Brucella spp.

Order: *Rhizobiales*
Family: *Brucellaceae*
Genus: *Brucella*

Bacteria

anses
French agency for food, environmental and occupational health & safety

Characteristics and sources of *Brucella*

Main microbiological characteristics

Brucella is the agent responsible for brucellosis, a contagious infectious animal disease with global distribution that can infect humans.

Brucella is a facultative intracellular Gram-negative coccobacillus, 0.5 to 0.7 µm in diameter and 0.5 to 1.5 µm in length. The cells are non-motile and do not form conventional flagella, capsules, or spores. Bacteria of the genus *Brucella* are strictly aerobic, but certain strains require a CO_2-enriched atmosphere (5 to 10%) in order to grow (see table 1).

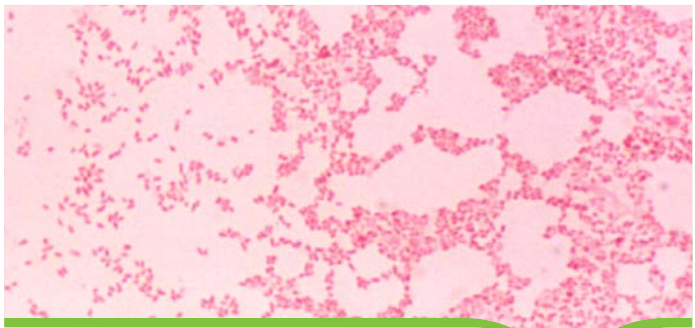
Table 1: Growth characteristics of *Brucella*

Parameters	Growth	
	Optimum	Extremes
Temperature	34°C	20-40°C
pH	6.6-7.4	5.8-8.7
NaCl	0.4%	4%
CO_2	5-10%	

The genus *Brucella* is comprised of ten species, classified according to their pathogenicity and host preferences (reservoir), seven of which can be isolated from land mammals: *B. abortus, B. melitensis, B. suis, B. canis, B. ovis, B. neotomae et B. microti*. The first three are also subdivided into biovars. Two species *(B. ceti* and *B. pinnipedialis)* have also been identified in marine mammals. *B. inopinata* was recently described in humans, with no known animal reservoir. Cases of human brucellosis have been attributed to 4 of the 7 *Brucella species* found in terrestrial mammals. *B. melitensis and B. suis* (except biovar 2) are the most virulent species, followed by *B. abortus and B. canis*. *Brucella ovis, B. neotomae* and *B. microti* are not reported to be pathogenic in humans. A few likely cases of human infection due to a marine mammal strain of *Brucella* have however been described.

Sources of the hazard

The main animal reservoirs of *Brucella* are cattle *(B. abortus)*, sheep and goats *(B. melitensis)* and domestic pigs *(B. suis)*. *Brucella* strains have also been isolated in other domestic species (camelids, water buffalo, reindeer, yaks, etc.) and in numerous ruminant species and other wild terrestrial mammals (bison, deer, hares, caribou, wild boar, chamois, ibexes, etc.). Wildlife can sometimes be a reservoir for *Brucella* with a possibility of accidental transmission to domestic ruminants (cases such as the bison in the USA and the ibex and wild boar in France, for example). *Brucella* strains have been isolated in marine mammals - cetaceans (rorquals, dolphins, porpoises, etc.), pinnipeds (seals, sea lions, walruses, etc.) and otters in particular.



Brucella melitensis **(Gram stain)**
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Infected animals release contaminated substances into the environment (gravid uterine tissue, vaginal secretions, urine, milk, sperm, suppuration matter). The survival of *Brucella (abortus* and *melitensis)* in the environment is promoted by humid conditions and low temperatures (≤ 4°C). *Brucella* can survive for over two months in water at 20°C, two months in soil and on cool, moist pastures, and up to eight months in liquid manure, as well as several months in dry substrates (hay, dust, fencing, etc.).

Transmission routes

Brucellosis is a zoonosis. Humans can be contaminated:

- by consuming contaminated food (mainly raw milk and dairy products);
- by contact of the skin - even apparently healthy skin - or the digestive, conjunctival or nasopharyngeal mucous membranes with infected animals or matter produced by them (mainly genital secretions, aborted foetuses or placentas, but also infected organs such as the liver, spleen or udder in particular, or contaminated manure or wool).

People working in direct contact with infected animals - breeders, veterinarians, A.I. technicians, slaughterhouse and rendering plant personnel - are those most highly exposed to infection. *Brucella* is also one of the agents most often responsible for contamination in laboratories, often through aerosolisation. A few rare cases of infection were observed during handling of vaccine strains (spattering on lips or in the eye, accidental inoculation).

Brucellosis transmission between humans, and sexual transmission in particular, although often mentioned in the literature, has never actually been proven.

Recommendations for primary production

- **Prevention of human brucellosis depends mainly on programmes to control and eradicate the disease in farm animals. The goal of these programmes is to reduce the prevalence of infection in herds through implementation of health control and/or medical measures (animal vaccination), and when possible to attain eradication of the disease, initially farm by farm, and then on a regional and national level.**
- **In infected areas, precautions must be taken on an individual basis by all those who, through their work, are in contact with infected matter or animals, or who are exposed to aerosols from them. These precautions include: hand washing, wearing gloves, masks and goggles, and a ban on smoking, drinking or eating in the workplace.**

Human foodborne disease

Nature of the disease

The human form of the disease is initially asymptomatic in 90% of cases, but this initial clinical silence is not an indicator of the subsequent expression of the disease. When no early diagnosis or treatment is made, the classical form of brucellosis progresses in three stages, each of which may remain paucisymptomatic or even mute (see table 2):

1. Primary-invasion **acute brucellosis** in its septicaemic form
2. Secondary post-septicaemic phase **(subacute or localised brucellosis)**
3. **Chronic phase**

Susceptible population(s)

Currently, there is no information available confirming that certain populations are at higher risk of severe infection or complications (arthritis, endocarditis, splenic/hepatic abscess, meningitis, encephalitis, etc.).

However, the only described cases of human infection by *B. suis* biovar 2 involved people with serious comorbidity inducing a heightened susceptibility to infections.

Dose-effect(1) and dose-response(2) relationships

While the infectious potential of *Brucella* is high, especially through aerial transmission, the infectious dose is closely linked to the individual susceptibility of the exposed individuals. Ten to 100 colony-forming units or less may be enough to cause the disease in humans.

(1) Relationship between the dose (quantity of microbial cells ingested during a meal) and the effect in the individual.
(2) For a given effect, relationship between the dose and the response, i.e. probability of the effect manifesting itself in the population.

Epidemiology

In France, surveillance of human brucellosis, a mandatory notifiable disease, was reorganised in 2002 and is now based on joint action by the French Institute for Public Health Surveillance (InVS) and the National Centre of Reference (NCR) for *Brucella* (ANSES & Inserm/CHU Nîmes) reporting to the Ministry of Health. *Brucella* strains from humans are sent to the NCR for identification, phenotyping and/or molecular typing, which can help to confirm cases and determine their origin. Brucellosis is a disease with worldwide distribution and significance. Only a few world regions are brucellosis-free with regard to domestic animals (except for rare accidental outbreaks in free-range pig farms). These regions include Northern, Central and Eastern Europe, Australia, Canada, Japan and New Zealand. The disease is still found in Northern Ireland, in all of Mediterranean Europe (Spain, Greece, Southern Italy, Portugal) and in the Balkans.

In France, the number of human brucellosis cases has been constantly falling for the last 30 years: over 800 cases in 1978, 77 in 1997, and 28 in 2012, representing an incidence of 0.04/100 000 inhabitants. From 2008 to 2011, 20 cases per year on the average were reported by the InVS. Most of the human brucellosis cases diagnosed currently in France are imported, mainly from Northern Africa, the Iberian Peninsula and the Balkans and Turkey, due to exposure in the country or consumption of a contaminated imported product. Indigenous cases are linked either to reactivation of a past infection or to laboratory contamination during manipulation of strains from patients (at least 13 cases between 2002 and 2012). In 2012, an outbreak with two recent infections (diagnosed in 2012 and 2013) was due to eating unripened cheese made from raw milk from a herd of infected dairy cows in Haute Savoie. In 2013, a cluster of 6 possible cases(3) was reported in tourists after eating cheese in Corsica. However, the diagnosis of *Brucella* infection in these individuals was not clearly established and contamination of the cheeses could not be confirmed.

Role of food

Main foods to consider

In countries affected by brucellosis, the main foods responsible for human brucellosis are raw milk and dairy products made from raw milk (unripened or medium-ripe cheese, butter, ice cream).

Eating undercooked contaminated offal or fruits and vegetables grown in soil treated with contaminated manure can also cause *Brucella* infection.

The contamination of fresh dairy products involves mainly fresh cheeses(4), incriminated in 60% of food exposure cases between 1998 and 2000. These mainly concern raw cheeses made using the milk of goats infected with brucellosis.

In raw milk, Brucella survives for 24 h at 25-37°C and 48 h at 8°C. The minimum fermentation time required for total destruction is not known but it is conventionally estimated that two months is sufficient.

(3) According to the definition of "case" in mandatory notification: "Possible case of brucellosis: detection of high antibody titre in a single serum".
(4) Unripened cheese which has undergone mainly lactic fermentation and which contains living flora at the time of sale to the consumer.

Table 2: Characteristics of the disease

Mean incubation period	Target population	Main clinical forms/symptoms	Duration of symptoms	Complications	Asymptomatic forms
1-3 weeks (up to several months)	All age groups Especially individuals subject to occupational exposure (in this case, mainly male adults)	**Primary-invasion acute brucellosis in its septicaemic form** - Undulant fever: profuse sweating, joint/muscle pain, fatigue, faintness, headache - General influenza-like symptoms.	Variable when no treatment is administered, regardless of the form. With appropriate treatment, the acute form disappears in a few days, while subacute forms can persist over several months.	**Subacute or localised brucellosis** (20-40% of cases): Local manifestations, often osteo-articular in nature (especially the vertebral column and the sacroiliac joint), but can also be genital, meningeal, hepatosplenic, cardiac, pulmonary, cutaneous and ophthalmic. Endocarditis is rare (< 2%) but has a high fatality rate (approx. 80%) **Chronic brucellosis** (non-systematic): Sites with torpid evolution[a] (articular, visceral).	Yes (probably the most common)

[a] evolving without any acute manifestations and no clear positive or negative changes.

Inactivation treatments in industrial environments

Table 3: Industrial inactivation treatments for *Brucella* spp.

Disinfectants	Effects of temperature
Sensitive to all the disinfectants authorised in the agrofood sector, as long as the recommendations for use are followed.	Pasteurisation (63°C - 30 minutes, 72°C - 15 seconds) is an effective heat treatment for *Brucella inactivation* ($D_{66.5}$ = 1.8 - 2.5 seconds).
High pressure	**Ionisation**
Data not available	10 kGy treatment causes inactivation of *Brucella abortus* biovar 1 in 500 mL of colostrum ($0.5x10^5$ UFC/ml before irradiation)

Monitoring in food

There is no food monitoring for *Brucella,* but in herds not officially recognised, or not recognised, as being brucellosis-free, measures are taken to prevent foodborne human infections including pasteurisation for cow and buffalo milk, and pasteurisation or ripening for at least two months for goat's and sheep's milk (Regulation (EC) no. 853/2004). The collection or processing of milk from animals showing clinical signs of brucellosis is also forbidden. There is no standardised method for *Brucella* detection in foods. The reference detection methods for the animal health sector are described in two AFNOR standards:

- AFNOR standard U 47-105: "Méthodes d'analyse en santé animale – Recherche et Identification des *Brucella* spp., autres que *B. canis* et *B. ovis*" (Analytical methods in animal health – Detection and Identification of *Brucella* spp., other than *B. canis* and *B. ovis*)
- AFNOR standard U 47-109: "Méthodes d'analyse en santé animale – Recherche et Identification de *Brucella ovis*" (Analytical methods in animal health – Detection and Identification of *Brucella ovis*)

Recommendations for operators

- **Controlling food-based *Brucella* contamination involves either the pasteurisation or sterilisation of milk, or the use of raw milk from herds officially declared brucellosis-free.**
- **In infected areas, precautions must be taken on an individual basis by all those who are in contact, through their work, with infected matter or animals, or who are exposed to aerosols from them. These precautions include: washing hands, wearing gloves, masks and goggles, and no smoking, drinking or eating in the workplace.**

In the laboratory, stringent biosafety measures must be implemented for the handling of human and animal samples suspected of being infected with *Brucella* and are imperative for the handling of *Brucella* itself, which does not preclude adherence to the basic good practices to be applied in all circumstances, since many cases are not suspected until laboratory confirmation is received.

Domestic hygiene

Recommendations to consumers

- **In general, when no information is provided on the health status of a country, non-pasteurised or unsterilised dairy products should not be consumed.**

References and links

General references

- French Agency for Food, Environmental and Occupational Health & Safety (2015) ANSES Opinion of 31 October 2012 on the survival of *Brucella* in dairy products. https://www. anses.fr/sites/default/files/documents/BIORISK2012sa0115.pdf
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- EFSA/ECDC The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2012 (currently in validation – cf. http://www.efsa.europa.eu/fr/topics/topic/zoonoticdiseases.htm)
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- Mailles A, Vaillant V (2007). Etude sur les brucelloses humaines en France métropolitaine, 2002 - 2004. Saint-Maurice : Institut de veille sanitaire, 2005 ; available at: http://www.invs.sante.fr/pmb/invs/(id)/ PMB_4331 (consulté le 18 février 2014).
- World Organisation for Animal Health (OIE) (2013) Brucellosis: recent developments towards 'One Health', G. Plumb, S. Olsen & G. Pappas; Ed.: Scientific and Technical Review 32 (1), 235 pp.
- Pappas G, Papadimitriou P, Akritidis N, Christou L, Tsianos EV (2006). The new global map of human brucellosis. Lancet Infect Dis.; 6:91-99.

Useful links

- National Centre of Reference for human brucellosis: Université Paris- Est/ ANSES, Animal Health Laboratory, Bacterial Zoonoses Unit (Maisons-Alfort); EU Reference Laboratory for Brucellosis, National and OIE/FAO Reference Laboratory for Animal Brucellosis
- NCR affiliated laboratory (serology): INSERM U1047/Laboratoire de bactériologie, CHU CAREMEAU (Nîmes)
- http://www.invs.sante.fr/Dossiers-thematiques/Maladies infectieuses/ Zoonoses/Brucellose
- http://www.oie.int/en/for-the-media/animal-diseases/animal disease- information-summaries/
- http://www.anses.fr/fr/search/site/brucellose?page=1&iso1=fr&iso2=en
- http://ansm.sante.fr/Dossiers/Micro-organismes-et-toxines hautement- pathogenes-MOT/Micro-organismes-et-toxines hautement- pathogenes-MOT/(offset)/0
- http://www.cdc.gov/brucellosis/