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Recommendations

Guidelines for the management of accidental exposure to *Brucella* in a country with no case of brucellosis in ruminant animals

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1. Objectives

Confirmed exposure of healthy individuals to *Brucella* is regularly reported, and every time healthcare professionals wonder about the optimal management. Infected individuals are usually biology laboratory personnel (medical, veterinary, research), travelers, and professionals from the agricultural sector.

The present guidelines aim to maximize post-exposure management of patients: proper use of potential antibiotics, adequate use of biological examinations, monitoring.

Malicious exposure is not addressed in the present guidelines as it has already been addressed in the NRBC-E plan.

2. Method

The topic of the present guidelines has been validated by the guideline committee of the French Infectious Disease Society (French acronym SPILF).

The list of questions was compiled by the scientific committee, which was made of experts appointed by partner scientific societies and ad hoc institutions.

Bibliographers performed a literature search on PubMed using the following search terms: brucellosis, *Brucella*, congenital infection, complication, laboratory-acquired infection, laboratory exposure, post-exposure prophylaxis, immunodeficiency, antibiotic resistance.

Publication analysis could not be performed using the GRADE method because of the low level of scientific evidence of the various studies.

Writing of the present guidelines was performed by the scientific committee, and the text was approved by a reviewing group made of practitioners appointed by the partner scientific societies.

3. Guidelines

3.1. Analysis of the situation

3.1.1. General information about Brucella sp. [1]

Brucella are bacteria classified in the third group of pathogenic biological agents (R. 4421-3 of the French employment code). As such, they should be manipulated in level III laboratories [2,3]. They are also included in the list of infectious agents targeted by the French executive order on microorganisms and toxins [4]. Any clinical suspicion should therefore be notified to the biology laboratory before sending biological samples for diagnostic testing, because of the high risk of transmission to laboratory personnel. Adequate prevention measures are thus required.

France has officially not been reporting any case of bovine brucellosis since 2005, and the last cases of brucellosis in ovine animals

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and goats were diagnosed in 2003. Surveillance is still ongoing for these species for the early detection of reemergence [5]. However, *Brucella suis* biovar 2 infections are still being reported in France in wild boars and jackrabbits. This bacterium is known to be poorly pathogenic to humans, and about 10 cases have been reported so far [6]. *Brucella* cases in aquatic animals (*Brucella ceti* and *Brucella pinnipedialis*) are also reported in France and worldwide. Human cases transmitted by *Brucella*-infected aquatic species are very rare; they are not addressed in the present guidelines.

Brucellosis is a non-specific clinical bacteremia with potential secondary septic localizations in various organs (joints, bones, liver, spleen, and rarely the brain). Relapses may be observed because of bacterial persistence in tissues.

Initial clinical disease occurs from a few days to a few weeks after exposure, but is not always diagnosed because of general symptoms or atypical presentations. Brucellosis diagnosis may be established a long time after infection (up to several years later) with subacute or chronic presentation.

No bacterial resistance has yet been observed to the commonly used antibiotics. The recommended treatment of brucellosis in France is therefore based on a combination of rifampicin and doxycycline for six weeks. Antibiotic susceptibility testing is not required for patient's management.

3.1.2. Frequency and epidemiology. Is brucellosis a public health problem in metropolitan France?

A total of 419 cases of brucellosis were diagnosed and reported in France from 2001 to 2017 (Santé publique France [SPF] data, http://www.santepubliquefrance.fr/), *i.e.* 17 to 41 cases a year. *Brucella melitensis* is most frequently isolated. Most cases diagnosed in France are imported cases (76%).

Domestic cases (excluding late reactivation of a former contamination) only rarely involve people who have been in contact with farm animals (cows, sheep, goats) in France or people eating raw milk dairy products [7]. The last native case was reported in 2011 when brucellosis reemerged in Haute Savoie department from wildlife animals [8].

Travelers and biology laboratory personnel are at highest risk of *Brucella* infection in France. A total of 22 cases of brucellosis were diagnosed and reported in France from 2001 to 2017 in biology laboratory personnel. Twenty of these cases had been contaminated by manipulating samples of imported cases, and the two remaining cases by manipulating samples from a laboratory technician who had been contaminated when handling samples from an imported case. For each contamination case identified at the laboratory, several colleagues exposed to the same samples were identified and monitored.

3.1.3. Contamination sources and transmission modes

Brucellosis reservoir is exclusively animal. Domestic ruminant animals (goats, bovine animals, and ovine animals) are the main source of bacterial infections in humans worldwide, but other domestic or wild animals may be infected (camelids, deer, pigs).

Animal brucellosis is usually a genital infection in females and leads to abortions, but orchitis or joint presentations may sometimes be observed.

B. melitensis mainly infects ovine animals and goats while *Brucella abortus* is mainly observed in bovine animals. However, *B. suis* is more frequently reported in pigs (except for *B. suis biovar 2* which is enzootic in boars and jackrabbits).

Humans can contract brucellosis through the digestive, mucocutaneous, respiratory, or tear duct routes. Airborne transmission of brucellosis is favored by *Brucella* sp.'s ability to disseminate by aerosols [9].

3.1.3.1. Contacts with animals/consumption of at-risk products. The major source of contamination for humans worldwide is direct contact with infected animals (goats, bovine and ovine animals), especially contact with aborting products, or consumption of raw milk products made with contaminated milk (milk and medium-ripe cheese). More rarely, contamination may occur after consumption of poorly cooked offal from infected animals. At-risk individuals are those exposed to animals or eating contaminated products in countries where animal brucellosis is not under control.

3.1.3.2. Occupational/nosocomial exposure. Occupational exposure to *Brucella* is observed in people with contact with infected animals in at-risk countries (livestock farmers, veterinarians, personnel of slaughterhouse). Another source of frequent occupational contamination is the handling of bacterial cultures from infected patients or animals: biologists, laboratory technicians, researchers [10,11].

Brucellosis is the most frequently declared laboratory-acquired infection worldwide [12].

Cases of brucellosis acquired in microbiology laboratories involve cases due to accidental exposure, consequence of a lack of prior clinical information, handling of samples outside of level II biosafety cabinets, material defects, falling of samples or bacterial cultures on the ground leading to container breakage [13,14].

Factors contributing to laboratory exposure are:

- absence of clinical suspicion or lack of suspicion notification to the biologist; thus leading to minor or insufficient precaution measures implemented, mainly handling of clinical samples or bacterial cultures outside of level II biosafety cabinets;
- microbiological diagnosis delay (e.g., identification error or no identification by the automated device), potentially leading to inadequate microbiological procedures [15].

3.1.4. Characterization and hierarchization of the transmission and infection risk

Public Health England [16] defines three risk levels depending on exposure:

- risk level = none: handling of *Brucella* isolates in a Class II biosafety cabinet using BSL-3 precautions (BioSafety Level 3); microbiology administrative staff handling sample request forms, but not handling opened samples; exposure to a biological sample for other non-microbiological tests (ionogram, complete blood count, etc.) ;
- risk level = low: all persons present in the laboratory room at distance more than 5 feet from activity, at the time of manipulation of *Brucella* isolates on an open bench, but who do not have high risk exposures as defined below ;
- risk level = high: person working without using BSL-3 precautions, or present within a 5 ft. radius, when performing activities on *Brucella* isolates (sniffing or opening culture plate, mouth pipetting specimen material containing *Brucella*), or all persons present in laboratory room in case of widespread aerosol-generating procedures (e.g., breakage of tube containing specimen).

American guidelines from the CDC [17] take into consideration the same criteria as the British guidelines, but they also consider the sample nature and suggest a more complex risk scale.

3.1.5. Diagnostic tools. Performances and predictive values.

3.1.5.1. Direct diagnosis.

3.1.5.1.1. Culture. Culture isolation of *Brucella* is the reference technique for confirming brucellosis diagnosis. The bacterium is most often isolated from blood culture or from an infected site

sample (abscess, joint fluid, spinal disc, etc.). Gram coloration identifies Gram-negative coccobacilli.

Culture of these bacteria takes time (>48 hours, except for *Brucella microti*, *B. inopinata*, *B. suis* biovar 5) and can only be performed on enriched agar media and under strict aerobic conditions. Bacterial colonies are smooth, translucent, non-hemolytic, with regular edges. They are catalase-positive, mainly oxidase-positive and exhibit urease and nitrate reductase.

Identification of colonies by mass spectrometry (MALDI-TOF) can be performed at the genus level. One must check beforehand that the level-3 pathogen database is available to avoid identification mistakes.

3.1.5.1.2. Molecular biology. PCR is a sensitive and specific technique for the diagnosis of brucellosis. It can be performed using bacterial colonies, whole blood, buffy coat, or serum at the acute bacterial phase, as well as from tissue, pus, or cerebrospinal fluid (CSF) biopsies.

PCR performs highly well with bacterial cultures. Its sensitivity and specificity are far lower with the other types of sample. Most PCR techniques are genus-specific. Species identification may be performed with multiplex PCR techniques used by the National reference center [18,19]. They are particularly useful for the diagnosis of acute brucellosis when antibiotics have already been prescribed, thus preventing bacterial growth in cultures, and for localized presentations of brucellosis for which PCR sensitivity is higher than culture sensitivity [20,21].

Various PCR techniques enable *Brucella* sp. identification. Amplification of the gene encoding for the 16S ribosomal RNA followed by sequencing leads to species identification within the *Brucella* genus. However, 16S sequences available from the databases may lead to identification mistakes [22–24]. Other targets are the *bcsp31* gene encoding for a 31-kDa protein, and the insertion sequence *IS*711 as several of its copies are present by genome.

3.1.5.2. Indirect diagnosis. Serology is not recommended as the first-line test. Several techniques must indeed be used simultaneously, because of its lack of specificity and of a very low positive predictive value (PPV) while the incidence and prevalence of brucellosis in France is low. False positive results are therefore frequently obtained. Serological cross-reactions are due to antigenic similarities with other bacteria, especially with Gram-negative bacteria (*Francisella tularensis, Yersinia enterocolitica O9, Vibrio cholerae*). Autoimmune diseases also contribute to false-positive results.

IgM can be detected from Day 10 following clinical sign onset. IgG can then be detected, and titers of both classes (IgM and IgG) rise during the acute phase of the disease. IgG levels then highly increase, especially at late phases of acute infection.

During the chronic phase, IgM disappear while IgG persist. However, acute brucellosis cannot be distinguished from chronic brucellosis based on the type of antibodies, because of individual kinetic variability.

3.1.5.2.1. Rose Bengal test. Rose Bengal test is a rapid screening method by agglutination on a slide. Early positive result is observed (two to three weeks) and sensitivity is very high (>95%) [21,22,25]. It is the most interesting screening technique for brucellosis suspicion because of its ease of use, rapidity, and sensitivity [25].

3.1.5.2.2. Wright's seroagglutination test. It is the reference method recommended by the WHO for brucellosis diagnosis in endemic countries. It can detect antibodies to one of the bacterial antigens (s-LPS). Antibody titers at 80 are currently considered the positivity threshold in non-endemic countries [26–28]. Antibody titers decrease within 4 to 8 months. Similar to other tests, this test lacks specificity. *Yersinia* serology must therefore be performed in case of a positive result to Wright's test, or the brucellosis diagnosis must be confirmed by the National reference center (see below).

3.1.5.2.3. Other serological techniques. They are mainly performed by the National reference center. They include indirect immunofluorescence (IIF) assay, immunocapture technique, and enzyme-linked immunosorbent assay (ELISA). These methods can identify at best the infection stage by evaluating the various antibody isotypes.

IIF is highly sensitive and more specific than the other agglutination techniques. Its interpretation is however subjective and requires the staff to be trained.

The immunocapture technique detects IgG and IgA agglutinating antibodies [23,24].

ELISA is particularly interesting for chronic, complicated, or localized brucellosis when the other tests are negative [19,25] in non-endemic areas where any positive result is suspicious [29].

3.2. Management of at-risk patients

3.2.1. How should we define risk?

The working group recommends performing a global risk assessment with no distinction between risk levels as it is a complicated analysis that is often performed retrospectively (Fig. 1). Such analysis is anxiety-provoking for people not included in the risk category associated with a potential treatment prescription, and one is tempted to overestimate the risk to confirm treatment decisions. It is thus recommended to categorize the risk as existing risk or non-existing risk.

The working group suggests using a risk assessment based on circumstances of exposure.

3.2.1.1. Laboratory activities for human, animal, or research biological diagnosis. Risk exposure to *Brucella* is defined by:

- inhalation or direct contact with the skin or mucous from a culture or from high inoculum (nutrient broth containing growth by multiplication of the infecting strain), i.e. opening culture bottles outside of level II biosafety cabinets, for everyone less than 1.5 meters away from the culture bottle or tube (or blood culture vial) at the time of opening and without respiratory protection (FFP2 or FFP3 masks) during the potential exposure period. Sniffing cultures for identification purpose by smell is included in this hypothetical case, and must not be done in laboratories;
- pregnant women and immunocompromised people are at higher risk of developing the disease, even when the inoculum is low (if they do not wear FFP2 masks);
- all individuals present in the room when a tube containing the *Brucella* culture breaks, and who do not wear FFP2 or FFP3 masks during the potential exposure period, are at risk of exposure. This type of accident contributes to dissemination and contact with the *Brucella* strain, even more so in case of liquid medium cultures as they are more concentrated and contain higher inoculum.

The risk is insignificant with all other biological samples (blood, abscess, joint fluid, CSF, etc.), including with the initial sample (other than the blood culture vial) leading to positive culture.

3.2.1.2. Travelers. Consumption of dairy products made from raw milk or contact with farm animals in travelers or people living in enzootic countries for brucellosis should not be considered a *Brucella* contamination risk requiring specific post-exposure management. Contamination of animals or food products is very rarely documented, and the contamination risk of these individuals is the same as the one incurred by the general population of the country concerned.

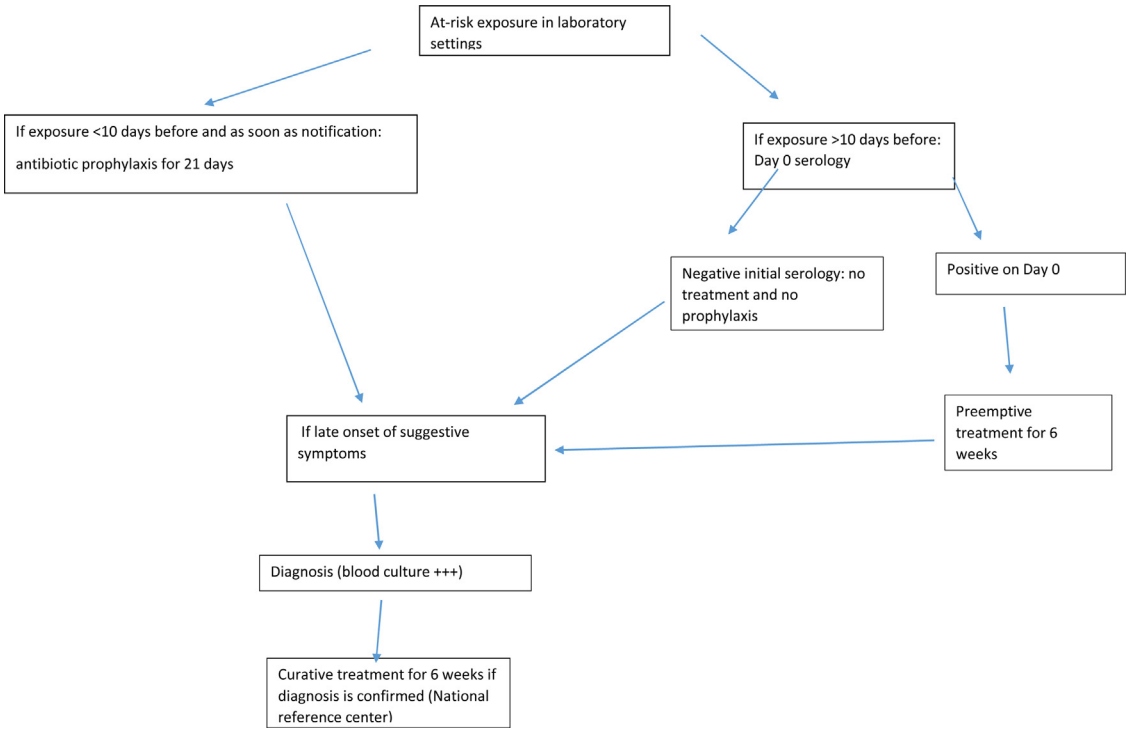

Fig. 1. Flow chart for the management of people exposed to *Brucella* in laboratory settings.

Exceptions can be made for co-exposed people to a confirmed brucellosis case returning from an enzootic country. This concerns travelers who consumed the same products or who have been in contact with the same animals as the index case. This situation however requires exposures of each individual to be checked.

3.2.1.3. Farmers, veterinarians, and slaughterhouse staff exposed to animals infected with Brucella *without adequate personal protective equipment or people consuming dairy products from such animals in the French context of brucellosis reemergence.* These situations include those similar to the one observed during brucellosis reemergence in the French alps in 2011 [8].

Brucellosis diagnosis in animals or detection of *Brucella* in milk or dairy products must be confirmed by formal methods, and exposure must be characterized for the risk to be considered real. The management and follow-up for contamination in biology laboratories must in these cases be implemented.

3.2.1.4. Boar and jackrabbit hunters exposed to B. suis *biovar 2.* Hunters may be exposed to *B. suis* biovar 2, especially during cutting up or disemboweling of jackrabbits and boars. Hunted animals are rarely tested outside of punctual surveillance programs, and these exposures often go unnoticed. These exposures are probably frequent, but they rarely lead to contaminations and diseases in humans because of the poorly pathogenic nature of this bacterial species to humans. They should not be considered risk exposures requiring prophylaxis initiation.

However, game meat may carry other bacteria or viruses that are pathogenic to humans. General prevention measures for infectious diseases, such as wearing gloves for cutting up or disemboweling, and meticulous cleaning of knives must always be recommended.

3.2.2. Who should be prescribed antibiotic prophylaxis? And how?

The present and next chapters distinguish prophylaxis prescribed to an healthy individual following risk exposure without biological diagnostic testing, and preemptive treatment prescribed to an healthy individual presenting with an asymptomatic infection documented by a biological test confirmed by the National reference center.

3.2.3. Indications

Antibiotic prophylaxis may be proposed to at-risk individuals, as early as risk exposure identification and up to 10 days after exposure (theoretical time for antibody production in case of infection, see below).

3.2.3.1. Antibiotics and treatment duration for prophylaxis. The oral route should be favored.

Doxycycline (200 mg once daily, as one intake) + rifampicin (600 mg once daily, as one intake), for 21 days.

Specialist's advice is required for pregnant women.

Children aged below 8 years: co-trimoxazole (30 mg/kg of sulfamethoxazole/day as two intakes) + rifampicin (15 mg/kg/day as one intake), for 21 days.

3.2.4. Who should be prescribed preemptive antibiotic treatment?

When the risk exposure took place more than 10 days before the consultation, antibodies are theoretically already present in case of infection. It is thus recommended to look for these antibodies to determine whether the patient was infected. Positive blood test results should be confirmed by the National reference center.

- Positive serological results confirmed by the National reference center should be linked to the risk exposure reported by the asymptomatic infected individual (mainly laboratory contamination), considering the very low incidence of brucellosis in France, and thus the very low prevalence of antibodies in the French general population.

- Antibiotics are not recommended in case of asymptomatic seroconversion in at-risk individuals who received adequate prophylaxis.
- If the positive serology is confirmed by the National reference laboratory in at-risk asymptomatic individuals who did not receive adequate prophylaxis after exposure, antibiotics are recommended.
- Molecules and dosages are similar to those suggested for prophylaxis, but for 6 weeks rather than the 21 days recommended for prophylaxis.

3.2.4.1. Justification. These recommendations for treatment and prophylaxis are based on a low level of scientific evidence from a few publications. An American literature review published in 2013 [30] compiled 28 reports including a total of 167 individuals working in laboratories and exposed to *Brucella*, mainly *B. melitensis* (80%). Seventy-one laboratory technicians developed brucellosis, confirmed by seroconversion ($n = 68$; 96%) and/or positive culture ($n = 47$; 66%). The 82 laboratory technicians exposed to a "high" risk had a 9.3-fold increased risk of developing brucellosis, compared with those deemed to be at "low" risk. Among the 167 exposed laboratory technicians, 34 received post-exposure prophylaxis (PEP). Thirty-three had been classified in the high-risk category. None developed brucellosis. Among the 82 laboratory technicians exposed to a high risk, those who received PEP had a 0.009-fold risk of developing brucellosis, compared with those who did not (95% CI, 0–0.042; $P < 0.0001$). Eight of 34 laboratory technicians receiving PEP had to discontinue treatment because of adverse effects.

3.2.5. Which follow-up modalities after exposure?

No serological follow-up is required for people who received preemptive or prophylactic treatment because of the impossible interpretation of results.

For occupational exposure and people wishing to document claims for compensation, it is recommended—although not required—to perform serological follow-up. This follow-up should be performed on Day 0 (on the day of notification), and then on Month 2 and Month 6. These tests must be performed by the National reference center.

In case of symptom onset evocative of brucellosis after exposure, with or without prophylaxis, the patient becomes a suspect of brucellosis and should not be managed as part of the post-exposure management protocol. Brucellosis diagnosis by blood cultures or PCR is highly recommended, and all brucellosis suspicions must be reported to the medical analysis laboratory.

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