

Brucella spp.

Nature and habitat

Main microbiological characteristics

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 flagella, capsules or spores.

The *Brucella* genus includes eight species, classified according to pathogenic potency and preferential host (reservoir), 6 of which can be isolated in land mammals: *B. abortus*, *B. melitensis*, *B. suis*, *B. canis*, *B. ovis* and *B. neotomae*. The first three are also subdivided into biovars. Two species (*B. cetaceae* and *B. pinnipediae*) have also been identified in marine mammals. Bacteria in the *Brucella* genus are strictly aerobic but some strains require a CO₂-rich atmosphere (5 to 10%) to grow. The optimum pH for growth ranges between 6.6 and 7.4. The optimum temperature for growth is 34°C, with the majority of strains developing at between 20 and 40°C on appropriate medium.

Zoonotic character⁽¹⁾

Brucellosis is an infectious and contagious disease in animals that can be transmitted to humans and has a global distribution. Cases of human brucellosis have been attributed to 4 of the 6 *Brucella* species encountered in land mammals. *B. melitensis* and *B. suis* are the most virulent species, followed by *B. abortus* and *B. canis*. *Brucella ovis* and *B. neotomae* are not reported to be pathogenic for humans. A few probable cases of human infection linked to a strain of *Brucella* in marine mammals have been reported however. *Brucella* is categorised as a group III biological risk for humans or animals and is included in the list of potential bioterrorism agents (group B, second highest priority agents).

Reservoir

The main animal reservoirs for *Brucella* are domestic cattle (*B. abortus*), sheep and goats (*B. melitensis*) and pigs (*B. suis*). Strains of *Brucella* have also been isolated in other domestic species (camelidae, water buffalo, reindeer, yak, etc.) and in numerous species of ruminants, suidae and wild land-living carnivores (bison, deer, hares, caribou, wild boars, etc.).

Strains of *Brucella* have been isolated in marine mammals, in particular cetaceans (humpback whales, dolphins, porpoises), pinnipedia (seals, sea lions, walruses) and otters.

Infected animals excrete contaminated substances into the environment (gravid uterus contents, vaginal secretions, urine, milk, sperm, suppuration products, faeces). The survival of *Brucella* (*abortus* and *melitensis*) in the environment is promoted by moist conditions and low temperatures. *Brucella* can survive for more than two months in water at 20°C, two months in soil and on fresh pasture in moist atmospheres, up to eight months in slurry and several months in dried substrates (hay, dust, pen gates, etc.).

Human disease

Symptomatic and asymptomatic forms

Human infection is initially asymptomatic in 90% of cases but the initial clinical silence is not an indicator of the subsequent expression of the disease. Brucellosis conventionally involves 3 phases, which may each remain paucisymptomatic or even mute:

- 1. acute septicaemic brucellosis following primo-infection:** this generally occurs after an incubation period of 1 to 3 weeks (incubation periods of several months nonetheless being possible) and classically presents in the form of "undulant fever" (undulant fever, profuse sweating, joint/muscle pain, fatigue, malaise, headaches) or general flu-like symptoms;

(1) Zoonosis: an infectious disease that can be transmitted from vertebrate animals to humans and vice-versa under natural conditions.

2. Secondary post-septicaemic phase (sub-acute or localised brucellosis): this may reveal the infection. It is marked by isolated or multiple focalisations (20 to 40% of cases, especially if the acute phase was treated late or was not diagnosed). The locations are usually osteo-articular (especially the spine and sacro-iliac joint), but also genital, or even meningeal, hepatosplenic, cardiac, pulmonary, cutaneous and ophthalmic;

3. Chronic phase: not systematic, this can develop a long time after contamination and be the revealing phase if the initial expression was not apparent. It presents in two forms: general and subjective symptoms, inducing chronic fatigue (physical and intellectual fatigue, depression, etc.) or focalisations (joints, internal organs) with a torpid course. Serious forms such as endocarditis are exceptional (less than 2%). However, the death rate from the resulting complications is very high (in the region of 80%).

Human contamination methods other than by food

Contamination usually occurs following contact with the skin (even apparently healthy) or mucous membranes (gastrointestinal, conjunctival and naso-pharyngeal) of infected animals and their products (mainly genital secretions, foetuses and placentas, but also infected organs, liver, spleen, udder in particular, and contaminated manure or wool).

The most exposed individuals are those working in contact with infected animals: livestock farmers, vets, inseminators, slaughterhouse or rendering plant workers. *Brucella* is also one of the agents most frequently responsible for contamination in the laboratory, often via aerosolisation. A few rare cases of infection have been observed following the use of vaccine strains (projection onto the lips or the conjunctiva, accidental inoculation).

Risk of secondary human-to-human transmission

It is widely accepted that human-to-human transmission of brucellosis, in particular sexual transmission, does not exist.

Exposed population

The populations exposed are professionals in contact with reservoir mammals and their products, or populations consuming unpasteurised milk or products made with unpasteurised milk.

High-risk population

No high-risk population.

Dose-effect⁽²⁾ and dose-response⁽³⁾ relationships

The infective power of *Brucella* is high, particularly by the airborne route, since 10 to 100 bacteria are sufficient to cause the disease.

Diagnosis

- Positive blood culture for *Brucella* (or positive culture from CSF or infected tissue)
 - Serology: Wright's seroagglutination test, buffered plate antigen or Bengal rose test (BPA), indirect immunofluorescence, ELISA, complement binding.
- The specificity of these tests is low (cross reactions with *Yersinia enterocolitica* O:9, *Francisella tularensis*, etc.) and their positive predictive value is therefore low in zones with a low prevalence in animals, such as France.
- Demonstration by PCR from blood or other samples.

Medical treatment and prevention

Antibiotic treatment:

- doxycyclin and rifampicin: the recommended treatment in the majority of cases;
- combination of trimethoprim-sulfamethoxazole and rifampicin or combination of doxycyclin and gentamicin when the above treatment cannot be given.

No human vaccine against brucellosis is currently authorised in the European Union or the majority of developed countries.

Prevalence, annual incidence. Epidemic character

Brucellosis is an infection with a global distribution and importance. Only a few countries are entirely free of brucellosis in domestic animals (with the exception of rare accidental outbreaks in pigs farmed outdoors): northern, central and eastern Europe (Germany, Austria, Benelux, France, the United Kingdom, CEEC, Scandinavia and Switzerland) and Australia, Canada, Japan and New Zealand. It is still present in Ireland and throughout Mediterranean Europe (Spain, Greece, Italy, Portugal) and the Balkans.

On a worldwide scale, there are two epidemiological situations for human brucellosis: indigenous infections which are common in enzootic countries and rare infections amongst travellers from countries free of animal brucellosis. In France, the number of cases of human brucellosis has been

(1) Relationship between the dose and the effect in an individual.

(2) For a given effect, relationship between the dose and the response in the population.

steadily falling for 30 years: more than 800 cases in 1978, 77 in 1997, 36 in 2002, i.e. an incidence of 0.05/100,000 inhabitants. Between 1 June 2002 and 31 May 2004, 72 cases of brucellosis (50 definite and 22 probable) were recorded by the InVS (Institute for Health Monitoring). The majority of cases of human brucellosis diagnosed in France today are imported, primarily from Portugal, Spain, Algeria or Turkey due to exposure in the country or consumption of a contaminated imported product. Indeed, brucellosis is still endemic in certain countries in Southern Europe and around the Mediterranean, such as Italy (0.7), Spain (1.4), Portugal (0.4) and Greece (2.0) - (human cases/100,000 in 2004, EFSA data). Nonetheless, a significant proportion of French human cases are linked to contamination in the laboratory when handling strains from previous cases.

Role of food

Foods involved

The main foods responsible for human brucellosis are unpasteurised milks and products containing unpasteurised milk (immature cheese, butter, ice cream).

The consumption of undercooked contaminated offal (very rarely meat) or of fruit or vegetables grown on soil treated with contaminated manure can also be a source of *Brucella* infection.

Conditions leading to contamination, development and survival of the microorganism in food

Contamination of fresh dairy products mainly concerns "fresh" cheese (cheese made by lactic coagulation), which was incriminated in 60% of food exposures between 1998 and 2000. These mainly concern unpasteurised cheeses made using the milk of goats infected with brucellosis.

In unpasteurised milk, *Brucella* survives for 24 h at 25-37°C, 48 h at 8°C and at least 2.5 years at 40°C. Survival in matured fermented cheeses appears to be relatively short. The minimum fermentation time required for total destruction is not known but it is conventionally estimated that 3 months is sufficient. In "fresh" cheeses, *Brucella* may persist for much longer, the strictly lactic fermentation and short drying duration promoting its survival.

Control measures in the food sector

Good hygiene practices

Controlling contamination with *Brucella* through food requires either pasteurisation or sterilisation of milk, or the use of unpasteurised milk that comes from herds officially recognised as being brucellosis-free.

Individual precautions should be taken by anyone working in contact with infected products or animals: handwashing, wearing of gloves, masks and safety glasses, not smoking in workplaces.

Characteristics of physical, chemical and biological purification treatments

Brucella species are sensitive to temperature, moisture and pH.

Temperature: pasteurisation (63°C – 30 minutes, 72°C – 15 seconds) is an effective treatment for *Brucella* (D66.5 = approximately 1.8 – 2.5 seconds).

Disinfectants: *Brucella* is sensitive to numerous disinfectants – sodium hypochlorite, 70% ethanol, iodine and alcohol solutions, glutaraldehyde, formaldehyde – but is considered to be not very sensitive to quaternary ammoniums.

Monitoring in food

Community legislation (Regulation 853/2004) makes it compulsory to heat-treat milk from herds not officially recognised as being brucellosis-free. It also stipulates the exclusion of collection and processing of milk from animals infected with brucellosis.

In France, monitoring of human brucellosis, a compulsorily notifiable disease, has been reorganised since 2002 and is based on the joint action of the Institut de veille sanitaire (Health Monitoring Institute, the Centre National de Référence (National Reference Centre) for *Brucella* (Afssa – French Food Safety Agency) and the laboratory associated with the CNR (National Research Centre) (CHU Grenoble) under the aegis of the Ministry of Health. Rather than challenging eating habits such as the consumption of products made from unpasteurised milk, prevention is based on control of infection (screening and slaughtering of infected herds, with, if necessary, vaccination in zones with a high prevalence) in farm animals, principally cattle, sheep and goats. These measures have led to an improvement in the health qualities of unpasteurised milk and the reduction of contact between humans and infected animals. Strains of *Brucella* of human origin must be systematically sent to the CNR for identification and typing.

The reference detection methods for animal health 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*).

Domestic hygiene

There are no specific domestic hygiene recommendations. However, hygiene rules must be complied with on the farm:

- Systematic handwashing (potable water and soap):
 - after contact with animals, waste or animal excrement,
 - before meals, breaks, at the end of the working day;
- No eating, drinking or smoking in the workplace;
- In the event of an open wound, wash, soap, then rinse. Disinfect and cover with a waterproof dressing;
- If projection in the eyes, immediately rinse with potable water;
- Regular washing of work clothes;
- Change clothes at the end of the day.

Links

References

- EFSA Zoonoses report 2004 (currently being validated)
- Cutler, S. J., A. M. Whatmore, et al. (2005). "Brucellosis-new aspects of an old disease" J Appl Microbiol. 98: 1270-81.
- Garin-Bastuji, B., F. Delcuelle, 2001.** Les brucelloses humaine et animales en France en l'an 2000. Situation épidémiologique – Programmes de contrôle et d'éradication. Méd. Mal. Infect., 31 Suppl 2: 202-216.
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- Maurin, M. (2005).** "La brucellose à l'aube du 21^e siècle" Med. Mal. Inf. 35: 6-16.

Institut de veille sanitaire (2000). "La brucellose humaine de 1998 à 2000" Surveillance nationale des maladies infectieuses: 199-201.

Alexandra Mailles et Véronique Vaillant. Les brucelloses humaines déclarées en France en 2001 et 2002 [online]. In: Surveillance nationale des maladies infectieuses, 2001-2003. Saint-Maurice: Institut de veille sanitaire, 2005; available on: <http://www.invs.sante.fr/publications/2005/snmi/brucellose.html> (consulted 18 December 2006).

Institut de veille sanitaire (2006). Étude sur les brucelloses humaines en France métropolitaine, 2002 – 2004. (Report currently being validated).

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National reference centre for human brucellosis:

Afssa Bacterial Zoonoses Unit; Community Reference Laboratory for Brucellosis, National and OIE/FAO Reference Laboratory for animal brucellosis.
Dr Bruno GARIN-BASTUJI

Laboratory associated with the RNC (serology):

Bacteriology Laboratory, CHU de Grenoble.
Prof. Max MAURIN

Web links

- <http://www.oie.int/hs2/report.asp?lang=fr>
- <http://data.euro.who.int/cisid/?TabID=10900>
- <http://www.afssa.fr/ftp/afssa/fiches/sa/index.htm>
- <http://afssaps.sante.fr/htm/10/piratox/indpira.htm>
- <http://www.inma.fr/telechar/zoonoses/Brucellose.pdf>
- <http://www.invs.sante.fr/surveillance/brucellose/default.htm>
- <http://www.phac-aspc.gc.ca/msds-ftss/msds23f.html>

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