Epidemiological Survey for *Toxoplasma gondii*, *Chlamydia psittaci* var. *ovis*, *Mycobacterium paratuberculosis*, *Coxiella burnetii*, *Brucella* spp., Leptospirosis and Orf Virus among Sheep from Northern Districts of Japan

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ABSTRACT. A serological survey was carried out in the northern prefectures of Hokkaido, Iwate and Aomori in Japan, for the presence of antibodies against *Toxoplasma gondii*, *Chlamydia psittaci* var. *ovis*, *Mycobacterium paratuberculosis*, *Coxiella burnetii*, *Brucella* spp., Leptospirosis and Orf virus (ORFV). Out of 267 samples tested, highest overall prevalence (28.78%) was found for *T. gondii*. The 12.59% of tested sheep were positive for *C. psittaci* var. *ovis*. A total of 8.67% were found to be seropositive for *C. burnetii*. Levels of these infections were found in all three prefectures. Seroconversion to ORFV was detected in Hokkaido and Iwate Prefectures (2.57%). Animals were positive only for *L. ballum* (1.50%), in Hokkaido and Aomori Prefectures. No animals tested positive for *Brucella* spp. and *M. paratuberculosis*.

KEY WORDS: Japan, northern prefectures, sheep, zoonosis.

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The limited sheep population (11,000 heads, data from the Japan Livestock Industry Association, 2000) represents a niche sector in Japan. However, it is integrated into the epidemiology of zoonotic agents. Furthermore, sheep are also farmed with other domestic species, such as cattle, another susceptible species involved in diffusing pathogens with zoonotic potential. At present, in Japan, only *Caprine Arthritis Encephalitis* virus, Scrapie, *Mycoplasma ovipneumoniae* and Border disease have been examined in small ruminant populations [7–9, 14].

In order to explore the presence of pathogens with recognized zoonotic potential and to obtain a preliminary picture of their current epidemiology, a serological survey of antibodies against *Brucella* spp., *Toxoplasma gondii*, *Chlamydia psittaci* var. *ovis*, *Mycobacterium paratuberculosis*, *Coxiella burnetii* (Q fever), orf virus (ORFV - contagious pustular dermatitis or ecthyma) and Leptospirosis was carried out. This was to determine their prevalence in the northern prefectures in Japan, Hokkaido, Aomori and Iwate, where the majority of the Japanese sheep, a total of 4,775 sheep (43%), are bred. The sampling activities were supported also

by veterinarians of the Agricultural Mutual Aid Association (NOSAI). Details of the sampling methodology and descriptions of the flocks were reported previously [7, 8].

Screening for anti-*Brucella* spp. antibodies was performed using a rapid serum agglutination test (Rose Bengal Plate Test, RBPT) using *B. abortus* biovar 1 strain 99 antigen covering *B. melitensis* and *B. abortus*, in accordance with the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the World Organisation for Animal Health-Office International des Epizooties (OIE) [26]. Antigen and control sera were from Istituto Zooprofilattico Sperimentale Abruzzo and Molise (IZSA&M) in Teramo, Italy.

A complement fixation test (CFT) for the detection of antibodies against *C. burnetii* was used [2]. Q fever antigen was from Siemens (Germany), and positive and negative reference controls were from IZSA&M.

The presence of antibodies against *Chlamydia psittaci* var. *ovis* was determined by using CFT as described by Donn *et al.* [4]. Antigen and positive and negative reference controls were from IZSA&M.

CFT was performed as a confirmatory test for the screening for anti-*Brucella* spp. antibodies, using *B. abortus* biovar 1 strain 99 antigen covering *B. melitensis* and *B. abortus*, in accordance with the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals of the OIE [26]. Antigen and control sera were from IZSA&M.

The testing for the presence of neutralizing antibodies against leptospirosis was performed using a microscopic agglutination test (MAT) as described by the Manual of Diag-

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nostic Tests and Vaccines for Terrestrial Animals of the OIE [27]. Antigens selected for use in the MAT included 8 strains (*L. ballum*, *L. bratislava*, *L. canicola*, *L. grippotyphosa*, *L. copenhageni*, *L. hardjo*, *L. pomona* and *L. tarassovi*) provided by the Istituto Zooprofilattico Sperimentale Lombardia and Emilia Romagna (IZSL&ER, Italy). Positive reference controls for each strain (IZSL&ER) were included in the test.

To detect antibodies against ORFV, an indirect enzyme-linked immunosorbent assay (ELISA) was used [3]. Antigen and control sera were from IZSA&M. The whole organism antigen and a horseradish peroxidase-labelled secondary antibody were used to perform the test. Briefly, a 96-well microtiter plates were coated at 4°C overnight with 100 µl/well of positive and negative antigens diluted in 0.05 M carbonate buffer (pH 9.6). The plates were then washed 3 times with phosphate buffer solution containing 0.05% Tween 80 (PBST), pH 7.4. One hundred microliters of each sera diluted in PBST containing 1%, bovine serum albumin (BSA), pH 7.4, were added in duplicate to positive and negative antigen wells. The plates were then incubated for 1 hr at 37°C. After washing as described above, wells were filled with 100 µl of an appropriate dilution of horseradish peroxidase-labeled rabbit anti-sheep IgG (Sigma-Aldrich, St. Louis, MO, U.S.A.) in PBST containing 1% of BSA. Following incubation for 1 hr at 37°C and washing 3 times with PBST, wells were filled with 100 µl substrate solution containing 1 mM ABTS (2,2'-azino-bis-[3-ethylbenzothiazoline-6-sulphonic acid]) and 4 mM H₂O₂ in 50 mM sodium citrate, pH 4.0, and the plates were shaken for 20 min at room temperature. The reaction was stopped by addition of 50 µl of sodium azide. The absorbance values of the samples were read at 405 nm. The cut-off value considered was 0.215. This was calculated by adding 3 times the standard deviation (SD) value to the mean optical density (OD) value obtained from 140 negative sera collected from genetically selected animals in an ORF-free flock [3].

Agar gel immunodiffusion (AGID) was used for the detection of antibodies against *M. paratuberculosis*. Briefly, 0.75% agarose was dissolved in 0.85% NaCl solution and buffered to pH 9.0 with 0.01 M tris hydroxymethyl amino-

methane. Gel was poured in plates on a 4-mm-thick layer with wells of 5 mm in diameter in a hexagonal pattern of 6 peripheral wells for serum samples and the positive control (Allied Monitor Inc., Fayette, MO, U.S.A.) and a central well for the antigen (Allied Monitor Inc.). Plates were examined after 24 and 48 hr of incubation. The appearance of precipitation lines of identity with the control serum lines before or at 48 hr was considered positive. Absence of any precipitation line was recorded as a negative test result.

An indirect immunofluorescence (IIF) antibody assay was used for the detection of antibodies against *T. gondii*. Glass microscope slides with circular areas covered with the RH Sabin strain of *T. gondii* (Biomérieux, Marcy-Étoile, France) grown in mice were used. Four different dilutions of serum ranging from 1:40 to 1:320 were made in phosphate-buffered saline (0.01 M PBS, pH 7.6). Positive and negative controls sera (Fuller Laboratories, Fullerton, CA, U.S.A.) were included in each test. Twenty microliters of the diluted serum was incubated with the antigen preparation for 30 min at 37°C in a humid chamber. After incubation, the slides were washed with PBS and dried. Then, 20 µl of the fluorescein isothiocyanate-conjugated rabbit anti-sheep IgG (Sigma-Aldrich), diluted 1:40, was added onto the slides and incubated for 30 min at 37°C in a humid atmosphere. After a second washing with PBS, the slides were prepared with buffered glycerine (pH 9.5), mounted with coverslips and examined using a fluorescence microscope using 40× objective lenses. A reaction with a serum dilution 1:40 was considered reactive, and the final titer was the last dilution that still showed fluorescence in the periphery of the parasites.

Results of serological screening for antibodies to the considered pathogens are summarized in Table 1. The highest percentage of seropositive animals showed an immune reaction to *T. gondii* (28.78%) in 11 flocks from all the three considered prefectures, with prevalence at flock level being up to 72.72% and the titers ranging from 1:40 to 1:160 (Table 2). Positive sera originated from 69 ewes and 7 rams. Fifty-five Suffolk and Suffolk crossbreeds, 6 crossbreeds Southdown x Poll Dorset, 9 Corriedale, 3 Black, 2 Cheviot and 1 Romanov were affected.

Table 1. Results of serological screening for antibodies to Leptospirosis, *Toxoplasma gondii*, *Chlamydia psittaci* var. *ovis*, *Brucella* spp., *Mycobacterium paratuberculosis*, Q fever and orf virus in sheep from prefectures of northern Japan

	Positive	% Positive*	Negative	NE	Anticomplement	Total
<i>T. gondii</i> (IIF)	76	28.78	188	3	-	267
<i>C. psittaci</i> var. <i>ovis</i> (CFT)	33	12.59	229	3	2	267
Q Fever (CFT)	21	8.67	221	3	22	267
Orf virus (ELISA)	6	2.57	227	34	-	267
Leptospirosis (MAT)						
Tested for: <i>L. australis</i> , <i>L. ballum</i> , <i>L. canicola</i> , <i>L. grippotyphosa</i> , <i>L. icterohaemorrhagiae</i> , <i>L. pomona</i> , <i>L. tarassovi</i> , <i>L. sejiro</i>	4	1.50	262	1	0	267
<i>L. ballum</i>						
<i>Brucella</i> spp. (RBPT, CFT)	0	0	267	0	0	267
<i>M. paratuberculosis</i> (AGID)	0	0	267	0	-	267

NE: Not executed due to insufficient aliquots for testing.

*Percentage computed excluding samples resulting anticomplement or not tested due to insufficient serum quantity.

Seroconversion against *C. psittaci* var. *ovis* (12.59%) was detected in 10 flocks in the 3 prefectures. Positive sera originated from 31 ewes and 2 rams: 12 Suffolk, 17 crossbreeds (mainly Suffolk x Cheviot and Suffolk x Corriedale), 1 Corriedale, 1 Cheviot and 2 Black. The average incidence of seropositive animals in individual herds ranged from 5.0% to 33.33% in the ten sampling groups from seropositive flocks. Titers ranged from 1:16 to 1:256 (Table 3).

Using CFT, sera from 21 animals (8.67%), out of 242 sera examined, were observed to be positive for anti-*C. burnetii* immunoglobulins (Table 1). Positive sera originated from 8 flocks from Hokkaido and 1 flock each from both Iwate and

Aomori Prefectures. The average incidence of seropositive animals in individual herds ranged from 5% to 29% in the 10 sampling groups from seropositive flocks. The obtained titer with CFT was 1:8 in all tested positive sera. The seropositive animals included all ewes and 1 ram, but of different breeds, including Suffolk, Cheviot, Corriedale and crossbreed animals.

Antibodies against ORFV were reported in 3 flocks from Hokkaido and Iwate. The 6 positive animals were all Suffolk ewes of 5 to 7 years of age. Antibodies against *L. ballum* were reported in 4 Suffolk ewes (from 4 to 8 years of age) from 2 flocks from Hokkaido and Aomori Prefectures, re-

Table 2. Indirect immunofluorescence (IIF) test. Results of serological screening for antibodies to *Toxoplasma gondii* in sheep from prefectures of northern Japan

Flock No.	Prefecture	IIF titer				NE	Total /flock
		Negative	40	80	160		
1	Hokkaido	11	5	3		1	20
2	Hokkaido	10	3	7			20
3	Hokkaido	16	3	1			20
4	Hokkaido	19		1			20
5	Hokkaido	17	1	2			20
6	Hokkaido	14	1	4	1		20
7	Hokkaido	10	5	5			20
8	Hokkaido	6	7	7			20
9	Hokkaido	20					20
10	Hokkaido	3	3	5			11
11	Iwate	20					20
12	Iwate	9	1	8	1	1	20
13	Aomori	20					20
14	Aomori	13		2		1	16
Total		188	29	45	2	3	267

NE: Not executed due to insufficient aliquots for testing.

Table 3. Complement fixation test (CFT). Results of serological screening for antibodies to *Chlamydia psittaci* var. *ovis* in sheep from prefectures of northern Japan

Flock No.	Prefecture	CFT titer						Anticomplement	NE	Total /flock
		Negative	16	32	64	160	256			
1	Hokkaido	14	4	1					1	20
2	Hokkaido	17	3							20
3	Hokkaido	19	1							20
4	Hokkaido	20								20
5	Hokkaido	15	4	1						20
6	Hokkaido	18	2							20
7	Hokkaido	20								20
8	Hokkaido	17	2	1						20
9	Hokkaido	18	2							20
10	Hokkaido	10							1	11
11	Iwate	15	5							20
12	Iwate	12	3	2			1	2		20
13	Aomori	20								20
14	Aomori	14	1						1	16
Total		229	27	5			1	2	3	267

NE: Not executed due to insufficient aliquots for testing.

spectively.

The prevalence of the different infections was found to vary between prefectures (Table 4). Prevalence of antibodies to *T. gondii*, the most diffuse pathogen in this study, was relatively high in Hokkaido and Iwate Prefectures (33.16% and 25.64%, respectively) when compared with the prevalence detected in animals from Aomori Prefecture (5.71%). The highest percentage of positive sheep for *C. psittaci* var. *ovis* was reported in Iwate Prefecture (28.94%). The highest prevalence for *C. burnetii* was found in Aomori Prefecture (17.24%). The prevalences of ORFV and *L. ballum* were low (not exceeding 3.18% and 2.77%, respectively) and differently distributed, with both being absent from Aomori and Iwate Prefectures, respectively.

Comparison between different age categories and the percentage of seropositive sheep showed that age ranged from 1 to 10 years only in the case of sheep infected with *C. burnetii* (Table 5). For most of the pathogens, no seroconversions were present in animals at 1 year of age, and seroconversion was infrequent after 8 years of age. Seroprevalence usually raises with age, although this was not reported in this study. This might be related to the low number of samples from animals more than 8 years of age (three 9-year-old animals, six 10-year-old animals and one 12-year-old animal), and

culling of old animals.

All the 267 collected samples were tested for *Brucella* spp. antibodies. Three samples (H8/17, H9/1 and H9/9) were positive in the RBPT, which was used as qualitative screening test. Sera originated from two different flocks from Hokkaido. Complement fixation was used as a confirmation test. None of the sera were confirmed to be positive for *B. melitensis* and *B. abortus*, showing titers <20 UI. Similarly, none of the tested animals were positive for antibodies against *M. paratuberculosis*.

This survey demonstrated positiveness for antibodies to *T. gondii*, *C. psittaci* var. *ovis*, *C. burnetii*, *L. ballum* and ORFV in sheep flocks in the northern prefectures of Japan.

Concerning brucellosis, to date, Japan is officially free from the disease in domestic animals. Only rare cases of *B. abortus* have been reported in cattle in 1992, 2002 and 2008 [28]. *B. suis* has never been reported in Japan. In 2006, the first serological survey in wild animals revealed 7.8% positiveness for antibodies against *Brucella* spp. in the Japanese wild boar (*Sus scrofa leucomystax*) from Shikoku region, in southern Japan [25]. With reference to small ruminants, the last occurrence of *B. melitensis* in Japan was reported in 1949. *B. abortus* has never been reported in sheep and goats. Five zookeepers in the City of Kawasaki developed brucel-

Table 4. Comparison between different prefectures of northern Japan for the percentage of sheep positive for antibodies to some pathogens with zoonotic potential. No animals reacted to *Brucella* spp. and *Mycobacterium paratuberculosis* antigens

Prefecture	% Positive				
	<i>Toxoplasma</i>	<i>Chlamydia</i>	Q fever	Orf	Leptospirosis <i>L. ballum</i>
Hokkaido	33.16	11.11	8.47	3.18	1.57
Iwate	25.64	28.94	2.77	2.50	0
Aomori	5.71	2.85	17.24	0	2.77
Total	28.78	12.59	8.67	2.57	1.50

Table 5. Comparison between different age categories for the percentage of sheep positive for antibodies to *T. gondii*, *C. psittaci* var. *ovis*, *C. burnetii*, *L. ballum* and orf virus. Percentage computed excluding samples resulting anticomplement or not tested due to insufficient serum quantity

Age category (years)	<i>T. gondii</i>	<i>C. psittaci</i> var. <i>ovis</i>	<i>C. burnetii</i>	ORFV	<i>L. ballum</i>
1	0	0	21.42	0	0
2	11.76	11.76	12.5	0	0
3	39.02	23.07	0	0	0
4	36.36	2.27	4.54	0	2.22
5	11.11	4.44	7.31	7.69	0
6	44.44	14.81	13.63	7.69	3.7
7	42.42	24.24	0	3.12	0
8	10	20	20	0	10
9	0	0	0	0	0
10	33.33	0	40	0	0
12	0	0	0	0	0
Unknown	38.09	23.80	20	0	0

losis in 2001 after attending the delivery of a baby moose (*Alces alces*). Subsequent investigations confirmed the infection in a goat at the zoo, but no information on isolation or characterization has been provided [6]. In this study, none of the sera tested were serologically positive for *Brucella* spp. This confirms the absence of the disease in sheep in the studied area in 2007 and corroborates the official free status of the country for brucellosis.

None of the sera tested were serologically positive to *M. paratuberculosis*. However, further epidemiological studies in small ruminant populations are needed, taking into consideration the presence of the pathogen in cattle in the country [17] and the importance in public health due to evidence of the potential role in the etiopathogenesis of Crohn's disease in humans, which is not strong but should not be ignored [24].

The present study showed positiveness for antibodies in sheep to well-known zoonotic diseases, i.e., toxoplasmosis, chlamydiosis and leptospirosis. In humans, the major concern for toxoplasmosis is congenital infection, causing severe sequelae in the infant (e.g., mental retardation, blindness and epilepsy). In Japan, a recent study reported 34.9% of pregnant women, from Hokkaido and Hyogo Prefectures, tested positive for *T. gondii* antibodies. It was likely that ingestion of raw or undercooked meat was the main risk factor for acute *T. gondii* infection in Japanese pregnant women. The incidence of congenital toxoplasmosis in Japan was estimated to be at least 0.0126% (1.26 per 10,000 births) [29]. Concerning ovine chlamydiosis, the risk of infection for humans is considered low, but pregnant women in particular should prevent contact with potentially infected small ruminants. Antibodies against *C. psittaci* var. *ovis* were reported in the majority of the tested sheep flocks. The observation of high titers up to 1:256 (Table 3) indicates the specificity of the seroconversion. However, further investigations will be necessary to clarify the epidemiology of the pathogen, taking into account that positive reactions with titers between 1:10 and 1:40 may relate to an infection with *C. pecorum* [20].

Concerning "akiyami" (autumn disease) (the Japanese name for leptospirosis), the disease was responsible for more than 200 reported human deaths annually in Japan until the 1960s, mostly in rice-field farmers. Since then, this number has decreased, and currently the annual incidence of leptospirosis in the country is low (<1 per 100,000) [23]. By 2003, leptospirosis had become a notifiable disease of the National Epidemiological Surveillance of Infectious Diseases (NESID), and fewer than 20 cases have been reported annually [18]. Serovars *Icterohaemorrhagiae*, *Copenhageni*, *Autumnalis*, *Hebdomadis* and *Australis* have been recognized as major causes of human leptospirosis [23], out of the 13 serovars of the causative *Leptospira* estimated by MAT in Japan [18]. In our survey, antibodies against a different serovar of *Leptospira* were detected, *L. Ballum*, which is almost exclusively associated with exposure to infected rats and mice [16].

Detection of antibodies against *C. burnetii* confirms the previous studies on the presence of Q fever in sheep in Japan, which was first reported by Htwe *et al.* [10]. The positive

rate (8.64%) in the present study showed a low pathogenic pressure in 2007 and was lower than the previously reported level (17.6%) of infection [10]. The reported lower prevalence was probably due to the use of the CFT instead of the more sensible IIF test previously used by Htwe *et al.* [10].

Other investigations on prevalence of coxiellosis in animal populations have been focused on cattle, particularly in dairy cattle with reproductive disorders [21], and have shown relatively high prevalences (58.9% and 60.4%). Reports indicated also serological evidence in wild animals [5]. Furthermore, cats are considered a significant source of *C. burnetii* responsible for human outbreaks [19].

In Japan, Q fever has been reportable since 1999. Every year, 7 to 46 clinical cases are reported in humans [19]. Atypical pneumonia due to *C. burnetii* among children has been reported, and the positive rate was 39.7% of positiveness among patients suffering from respiratory diseases [22]. The prevalence of *C. burnetii* antibodies in samples from adult humans showed overall seroconversion rates ranging from 8.6% to 16.5%, suggesting an occupational risk to humans living in close contact with animals [11].

Contagious pustular dermatitis infection in sheep in Japan was first reported in the 1940s [1]. Serological surveys demonstrated that 88.5% of sheep in Hokkaido were seropositive (AGID test) based on 52 tested samples [15]. An extensive outbreak of disease occurred in sheep in Hokkaido [13], and ORFV was isolated also in Iwate Prefecture [12]. Our study revealed a sensibly lower prevalence (2.57%) when compared with that reported by Kuroda *et al.* [15]. No human case has been reported in Japan so far.

The demonstration of circulation of some bacterial and viral zoonotic agents in sheep flocks in the northern prefectures of Japan, based on serological analysis, advanced the knowledge on pathogens affecting domestic sheep in Japan. Considering the zoonotic potential of these pathogens, further studies are needed. Awareness of professionals in the appropriate fields and consumers should be enhanced to ensure a preventive approach in public health. Furthermore, according to the OIE, brucellosis, chlamydiosis, paratuberculosis and Q fever are included in the list of notifiable diseases of importance to international trade [28].

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REFERENCES

1. Asakawa, Y., Imaizumi, K., Tajima, Y. and Endo, M. 1952. Studies on a contagious ecthyma-like disease observed among the sheep. *Jpn. J. Med. Sci. Biol.* 5: 475-486. [Medline]

2. Caporale, V., Nannini, D., Baldelli, R., Leori, G. and Semproni, G. 1986. Indagine siero-epidemiologica su alcune infezioni degli ovini in tre province italiane. *Atti S.I.P.A.O.C.* **6**: 113–129.
3. Casaccia, C., Piscicella, M., Petrini, A., Calzetta, G. and Capua, I. 1998. Allestimento e validazione di un kit ELISA per la diagnosi dell'ectima contagioso degli ovini. XIIIth S.I.P.A.O.C. National Congress, Palermo, Italy.
4. Donn, A., Carnieletto, P., Chiaracone, G., Ladu, M., Machell, J., Mandola, M. L., Ruii, A., Stancanelli, A. and Turilli, C. 1997. Standardizzazione della tecnica di fissazione del complemento per la dimostrazione di anticorpi anti-Chlamydia nel siero di sangue. *Il progresso veterinario* **52**: 125–128.
5. Ejercito, C. L., Cai, L., Htwe, K. K., Taki, M., Inoshima, Y., Kondo, T., Kano, C., Abe, S., Shiota, K., Sugimoto, T., Yamaguchi, T., Fukushi, H., Minamoto, N., Kinjo, T., Isogai, E. and Hirai, K. 1993. Serological evidence of *Coxiella burnetii* infection in wild animals in Japan. *J. Wildl. Dis.* **29**: 481–484. [Medline]
6. Emerging Infections network 2001. Japan – Brucellosis in zoo animals and humans. *EINet News Briefs Archive* **6** : 1 (depts. washington.edu/apecein/newsbriefs/2001/0007nb11.htm> Accessed 2 May 2012).
7. Giangaspero, M., Ibata, G., Savini, G., Osawa, T., Tatami, S., Takagi, E., Moriya, H., Okura, N., Kimura, A. and Harasawa, R. 2011. Epidemiological Survey for Border Disease virus among Sheep from Northern Districts of Japan. *J. Vet. Med. Sci.* **73**: 1629–1633. [Medline] [CrossRef]
8. Giangaspero, M., Nicholas, R. A. J., Hlusek, M., Bonfini, B., Osawa, T., Orusa, R., Tatami, S., Takagi, E., Moriya, H., Okura, N., Kato, K., Kimura, A., Harasawa, R. and Ayling, R. D. 2012. Seroepidemiological survey of sheep flocks from Northern Japan for *Mycoplasma ovipneumoniae* and *Mycoplasma agalactiae*. *Trop. Anim. Health Prod.* **44**: 395–398. [Medline] [CrossRef]
9. Horiuchi, M., Nemoto, T., Ishiguro, N., Furuoka, H., Mohri, S. and Shinagawa, M. 2002. Biological and Biochemical Characterization of Sheep Scrapie in Japan. *J. Clin. Microbiol.* **40**: 3421–3426. [Medline] [CrossRef]
10. Htwe, K. K., Amano, K., Sugiyama, Y., Yagami, K., Minamoto, N., Hashimoto, A., Yamaguchi, T., Fukushi, H. and Hirai, K. 1992. Seroepidemiology of *Coxiella burnetii* in domestic and companion animals in Japan. *Vet. Rec.* **131**: 490. [Medline] [CrossRef]
11. Htwe, K. K., Yoshida, T., Hayashi, S., Miyake, T., Amano, K., Morita, C., Yamaguchi, T., Fukushi, H. and Hirai, K. 1993. Prevalence of antibodies to *Coxiella burnetii* in Japan. *J. Clin. Microbiol.* **31**: 722–723. [Medline]
12. Inoshima, Y., Murakami, K., Yokoyama, T. and Sentsui, H. 2001. Genetic heterogeneity among parapoxviruses isolated from sheep, cattle and Japanese serows (*Capricornis crispus*). *J. Gen. Virol.* **82**: 1215–1220. [Medline]
13. Kanou, Y., Inoshima, Y., Shibahara, T., Ishikawa, Y., Kadota, K., Ohashi, S., Morioka, K., Yoshida, K. and Yamada, S. 2005. Isolation and characterization of a parapox virus from sheep with popular stomatitis. *JARQ* **39**: 197–203.
14. Konishi, M., Haritani, M., Kimura, K., Tsuboi, T., Sentsui, H. and Murakami, K. 2007. Epidemiological survey and pathological studies on Caprine arthritis encephalitis (CAE) in Japan. *Bull. Natl. Inst. Anim. Hlth.* **113**: 23–30.
15. Kuroda, Y., Yoshida, M., Shibahara, T., Matsui, T., Nakane, T., Hara, H., Inoshima, Y. and Sentsui, H. 1999. An epidemic of parapoxvirus infection among cattle: isolation and antibody survey. *J. Vet. Med. Sci.* **61**: 749–753. [Medline] [CrossRef]
16. Lindenbaum, I. 1982. Leptospirosis in Israel: epidemiological features and laboratory findings in 13 human cases due to *Leptospira ballum*. *Isr. J. Med. Sci.* **18**: 659–665. [Medline]
17. Momotani, E. 2012. Epidemiological situation and control strategies for paratuberculosis in Japan. *Jpn. J. Vet. Res. (Suppl)*: S19–S29. [Medline]
18. National Institute of Infectious Diseases - Infectious Disease Surveillance Center 2008. Leptospirosis in Japan, November 2003–November 2007. *IASR* **29**: 1–2.
19. Porter, S. R., Czaplicki, G., Maimil, J., Horii, Y., Misawa, N. and Saegerman, C. 2011. Q fever in Japan: an update review. *Vet. Microbiol.* **149**: 298–306. [Medline] [CrossRef]
20. Rodolakis, A. 2001. Chlamydiosis in goats. In: Recent Advances in Goat Diseases (Tempesta, M. ed.), International Veterinary Information Service, Ithaca.
21. To, H., Htwe, K. K., Kako, N., Kim, H. J., Yamaguchi, T., Fukushima, H. and Hirai, K. 1998. Prevalence of *Coxiella burnetii* infection in dairy cattle with reproductive disorders. *J. Vet. Med. Sci.* **60**: 859–861. [Medline] [CrossRef]
22. To, H., Kako, N., Zhang, G. Q., Otsuka, H., Ogawa, M., Ochiai, O., Nguyen, S. V., Yamaguchi, T., Fukushima, H., Nagaoka, N., Akiyama, M., Amano, K. and Hirai, K. 1996. Q fever pneumonia in children in Japan. *J. Clin. Microbiol.* **34**: 647–651. [Medline]
23. Victoriano, A. F., Smythe, L. D., Gloriani-Barzaga, N., Cavinta, L. L., Kasai, T., Limpakarnjanarat, K., Ong, B. L., Gongal, G., Hall, J., Coulombe, C. A., Yanagihara, Y., Yoshida, S. and Adler, B. 2009. Leptospirosis in the Asia Pacific region. *BMC Infectious Diseases* **9**: 147. [Medline] [CrossRef]
24. Waddell, L. A., Rajic, A., Sargeant, J., Harris, J., Amezcua, R., Downey, L., Read, S. and McEwen, S. A. 2008. The zoonotic potential of *Mycobacterium avium* spp. paratuberculosis: a systematic review. *Can. J. Public Health* **99**: 145–155. [Medline]
25. Watarai, M., Ito, N., Omata, Y. and Ishiguro, N. 2006. A serological survey of *Brucella* spp. in free-ranging wild boar (*Sus scrofa leucomystax*) in Shikoku, Japan. *J. Vet. Med. Sci.* **68**: 1139–1141. [Medline] [CrossRef]
26. World Organisation for Animal Health (Office International des Épizooties: OIE) 2010. p. 13. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE, Paris, Bovine Brucellosis, Chapter 2.4.3.
27. World Organisation for Animal Health (Office International des Épizooties: OIE). 2012. pp. 254–255. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, OIE, Paris, Leptospirosis, Chapter 2.1.9.
28. World Organisation for Animal Health (Office International des Épizooties: OIE) 2012. World Animal Health Information System (web.oie.int/wahis/public.php accessed on 2 May 2012).
29. Yamada, H., Nishikawa, A., Yamamoto, T., Mizue, Y., Yamada, T., Morizane, M., Tairaku, S. and Nishihira, J. 2011. Prospective study of congenital toxoplasmosis screening with use of IgG avidity and multiplex nested PCR methods. *J. Clin. Microbiol.* **49**: 2552–2556. [Medline] [CrossRef]