

## REVIEW

# Advances in the Understanding of *Coxiella burnetii* Infection in Japan

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**ABSTRACT.** Q fever is a zoonotic disease caused by a rickettsia *Coxiella burnetii*. Since its first description in 1937, the disease has been found to be present in most countries of the world. Serological evidences of Q fever in humans and coxiellosis in animals were reported in Japan in the 1950s, however, systematic studies of the disease did not begin until the report of isolation of *C. burnetii* from an acute Q fever patient in 1989. In addition to the extensive information about epidemiology of the disease, the understanding of Japanese isolates of *C. burnetii* is increasing rapidly in recent years. In this review, the epidemiology of the disease along with some characteristics of isolates of *C. burnetii* in Japan is summarized in five sections, i.e., coxiellosis, Q fever, modes of spread of the infection, laboratory diagnosis of the infection and some characteristics of Japanese isolates. This review includes some recent, unpublished data from our and other groups. — **KEY WORDS:** *Coxiella burnetii*, coxiellosis, epidemiology, Q fever.

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It is almost 60 years since its recognition as a clinical entity [6], but Q fever still remains as a serious problem in all parts of the world [1-4, 21, 22, 29, 31, 33, 35, 36, 44, 57]. The disease is caused by *Coxiella burnetii*, a microorganism widely distributed in nature and responsible for *Coxiella* infection in various mammals (including humans) and birds [3, 4, 29, 35, 36]. The microorganism is maintained in nature by two cycles that are essentially independent but occasionally overlapped. The basic cycle involves many species of wildlife and their ectoparasites, and the second cycle domestic animals (including companion animals). Among the ectoparasites, ticks are considered to be the natural primary reservoirs of *C. burnetii* responsible for the spread of the infection in wild animals and for transmission of *C. burnetii* from wild animals to domestic animals. The agent is then dispersed into the environment through such excretions of the animals as saliva and feces. Among domestic animals, cattle, sheep and goats are considered to be the main reservoirs of the agent responsible for infection of animals and humans. Infected female animals shed enormous numbers of *C. burnetii* through their decidua (placenta and birth fluids), colostrum and milk into the environment, where the resistant microorganism can remain viable over long periods of time. Thus, such excretions are potential sources of the infection in animals and humans via inhalation of infectious aerosol or airborne dust [3, 4, 7-9, 29, 34, 40, 61].

A very low prevalence of complement fixation antibodies to *C. burnetii* in humans and cattle was reported by Kitaoka [23, 24] and Omori *et al.* [52], respectively, in Japan in the 1950s: 22 (2.9%) of 756 slaughterhouse workers and veterinarians, and 11 (1.1%) of 983 cattle were *C. burnetii*-positive. In the following 40 years, no other serological surveys of Q fever in humans or animals were conducted. Systematic studies of Q fever did not begin until the report

of isolation of *C. burnetii* from an acute Q fever patient who became ill shortly after returning to Japan from Canada in 1989 [51].

It is the purpose of this review to assemble the results of all studies on the prevalence of *Coxiella* infection in animals and humans in Japan and to present some fresh information on Japanese isolates of *C. burnetii*, in an effort to provide a picture of the epidemiology of the disease in Japan.

**COXIELLOSIS** (the term coxiellosis is used for *Coxiella* infection in animals other than man)

## 1 WILD ANIMALS AND BIRDS

Serological evidence of *C. burnetii* infection has been found in wild animals from many areas of Japan. The enzyme-linked immunosorbent assay (ELISA) antibodies were found in 134 (26.2%) of 511 serum samples from 11 wild animal species in eight Prefectures (Hokkaido, Iwate, Aichi, Fukui, Gifu, Hyogo, Mie and Shiga) [11] (Table 1). High prevalence of the infection was observed in Japanese black bears (*Ursus thibetanus*) (28 out of 36; 77.8%), Hokkaido deer (*Cervus nippon yeoensis*) (42 out of 61; 68.9%) and Japanese deer (*Cervus nippon centralis*) (40 out of 72; 55.6%). Recently, Yasumoto *et al.* [75] found indirect immunofluorescence (IF) antibodies to *C. burnetii* in 72 (42.9%) of 168 Japanese deer (*Cervus nippon*), including 24 (52.2%) of 46 in Hokkaido and 48 (39.3%) of 122 deer in the Tohoku district. Evidence of *Coxiella* infection in wild rodents was shown in a recent study by Hirai *et al.* (manuscript in preparation), in which seven (24.1%) and three (10.3%) of 29 serum samples including 17 of wood mice (*Apodemus speciosus*), 10 of hime mice (*Apodemus argenteus*) and two of yachi mice (*Clethrionomys rufocanus*) were found to contain IF antibodies and to be polymerase chain reaction (PCR)-positive, respectively.

Table 1. Seroprevalence of *Coxiella* infection in animals and birds

Animals	Samples	% of positives	Authors
Healthy cattle	1,106	1.9	Kaplan and Bertagna (1951)
	983	3.9	Kitaoka (1954)
	329	29.2	Yoshiie <i>et al.</i> (1991)
	562	46.6	Htwe <i>et al.</i> (1992)
	1,501	25.4	Htwe <i>et al.</i> (1992)
	190	31.1	Nagaoka <i>et al.</i> (1994)
	198	16.2	Yamamoto <i>et al.</i> (1996)
Reproductive disorder cattle	102	84.3	Htwe <i>et al.</i> (1992)
	166	78.9	To <i>et al.</i> (1995)
	207	60.4	To <i>et al.</i> (1996)
Sheep	256	28.1	Htwe <i>et al.</i> (1992)
Goat	85	23.5	Htwe <i>et al.</i> (1992)
Dog	635	15.0	Htwe <i>et al.</i> (1992)
	591	9.6	Nguyen <i>et al.</i> (1996)
	81	9.9	Nagaoka <i>et al.</i> (1996)
	301	16.6	Kanda <i>et al.</i> (1996)
	274	0	Htwe <i>et al.</i> (1992)
Cat	100	16.0	Morita <i>et al.</i> (1994)
	150	15.3	Nguyen <i>et al.</i> (1996)
	101	6.7	Nagaoka <i>et al.</i> (1996)
	304	18.8	Kanda <i>et al.</i> (1996)
Pig	396	0	Htwe <i>et al.</i> (1992)
Chicken	1,589	2.0	To <i>et al.</i> (1996)
Quail	174	2.9	To <i>et al.</i> (1996)
Duck	158	2.2	To <i>et al.</i> (1996)
Bear	36	77.8	Ejercito <i>et al.</i> (1993)
Deer	133	61.7	Ejercito <i>et al.</i> (1993)
Hare	8	62.5	Ejercito <i>et al.</i> (1993)
Monkey	54	27.7	Ejercito <i>et al.</i> (1993)
Nutria	32	12.5	Ejercito <i>et al.</i> (1993)
Deer	168	42.9	Yasumoto <i>et al.</i> (1996)
Wild rodent	129	24.1	Hirai <i>et al.</i> (1997)
Crow	431	36.0	To <i>et al.</i> (1996)
Rock dove	201	6.0	To <i>et al.</i> (1996)

Table 2. Isolation of *Coxiella burnetii* from animals and wild birds

Origin		Prefectures	No. of samples	Isolation rate (%)	Authors
Cattle with reproductive disorder	Raw milk	Chiba, Shizuoka, Mie and Ehime	224	16.1	Htwe <i>et al.</i> (1995)
	Raw milk	Chiba, Shizuoka, Mie and Gifu	207	24.6	To <i>et al.</i> (1995)
Healthy cattle	Uterus swabs	Chiba and Ehime	61	21.3	Htwe <i>et al.</i> (1995)
	Raw milk	Shizuoka	47	36.3	Nagaoka <i>et al.</i> (1996)
	Udders	Gifu	50	8.0	Htwe <i>et al.</i> (1995)
	Fetus	Shizuoka and Mie	4	50.0	To <i>et al.</i> (1995)
Tick	<i>Ixodes</i> spp.	Gifu (2 pastures)	15	26.7	To <i>et al.</i> (1995)
Tick	<i>Ixodes</i> spp.	Toyama	16	75.0	Ishikura <i>et al.</i> (1997)
Dog	Sera	Shizuoka	6	100	Nagaoka <i>et al.</i> (1996)
Cat	Sera	Shizuoka	5	100	Nagaoka <i>et al.</i> (1996)
	(raised by Q fever patients)				
	Uterus swabs	Shizuoka	29	31.0	Nagaoka <i>et al.</i> (1996)
Crow	Sera	Gifu	5	100	To <i>et al.</i> (1996)
	Spleens	Gifu	5	100	To <i>et al.</i> (1996)
	Feces	Gifu	5	100	To <i>et al.</i> (1996)

Serological evidence of the infection was found also in 167 (19.4%) of 863 serum samples from 5 wild bird species from five prefectures (Hokkaido, Aomori, Tokyo, Mie and Gifu) by the microagglutination (MA) test [70] (Table 1). The bacteriological evidence of the infection was found in 37 (22.2%) of 167 wild birds by PCR. Furthermore, the microorganism was isolated from serum, spleen and stool samples from five jungle crows (*Corvus macrorhynchos*) (Table 2). Among wild birds, high prevalence of the infection was observed in carrion crow (*Corvus corone*) (37.0%), jungle crow (35.3%) and rock doves (*Columba livia*) (6.0%). Serological evidence of the infection among the birds was found in northern (Hokkaido), central (Tokyo, Chiba, Shizuoka, Aichi, Mie and Gifu) and southern (Fukuoka) Japan. Bacteriological evidence was found only in samples collected in central and southern Japan. The absence of serological or bacteriological evidence in some prefectures may be due partly to the small sample size and the location of sample collection. There was a tendency to high prevalence among birds living and/or feeding in close proximity to infected livestock.

Wildlife coxiellosis occurs in all parts of the world [3, 12, 56, 59, 81], but only a few cases of transmission of the disease from wild animals to humans were recorded. Such wild animals are bandicoots in Australia [3], Meriones and wild rabbits in Morocco [3], and wild rabbits in Canada [41]. It appears unlikely that wild animals and birds contribute a great deal to the epidemiology of the disease at present [3, 29]. The epidemiologic evidence presented above shows that many species of wild animals and birds are maintaining this agent. No information on the extent to which wild animals and birds transmit *C. burnetii* to humans and domestic animals in Japan is available at the present time. These wild animals and birds might be one of the less important links in the *C. burnetii* infection cycle.

## 2 DOMESTIC ANIMALS

Animals rarely seem to develop illness after *Coxiella* infection. Although the most common portal of the infection is believed to be the respiratory system, coxiellosis does not cause respiratory impairment in any animal species unlike that in humans. In chronic infection of adult animals, *C. burnetii* is not localized in the heart or liver. The most vulnerable site of *Coxiella* localization is the female reproductive system, including both the uterus and mammary gland, where damage is usually limited despite massive proliferation of *Coxiella*, but from where chronic shedding is the mechanism of spreading *C. burnetii* into the environment [1, 3, 4, 28, 29, 31].

Domestic ruminants are most frequently the source of human infection; some epidemiologists believe that the main reservoirs of *C. burnetii* responsible for human and livestock infection are small ruminants, while others believe that they are cattle [3, 30, 36]. Ovine coxiellosis seldom becomes chronic, while coxiellosis in cows is frequently endemic, a feature reflected by the fact that anti-*C. burnetii* antibodies in cows last longer than in other animal species. Prolonged excretion over many months or even years of *C. burnetii*

can be expected from lactating cows, but not in sheep. Goats, like sheep, have a predisposition to abortion, but like dairy cows, have that to chronic infection [3, 29].

### 1) Cattle

A large number of studies on the seroprevalence of the infection in cattle have shown that cows infected with *C. burnetii* in recent years have increased in numbers in Japan [13, 14, 16, 48, 67, 68, 76] compared with those reported by Kaplan and Bertagna [20], Kitaoka [24] and Omori *et al.* [52] in the 1950s (21 or 1.9% of 1,106 cattle; 38 or 3.9% of 983 cattle; and 11 or 1.1% of 983 cattle) (Table 1). The most comprehensive survey was conducted in 1992 by Htwe [15], who used the IF test to examine 2,063 healthy cows from 15 prefectures and 102 cows with reproductive disorders from two prefectures. She found that 29.5% of healthy cows and 84.3% of cows with reproductive disorders had *C. burnetii* antibodies. Yanase *et al.* [73] showed the prevalence and seasonal variation of infection with *C. burnetii* in dairy cattle in Hokkaido. Recent studies from our laboratory have shown also serological and bacteriological evidence of the infection in cows with reproductive disorders [67, 68]. The intensity of the infection appeared to vary from prefecture to prefecture, doubtless owing mainly to uneven sampling, nevertheless it can probably be concluded that cattle with reproductive disorders are much more often infected than are healthy ones.

The serological observations were confirmed by detection of *C. burnetii* in milk (16.8–37.0%), uterine fluid (21.3%) and aborted fetuses (50.0%) [48, 68] (Table 2). Thus, dairy cattle may be considered to be important reservoirs of the agent responsible for infection of animals and humans in Japan.

The rates of the infection found by Huebner and Bell [18] in cattle in California and by Lang [27] in cattle in Ontario seem to be higher than those described above; the milk from two-thirds of 63 dairies in the Los Angeles area contained *C. burnetii*, and the sera from four-fifths of 200 dairies in the western and central Ontario areas contained ELISA antibodies to *C. burnetii*. The reasons for these discrepancies may be explained by spatial, temporal, strain, and many other factors determining the prevalence of Q fever and animal coxiellosis as well as possible differences among laboratories and testing procedures.

### 2) Sheep and goats

Htwe *et al.* [16] found IF antibodies against *C. burnetii* in 72 (28.1%) of 256 sheep and 20 (23.5%) of 85 goats (Table 1). In both species, all serum samples were positive at such low titer levels as 1: 16 to 1: 32. These animals are known to play a role in the epidemiology of Q fever in some parts of the world [1, 3, 4, 25, 26, 29, 36]. Although serological evidence of the infection has been demonstrated in sheep and goats, the small number of the animals probably makes them of little importance as a cause of Q fever in this country.

### 3) Dogs and cats

Serological and bacteriological evidence of the infection has been found in dogs and cats in many parts of Japan [14,

16, 45, 48] (Tables 1 and 2, respectively). In 1992 Htwe *et al.* [16] failed to show even a single antibody positive among 247 cats in Ibaraki and Gifu Prefectures. Recently, Morita *et al.* [45] showed evidence of the infection in 16 (16.5%) of 97 domestic cats in 12 prefectures and suggested that the cats is one of the reservoirs of *C. burnetii* in Japan. These animals were found to act as a source of Q fever in the studies by Nagaoka *et al.* [48] and Hirai [14], in which *C. burnetii* was isolated from serum samples of six dogs and five cats, and from uterus swab samples of nine cats raised by Q fever patients.

The epidemiological importance of these companion animals has been shown in many parts of the world [3, 29, 36]. Especially, infected parturient cats are known to cause small outbreaks of Q fever [37–39, 53].

#### 4) Pigs and domestic birds

There is no evidence of *Coxiella* infection in pigs in Japan, although the information is scanty [16] (Table 1). There has been no substantial evidence showing that pigs serve as a source of Q fever, although the natural susceptibility of swine to *Coxiella* infection was demonstrated by the presence of antibodies to *C. burnetii* in their serum [33]. The role played by pigs in the epidemiology of Q fever is not known [29].

Recently, coxiellosis was serologically and bacteriologically diagnosed in domestic birds in Japan [70]. In four species of domestic birds, MA antibodies were found in 41 (2.1%) of 1,951 serum samples and PCR-positives in 17 (41.5%) of 41 serum samples. The infection was observed in 2.9% of 174 Japanese quail, in 2.2% of 158 puddle ducks and in 2.0% of 1,589 chickens (Table 1). Domestic birds might be one of the less important links in the *C. burnetii* infection cycle.

Birds are part of the host spectrum of *C. burnetii*. Infected domestic poultry can transmit the agent to humans through consumption of infected raw eggs or through fomites [29]. Seroconversion in humans living in close contact with infected birds was shown in India [55], but clinical Q fever was not mentioned in the report.

### 3 TICKS

Evidence of *Coxiella* infection in ticks was shown by To *et al.* [68], who isolated four strains of *C. burnetii* from 15 batches of *Ixodes* spp. collected from two pastures in Gifu Prefecture. Recently, Ishikura *et al.* and Takada *et al.* (personal communication) found evidence of the infection in 178 (42.6%) of 418 tick samples from Toyama Prefecture and 16 (17.6%) of 91 tick samples from Gifu Prefecture, respectively, as determined by PCR and/or isolation. Infected ticks are probably important in maintaining the cycle of *C. burnetii* infection. Ticks rarely transmit *C. burnetii* by bites to humans but do so to domestic animals frequently [3, 21, 29]. *C. burnetii* can, however, be emitted not only in the saliva but also in feces. Feces of infected ticks are a rich source of *C. burnetii*. The titer in the feces may reach  $10^{12}$  infectious organisms per gram [3, 29].

### Q FEVER

The epidemiology of *C. burnetii* infection in humans is closely linked to the epidemiology of coxiellosis in animals. In humans, the epidemiology of Q fever varies from country to country. The clinical manifestations of Q fever also vary from country to country and may reflect differences in the virulence of strains of *C. burnetii*, in the biology of the host (such as age and sex), in the route of infection and in the dose of the pathogenic agent [21, 31, 35, 40].

Q fever is more frequent in males than in females. This can be explained by the fact that Q fever has the characteristics of a professional zoonosis, i.e. it occurs more frequently in professions in close contact with domestic animals or animal products. However, on livestock farms, the risk of Q fever was related to contact with the farm environment rather than with animals. The high resistance of the agent to physical and chemical stress, enabling its survival for long periods in the environment and the transport of fomites over long distance by the wind or other means, certainly contributes to the spread of *C. burnetii* to many unexpected places [21, 31, 36, 40, 62]. However, the pattern of maintenance of *C. burnetii* in animal or tick hosts differs from one district of the world to another [21, 33,

Table 3. Seroprevalence of *Coxiella* infection in humans

Origin	Samples	% of positives	Authors
Meat-processing workers & veterinarians	756	2.9	Kitaoka (1954)
Veterinarians	9	22.2	Yoshiie <i>et al.</i> (1991)
Healthy humans (adults)	60	3.3	Htwe <i>et al.</i> (1992)
	275	22.2	Htwe <i>et al.</i> (1992)
Meat-processing workers	107	11.2	Htwe <i>et al.</i> (1992)
Adults with respiratory disorders	184	15.2	Htwe <i>et al.</i> (1992)
Adults with fever of unknown etiology	13	61.5	Nagaoka (1993)
Children with respiratory disorders	72	45.8	Hasegawa <i>et al.</i> (1996)
Children with flu-like symptoms	55	32.7	Nagaoka <i>et al.</i> (1996)
Children with atypical pneumonia	56	35.7	To <i>et al.</i> (1996)
Hospitalized patients (adults)	3,000	5.2	Nguyen <i>et al.</i> (1996)
Veterinary students	73	9.6	Furuya <i>et al.</i> (1997)
Adults with chronic respiratory disease	48	56.3	Furuya <i>et al.</i> (1997)

Table 4. Isolation of *Coxiella burnetii* from humans

Clinical manifestations	No. of samples	Isolation rate (%)	Authors
Acute Q fever (adult)	1	100	Oda <i>et al.</i> (1989)
Flu-like symptoms (children)	18	72.2	Nagaoka <i>et al.</i> (1993)
Fever of unknown etiology (adults)	24	37.5	Nagaoka <i>et al.</i> (1993)
Atypical pneumonia (adults)	16	18.8	Nagaoka <i>et al.</i> (1995)
Atypical pneumonia (children)	58	36.2	To <i>et al.</i> (1996)
Hospitalized patients (adults)	17	76.5	Hirai <i>et al.</i> (1997)

40]. In some countries the infection among domestic or wild animals results in considerable cases of infection among humans in contact with these animals, whereas in other areas little if any transmission to humans occurs [21, 33, 40].

The clinical manifestations of Q fever are readily divided into acute and chronic forms [35, 63]. The former usually occurs as a self-limited febrile illness, sometimes accompanied with pneumonia or hepatitis. One half of the infections with *C. burnetii* are asymptomatic [10]. The chronic form almost invariably means endocarditis, but vertebral osteomyelitis and granulomatous hepatitis may be other manifestations [54].

Serological and/or bacteriological evidence of *Coxiella* infection has been found in various groups of humans in many districts of Japan except in Hokkaido [14, 17, 48, 69, 76, 77] (Tables 3 and 4). These data showed high incidence of the infection in healthy humans living in close contact with animals and their products, e.g., in veterinarians, meat-processing workers, and in patients with respiratory disorders. However, information on Q fever among dairy cattle-farm workers, milk-processing workers, and butchers, and that on the possible dangers of dairy products (milk, cheese and butter) are not available at the present time.

The absence of reported cases of Q fever in Hokkaido, which is an agricultural area with a large number of cattle, is most probably due to the fact that active surveillance programs have not been performed, or to the likelihood that clinical Q fever cases have been undiagnosed or misdiagnosed as influenza, common cold, or pneumonia. Moreover, as stated by Raoult [5], Q fever is probably ubiquitously present since reservoirs are found everywhere (cattle, sheep, goats, cats and so forth) and the differences in prevalence are related mainly to different interests of the investigators.

Acute cases of Q fever have been reported in patients with atypical pneumonia [69], flu-like symptoms [48] or other manifestations of the respiratory tract [14] (Tables 3 and 4). Chronic cases of Q fever were shown in a retrospective study by Yuasa *et al.* [77] who found that four of 56 patients had chronic Q fever (endocarditis) by PCR. Recently, Hirai *et al.* (manuscript in preparation) succeeded in isolating 13 strains of *C. burnetii* harboring QpRS plasmid (associated with the chronic form of Q fever) from 17 patients with clinical manifestations of granulomatous

hepatitis, endocarditis, or arteritis. Sporadic cases of Q fever necro-lymphadenitis, cerebellitis, exanthem and so forth were shown by Hirai [14]. The above-mentioned data showed that infection with *C. burnetii* occurs in Japan often subclinically, but also that clinical Q fever is not uncommon in humans. It is important to know the ratio of subclinical (antibody-forming) to clinical infections among humans living in close contact with domestic animals or animal products, but such information is presently lacking.

A high prevalence of the antibodies in a population with no clinical Q fever may be due to a past acquisition of immunity, properties of the strain of *C. burnetii*, or the misdiagnosis [36]. Clinical Q fever is seldom reported among persons occupationally exposed to animals, although the antibodies were found to be rather common in such individuals in Japan. The foregoing data showed that children in Japan bore the brunt of Q fever; an observation most easily explained by postulating an apparent immunity in adults living in close contact with animals. Such immunity might be the result of childhood exposure. Analogous situations appear to exist in South Africa and in the Romney Marsh area of Great Britain, where most of the adult population are apparently immune and patients with Q fever are seen in South Africa- or Romney Marsh-borne children [20, 33].

#### MODES OF SPREAD OF THE INFECTION

Although infected wild animals and ticks may have been the original source of Q fever, from which the disease spread to domestic animals, yet it appears unlikely that these animals contribute much to the epidemiology of the disease at present. It is beyond doubt that animal coxiellosis is by now established to a certain extent in domestic animals and can maintain itself in them without the necessity of wild ones or ticks [3, 29].

Naturally, the spread of the infection in domestic animals depends on many factors, such as the population density of the animals, the system of rearing, the local manner in which an animal's parturition is handled, and other partly unknown elements. Mainly, the disease passes from animal to animal by inhalation of infectious aerosol or airborne dust.

There has been experimental and epidemiological evidences showing that the most important route by which

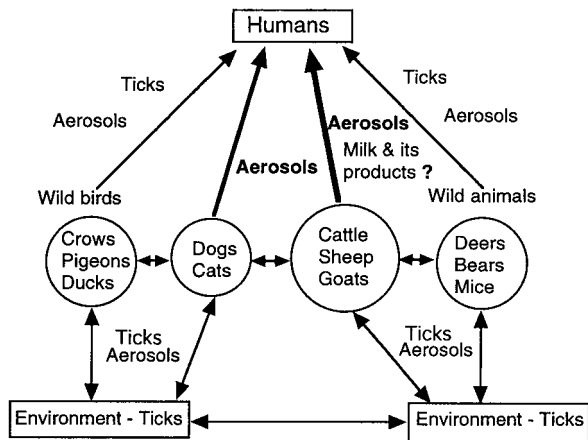


Fig. 1. Epidemiology of Q fever in Japan.

*C. burnetii* infects man is through inhalation of contaminated aerosol [3, 21, 36].

The exact modes of transmission of Q fever to humans and animals in Japan are not known. Serological and/or bacteriological evidence of the infection in veterinarians and in patients in Gifu and Shizuoka [14, 17, 48, 50, 69], where a rather high incidence of animal coxiellosis is reported [13, 15, 16, 49, 67, 68], together with data on detection of *C. burnetii* from dust samples collected from a barn having infected dairy cattle in the Hokkaido area [74] suggested that the aerosol mode is a very important route, if not the principal route, by which *C. burnetii* infects humans and animals. However, there is no evidence to rule out the roles of other modes (oral, percutaneous, vertical and person-to-person) in transmitting Q fever to humans or animals. The epidemiology of Q fever in Japan is suggested in Fig. 1. Further intensive research on modes of transmission of Q fever to humans is needed to elucidate the epidemiology of Q fever in Japan.

#### LABORATORY DIAGNOSIS OF *COXIELLA BURNETII* INFECTIONS

Q fever is difficult to differentiate clinically from a number of other febrile illnesses, such as influenza, brucellosis, leptospirosis, typhoid fever and psittacosis [19, 21, 40, 47, 72]. Thus, diagnosis of Q fever has been based exclusively on serological and/or bacteriological findings.

A wide variety of techniques are used to detect the *C. burnetii* antibodies in serum or the agent in infected materials (blood, urine, milk and tissue specimens). The serological techniques commonly used include MA, the IF test and ELISA [40, 71, 80]. The IF test is the best since it is simple to perform, and highly specific, and allows also detection of specific antibody classes (IgA, IgG, and IgM) to both phase I and II cellular antigens [40, 63]. Recently, we developed a rapid method for detection of *C. burnetii* antibodies using high-density particle agglutination, which

seems to be a promising tool for routine serodiagnosis of the infection because of its simplicity, sensitivity and specificity [49, 50]. The techniques commonly used for detecting the microorganism include the direct immunofluorescence test, isolation and PCR. PCR is the best since it is comparatively simple to perform, takes less time to test a large number of samples, and is also highly sensitive and specific [32, 46, 58, 60, 78].

Most of the samples for serological and bacteriological tests for *Coxiella* infection in humans and animals made by our laboratory, which is located in Gifu (central Japan), has been collected from the central part of Japan. However, we tested serum samples also from other parts of Japan, often for confirmation of the diagnosis of the infection. Whenever the test result is consistent with Q fever, we ask the provider of the sample to provide further samples of serum, blood, or other tissues for culture and epidemiological and clinical data for analysis. In our own experience, a patient is diagnosed as having acute Q fever if (i) his acute-phase serum is positive by PCR, (ii) his acute-phase serum contains IF polyvalent and IF IgM and/or IgG antibodies at titers of 1:32 or greater, (iii) there is a seroconversion, or (iv) there is a four-fold increase in the specific antibody titers between acute-phase and convalescent-phase sera with a suggestive clinical background, including fever of unknown origin, influenza-like symptoms, granulomatous hepatitis, pneumonia or meningoencephalitis. The individual is diagnosed as having a previous infection if his or her sample is positive in the IF test, but negative in PCR and/or there is no increase in specific antibody titers between the paired serum samples. These standards are used also to determine whether an animal has or previously had the disease. The presence of *C. burnetii* in milk or tissue samples is determined by PCR and/or the direct immunofluorescence test. The individuals are considered not to have the infection when their samples are negative in all three tests, irrespective of their clinical presentation. In some cases, isolation of *C. burnetii* from serum or other tissues provided a definite bacteriological diagnosis.

#### SOME CHARACTERISTICS OF JAPANESE ISOLATES

##### 1 PATHOGENICITY TO GUINEA PIGS

We investigated the pathogenicity of 59 isolates from cattle and three isolates from humans to guinea pigs [68]. The pathogenicity was evaluated by comparing the febrile response, the extent of splenomegaly, the enlargement of testicles, the presence of *C. burnetii* in the spleen and the antibody response. The pathogenicities of the 62 strains to guinea pigs are shown in Table 5. Based on the pathogenicity data, we divided the isolates into three groups: high (eight isolates), moderate (29 isolates) and low (25 isolates) pathogenicities for guinea pigs. The data suggest that many isolates in Japan have moderate or low virulence.

##### 2 GROWTH IN BUFFALO GREEN MONKEY CELLS AND L929 CELLS

The growths of 12 Japanese isolates of *C. burnetii* in

Table 5. Pathogenicities of Japanese isolates to guinea pigs

Degree of pathogenicity	Number of isolates	Febrile response <sup>a)</sup>	Spleno-megaly	Enlargement of testicle
High	8	+++	8	8
Moderate	29	++	16	17
Low	25	+	8	4

<sup>a)</sup>+++;  $40.5 \pm 0.5^\circ\text{C}$ , ++;  $39.4 \pm 0.5^\circ\text{C}$ , +;  $38.7 \pm 0.1^\circ\text{C}$ .

Buffalo Green Monkey (BGM) and L929 cells were compared with those of 13 reference strains in the same cells [64]. The Japanese isolates included three from human blood (307, 605, TK-1), and other nine from cow milk (1M, 3M, 27M, 60M, 82M), cow udder (53U), aborted bovine fetus (50F) or ticks (*Ixodes* spp.; 57T, 58T). The reference strains were Priscilla, Nine Mile, California 76, Bangui, Henzerling, Ohio 314, El Tayeb, Munich, G Q212, S Q 217, Ko Q219, MAN and ME. The 12 isolates grew far more slowly with less host damage than did the reference strains. The cytopathic effects (CPEs) of the 12 isolates were similar to one another in the BGM and L929 cells, but they were quite different from those of the reference strains. There was heterogeneity in the size and the appearance of both the vacuoles and the whole infected cells. All of the reference strains except Priscilla had an impressive CPE (the vacuoles were as big as the cells) while the 12 isolates showed moderate CPE. It is interesting that BGM cells infected with Priscilla became small and round. The reasons for these differences are not known.

### 3 BANDING AND IMMUNOBLOTTING PATTERNS OF PROTEINS AND LIPOPOLYSACCHARIDES

We investigated differences in SDS-PAGE and immunoblotting patterns of proteins and lipopolysaccharides (LPSs) between the Japanese isolates and the reference strains mentioned above. The protein profiles of the 25 isolates were strikingly similar to one another except for some small differences in the range below 33 kDa. These findings showed that the protein profiles of strains from a variety of sources differing clinically and geographically were not significantly different [66]. The immunoblotting patterns of the proteins were similar one another except for a noteworthy difference in the antigenicity of a 28-kDa polypeptide which was immunodominant in strains from milk, ticks and human cases of acute Q fever, but not immunogenic in strains from human cases of chronic Q fever (MAN, ME, Ko Q 229, S Q217, G Q 212 and Priscilla). Based on these data, we suggest that this polypeptide is a marker that distinguishes between the acute and chronic strains [66].

We found also that these isolates were grouped into four in the structure of LPSs based on the SDS-PAGE profiles [64, 65]. The LPSs of group 1 were identified in Japanese isolates and others from human cases of acute Q fever, milk and ticks. Three remaining groups were found in isolates from human cases of chronic Q fever. These LPSs shared many antigenic epitopes. The LPS profiles of 12 Japanese

isolates show association of these isolates with the acute form of Q fever [68].

### 4 MOLECULAR BIOLOGY

Studies on the plasmid types of *C. burnetii* showed that all Japanese isolates contained plasmid QpH1 [14]. This plasmid is 36 kb in size and was present at approximately three copies per cell.

Masuzawa *et al.* [42] showed that the levels of sequence similarity of 16S rRNA genes among Japanese isolates (including 1M, 27M, 50F, 58T and 605) and American isolates (Bangui, Ohio and G Q212) were higher than 99%.

Masuzawa *et al.* [43] found also that the pathogenicities of 10 strains of *C. burnetii* (Japanese strains 1M, 3M, 27M, 60M, 50F, 58T and 605 and prototype strains Bangui, Ohio and G Q212) to guinea pigs were unrelated to the presence of two putative virulence-related genes (*Cbmip*, *Coxiella burnetii* macrophage infectivity potentiator-like gene and *qrsA*, Q-agent regulatory sensor-like protein gene) that supposedly allowed the bacteria to survive in macrophages. In addition, they found that the sequence similarities of the *Cbmip* and *qrsA* genes were higher than 99% among these 10 strains.

Recently, analysis for sequence similarities of *comI* gene, which encodes a 27-kDa outer membrane protein of *C. burnetii*, from 10 Japanese strains and 11 prototype strains from our laboratory revealed that the levels of sequence similarity of 21 strains were higher than 98%, and those of 10 Japanese strains higher than 99% [79].

These results suggest that *C. burnetii* strains from a variety of sources being different clinically and geographically, including those from Japan, are very similar one another, and that the genus *Coxiella* contains only one species, *C. burnetii*.

### CONCLUDING REMARKS

The serological and bacteriological findings on *C. burnetii* infection in animals and humans have shown that the infection is widespread in Japan and suggest that domestic animals are important reservoirs of *C. burnetii*.

It is likely that the inhalation of contaminated aerosol is the most important route by which *C. burnetii* infects animals and humans in Japan.

The Japanese isolates of *C. burnetii* are associated with the acute form of Q fever. These isolates had various degrees of virulence (high, moderate and low), but are strikingly similar to one another and to reference strains of acute infection with respect to the structure and immunogenicity of proteins and LPSs and the nucleotide sequences of 16S rRNA, *comI*, *Cbmip* and *qrsA* genes.

This review is the first to describe the epidemiology of Q fever along with some characteristics of *C. burnetii* isolates in Japan, which could help to set up active surveillance programs to study the epidemiology and prevention of the disease.

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