXX, Westbrook, Maine, USA), following the manufacturers' instructions.

As for the flock production, the annual lambing rate was calculated as number of lambs born per ewes exposed to the ram and it was based on a lambing season occurring from February to April, with an exception being made for 1 farm where the reproductive cycle was related to 3 breeding seasons. The proportions of screened pathogens infection rate of the sampled animals were compared using the Pearson's correlation coefficients statistics in order to calculate the possibility of a relationship between the prevalence of infection and production parameters such as annual lambing rate, annual lamb mortality rate and annual adult mortality rate. Differences were considered to be significant at $P < 0.05$.

Results of serological screening for antibodies to RSV, PIV3 and IBR in sheep from each Prefecture of the Northern Japan are summarized in Table I. All the 267 sera were submitted to IBR testing. Not all the samples were applicable to serological tests for RSV and PIV3 antigens (Table I). Some sera showed cytotoxicity (indicated by cell death, probably caused by the sub-optimal condition of the samples) or they were not tested due insufficient serum quantity. All such samples ($n = 83$ for RSV and $n = 71$ for PIV3) were then excluded.

The SN test revealed 23 samples out of the 196 sera examined positive for anti-PIV3 immunoglobulins (Table I); this corresponds to a prevalence of 11.73%. At flock level positiveness ranged between 5.55% and 88.23%, whereas titres ranged from 1:8 to 1:256. PIV3 infection was detected in 5 out of the 14 sampled flocks. Levels of infection were found in flocks from Hokkaido and Iwate Prefectures, but not in the Aomori Prefecture. Four Suffolk, 1 Cheviot, 1 Corriedale, and 17 cross-breeds, mainly Suffolk × Cheviot, 1 rams and all the other ewes, were affected.

RSV infection was detected in 3 flocks, with an overall prevalence of 8.69%; 16 animals, out of 184 sera

Table I. Results of serological screening for antibodies to IBR, RSV and PIV3 in sheep from the Prefectures of Hokkaido, Aomori and Iwate in the Northern Japan.

	Positive	% Positive*	Negative	NE	Toxic	Total
IBR (ELISA)	0	0	267	—	—	267
RSV (SN)	16	8.69	168	64	19	267
PIV3 (SN)	23	11.73	173	69	2	267

NE = not executed due to insufficient aliquots for testing; * Percentage computed excluding samples resulting toxic or not tested for insufficient serum quantity.

examined, were observed to be positive for anti-RSV immunoglobulins (Table I). Positive sera originated from 2 flocks from the Hokkaido Prefecture and 1 flock from the Aomori Prefecture. None of the sera collected from the Iwate Prefecture were found to be positive. The percentage of positive sheep was 9.23% and 14.28% in Hokkaido and Aomori Prefectures, respectively. The average incidence of seropositive animals in individual herds was 13.33%, 26.66% and 55.55% respectively for the 3 sampling groups from sero-positive flocks. The obtained titres with SN were 1:256 in all tested positive sera. The seropositive sheep were all females and except for 1 ram. The sheep were of different breeds, 4 Suffolk, 10 Suffolk x Cheviot cross breeds, and 2 Romanov x Poll Dorset x Suffolk cross breeds.

The variation of prevalence of the different infections among the 3 Prefectures is reported in Table II. The analysis of the percentage of sheep

positive for antibodies to RSV and PIV3 per age categories showed that for both the pathogens no seroconversions were present in animals of 1 and 2 years of age and in animals older than 7 years. Seroconversion was related mainly to single infections. However, antibodies against RSV and PIV3 were simultaneously identified in 10 animals from the same flock (sample 5 from Hokkaido Prefecture).

The assessment of the possible impact of RSV and PIV3 infections on the production levels in the sampled flocks did not reveal a clear correlation with the reported levels of seropositive animals (Table III). However, with concern to the annual lamb mortality rate, it is noteworthy that in 4 flocks losses of 20% or more have been reported. In 3 out of 4 of these flocks animals were found seropositive to RSV. Although no diagnostic measures were in place and the observation was not statistically significant ($p = 0.05539$), this may suggest a relation of RSV infection with lamb mortality.

All the 267 samples collected were tested for IBR antibodies. None of the tested animals resulted serologically positive.

This survey demonstrates positiveness for antibodies to PIV3 and RSV in sheep flocks in the Northern Prefectures of Japan, where the majority of the Japanese sheep are bred. The survey also provides the first serological evidence of the occurrence of these diseases in sheep in the country. Interviews with farmers revealed that no previous investigations on these pathogens have been carried out in all of the randomly selected sampling units for this study,

Table II. Comparison of the 3 Prefectures in Northern Japan for the percentage of sheep positive for antibodies to the different respiratory viruses. No animals reacted to infectious bovine rhinotracheitis antigen.

	Positive (%)	
	PIV3	RSV
Hokkaido	14.92	9.23
Iwate	8.82	0
Aomori	0	14.28
Total	11.73	8.69

PIV3 = Parainfluenza virus type 3; RSV = Respiratory syncytial virus.

Table III. Comparison of different production parameters for the percentage of sheep positive for antibodies to Respiratory syncytial virus and Parainfluenza virus type 3.

Flock No.	Prefecture	RSV	PIV3	Annual lambing rate	Annual lamb mortality rate	Annual culling rate	Mortality rate among adults
1	Hokkaido	0	21.42	NR	NR	NR	5
2	Hokkaido	0	0	0.72	1.29	0	4.76
3	Hokkaido	0	0	1.62	3.46	14.77	9.2
4	Hokkaido	NE	NE	1.1	12.78	2.97	0
5	Hokkaido	55.55	88.23	1.61	20	11.73	8.33
6	Hokkaido	0	0	1.48	17.09	10.33	9.09
7	Hokkaido	0	0	1.58	16.92	6.66	2.22
8	Hokkaido	13.33	0	2.44	20.53	NR	NR
9	Hokkaido	0	5.55	1.23	0	0	0
10	Hokkaido	0	50	NR	NR	NR	10
11	Iwate	0	0	1.61	6.89	0	11.76
12	Iwate	0	17.64	1.38	9.83	24.03	4.8
13	Aomori	0	0	1.54	25.35	17.64	2.94
14	Aomori	26.66	0	1.14	21.87	0	9.09

NE = not executed due to insufficient aliquots for testing; NR = not recorded.

which then should be regarded as the first source of preliminary information on the epidemiology and distribution of such pathogens for the years 2007 and 2008.

The demonstration of respiratory virus circulation in sheep flocks in the Northern Prefectures of Japan, based on serological analysis, advanced the knowledge on pathogens affecting domestic sheep in Japan. These interesting findings deserve further evaluations in order to examine the full extent of the problem in small ruminant populations, taking into account that infections with PIV3 and RSV are characterized by a potential negative impact on animal health (1, 2), also indirectly, predisposing lambs to a severe pneumonia caused by several serotypes of *Pasteurella haemolytica* (enzootic pneumonia) (3, 10, 11, 12).

Furthermore, while ovine farming is a relatively minor sector in Japan - the population is constituted by 11,000 heads (according to the Japan Livestock Industry Association 2000) – it is worth considering that in some farms other domestic animals, i.e. cows, were housed close to sheep pens or had access to common pastures. A sheep flock (sample 7 from Hokkaido Prefecture) originated from a farm mainly focused on dairy cattle breeding, thus being in close contact with a herd of 700 black Japanese cows. Preventive measures should be carefully considered to avoid diffusion and impact on valuable breeding cattle farming. This is evident if one were to consider the potential adverse effects, both direct or indirect, on production of these pathogens detected in sheep and to take into account that in cattle PIV3 and RSV are among the main causes of respiratory disorders

(6) along with bovine diarrhoea virus (BVDV). The same goes for IBR, which is known to cause major welfare and economic problems in cattle, the potential for infection in sheep remains consistent when considering that the infection is present in cattle (8, 9) and in particular it is most frequent in Hokkaido, as indicated by reports from 2005 to 2011, which described up to 42 outbreaks in 2009 (14). The importance of sheep in the epidemiology of IBR remains limited, considering the lower capacity of spreading the virus (4). However, according to the World Animal Health Organisation (*Office International des Épizooties*: OIE), IBR is included in the list of reportable diseases of importance to international trade (13).

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References

1. Al Darraji A.M., Cutlip R.C. & Lehmkuhl H.D. 1982. Experimental infection of lambs with bovine respiratory syncytial virus and *Pasteurella haemolytica*: pathologic studies. *Am J Vet Res*, **43**(2), 224-229.
2. Cutlip R.C. & Lehmkuhl H.D. 1982. Experimentally induced parainfluenza type 3 virus infection in young lambs: pathological response. *Am J Vet Res*, **43**(12), 2101-2107.
3. Davies D.H., Dungworth D.L., Humphreys S. & Johnson A.J. 1977. Concurrent infection of lambs with parainfluenza virus type 3 and *Pasteurella haemolytica*. *NZ Vet J*, **25**(10), 263-265.
4. Galais-Duhamel C. 2006. Les herpèsvirus bovins chez les ruminants. Ph.D. thesis, National Veterinary School, Maisons-Alfort, France, 124 p. (<http://theses.vet-alfort.fr/telecharger.php?id=85> accessed on 20.09.2013).
5. Giangaspero M., Ibata G., Savini G., Osawa T., Tatami S., Takagi E., Moriya H., Okura N., Kimura, A. & Harasawa R. 2011. Epidemiological Survey for Border Disease virus among Sheep from Northern Districts of Japan. *J Vet Med Sci*, **73**(12), 1629-1633.
6. Hodgins D.C., Conlon J.A. & Shewen P.E. 2002. Respiratory viruses and bacteria in cattle. Chapter 12. In Polymicrobial diseases (K.A. Brogden & J.M. Guthmiller, eds.), ASM Press, Washington, DC. (<http://www.ncbi.nlm.nih.gov/books/NBK2480/#top> accessed on 20.09.2013).
7. Inaba Y., Tanaka Y., Sato L., Omori T. & Matumoto M. 1972. Bovine respiratory syncytial virus studies on an outbreak in Japan, 1968-1969. *Jpn J Microbiol*, **16**(5), 373-383.
8. Ogino H., Inui S. & Narita M. 1996. Demonstration of infectious bovine rhinotracheitis virus antigen by immunoperoxidase method in tissues of aborted bovine fetuses preserved for 25 years in paraffin blocks. *J Vet Med Sci*, **58**(5), 459-460.
9. Ogino H., Kaneko K., Nakabayashi D., Watanabe T & Murayama J. 1996. Pathology of bovine abortion and newborn calf death caused by dual infection with *Chlamydia psittaci* and infectious bovine rhinotracheitis virus. *J Vet Med Sci*, **58**(1), 67-70.
10. Rushton B., Sharp J.M., Gilmour N.J.L. & Thompson D.A. 1979. Pathology of an experimental infection of specific pathogen-free lambs with parainfluenza virus type 3 and *Pasteurella haemolytica*. *J Comp Pathol*, **89**(3), 321-329.
11. Sharp J.M., Gilmour N.J.L. & Thompson D.A. 1978. Experimental infection of specific pathogen-free lambs with parainfluenza virus type 3 and *Pasteurella haemolytica*. *J Comp Pathol*, **88**(2), 237-243.
12. Trigo F.J., Breeze R.G., Liggit H.D., Evermann J.F. & Trigo E. 1984. Interaction of bovine respiratory syncytial virus and *Pasteurella haemolytica* in the ovine lung. *Am J Vet Res*, **45**(8), 1671-1678.
13. World Organisation for Animal Health (Office International des Épidémiologies: OIE). 2012. OIE listed diseases 2012 (www.oie.int/en/animal-health-in-the-world accessed on 20.09.2012).
14. World Organisation for Animal Health (Office International des Épidémiologies: OIE). 2012. World Animal Health Information System (www.oie.int/wahis/public.php accessed on 20.09.2012).