

# Brucellosis in humans – etiology, diagnostics, clinical forms

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## Abstract

Brucellosis in humans is a zoonosis of greatly varied clinical image. It occurs on all inhabited continents. The course of the disease may be acute, sub-acute or chronic. The etiologic factors of brucellosis are small, aerobic Gram-negative rods of the genus *Brucella*, which currently contains ten species: *B. abortus*, *B. suis*, *B. ovis*, *B. melitensis*, *B. canis*, *B. neotomae*, *B. pinnipedialis*, *B. ceti*, *B. microti* and *B. inopinata*.

In humans, the disease is caused mainly by: *B. melitensis* as the most pathogenic species, followed by *B. suis*, whereas *B. abortus* is considered as the mildest type of brucellosis. The natural reservoir of the germ and the source of infection in humans are infected domestic animals, primarily cattle, sheep, goats, as well as wild animals. Infection in humans occurs by penetration through damaged skin, conjunctiva, and more rarely via the alimentary route by the consumption of infected products. Especially exposed are: veterinarians, veterinary technicians, insemination service employees, zoo technicians, farmers working on multi-herd farms (production cooperatives), e.g. cattlemen, also private farmers, employees of slaughter houses and meat processing enterprises. A basis for diagnosing brucellosis are serologic tests which allow the detection of antibodies occurring in response to infection, performed with the use of the following methods: agglutination test, complement fixation test, Coombs test, 2-mercaptoethanol agglutination test, and Burnet's intradermal allergy test which detects the state of hypersensitivity of the infected organism to *Brucella abortus* rods.

## Key words

brucellosis, *Brucella sp.*, occupational exposure, serologic diagnostics

## INTRODUCTION

Brucellosis (Lat. *Brucellosis* or *Abortus epizooticus*) is a chronic infectious bacterial disease affecting various species of domestic and wild animals, as well as humans. In humans, this disease is also called: Maltese fever, Bang's disease, undulant fever, or Mediterranean fever [1, 2].

In humans, the disease may cause many symptoms varying from mild flu-like to severe complications on the part of the nervous system, musculoskeletal system and the heart.

From 1 December 1980, the entire territory of Poland has been officially considered as free from cattle brucellosis. At that time, the percentage of infected animals was less than 0.5 %, while the percentage of infected farms – less than 0.2 %.

At present in Poland, no newly acquired infections are observed; however, single cases of brucellosis are noted in association with employment of workers at tending animals abroad, e.g. sheep shearing, or in Polish tourists visiting Mediterranean countries [3, 4].

Although the disease occurs worldwide, it is most prevalent in the countries which do not possess adequate standards developed in the area of public health and protection of animal health. The areas at high risk of brucellosis infection are the countries of the Mediterranean Sea Basin (Portugal, Spain, South France, Italy, Greece, Turkey, North Africa), also countries of South and Central America, Asia, Africa, the Caribbean and Near East.

## ETIOLOGIC FACTOR OF THE DISEASE

Brucellosis in humans is a zoonosis of a greatly varied clinical image, caused by small, aerobic, Gram-negative rods of the genus *Brucella*. After penetration into the body the bacilli proliferate in the lymphatic system, mainly in the lymph nodes, subsequently break through the protective barrier and penetrate into various organs [5].

The genus *Brucella* was discovered by David Bruce in 1887 [6] and currently consists of ten species (of many serotypes). These are:

1. *Brucella melitensis* – isolated in 1887 in Malta (hence called Malta fever) by David Bruce from the spleen of a soldier who died from acute brucellosis [6]. The species most pathogenic for humans.
2. *Brucella abortus* – causing abortions in cattle, for many years the main etiologic factor of brucellosis in animals and humans (Bang's disease) in Poland [7].
3. *Brucella suis* – causing the disease mainly in swine [7, 8], pathogenic also for humans [9, 10]. In Poland it was also isolated from wild hares [11].
4. *Brucella canis* – isolated from dogs, may also be the cause of illness in humans [12]. It was first described by Carmichael in 1966 [13] who isolated the bacillus from the placenta, fetuses and vaginal discharge of bitches that aborted their litters. The disease was earlier diagnosed in the United States in Beagle dogs [14].
5. *Brucella neotomae* – isolated in the United States from rats [15].
6. *Brucella ovis* – infects not only sheep, in Poland it was cultured from ram semen [16].
7. *Brucella marina* – *Brucella ceti* – found in sea mammals (whales, seals) in the Atlantic Ocean [17, 18, 19, 20].

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8. *Brucella marina* – *Brucella pinnipedialis* – also found in sea mammals [17, 18, 19, 20].

9. *Brucella microti* – isolated from the common vole (*Microtus arvalis*) in the Czech Republic [21], from soil in the same area years later [22], and from mandibular lymph nodes of wild red foxes (*Vulpes vulpes*) in Austria [23].

10. *Brucella inopinata* – isolated from a breast implant wound of a woman with clinical signs of brucellosis [24].

The above-mentioned species of *Brucella sp.* are pathogenic for many mammals (wild animals – including small rodents, breeding stock and domesticated animals), as well as sea mammals and birds. Breeding stock (cattle, sheep, goats, dogs and even poultry), and wild animals (rodents, hares, etc.) are both the reservoir and vector of the disease in humans [25]. Pathogenicity of 5 *Brucella* species for humans has been confirmed: *B. melitensis*, *B. abortus*, *B. suis*, *B. canis*, and recently *B. marina* [18]. The disease in humans is caused mainly by: *B. melitensis* as the most pathogenic species, followed by *B. suis*, while *B. abortus* is considered as the mildest type of brucellosis [25]. However, this 'mildness' should be approached very cautiously, because despite the progress in therapy, there occurred and still occur very severe cases of the disease in humans of *Brucella abortus* etiology, some of them ending in death. The older and middle-aged generation of Polish physicians also encountered such a severe course. It seems that the sequencing in recent years of *Brucella melitensis*, *B. suis* and *B. abortus* genomes [26], and the confirmation of their high similarity explains well the high pathogenicity of these 3 species for humans.

## SOURCES AND ROUTES OF INFECTION

A natural reservoir of the germs, and the source of infection in humans, are ill domestic animals, mainly cattle, sheep, goats and swine. Also, dogs, especially shepherd dogs, and wild animals: hares, wild rabbits, roe deer and foxes may play some role as reservoirs and spreaders of the germs [10].

Among humans, the highest exposure is observed among: veterinary doctors, veterinary technicians, insemination service employees, zoo technicians, farmers working on multi-herd farms (production cooperatives), e.g. cattlemen, also private farmers, employees of meat processing enterprises and the fodder processing company 'Bacutil'.

Humans are most frequently infected via:

1. damaged skin of the hands during the direct contact with infected placenta, aborted foetus or amniotic fluid while performing gynaecological procedures in cattle, while examining and flaying slaughtered animals;
2. mucous membranes (mucosa);
3. airways.

Infection with brucellosis may also occur while handling manure from infected animals. Infections via the alimentary route by the consumption of infected milk or dairy products are rare [7, 9].

## DIAGNOSTICS OF BRUCELLOSIS

Diagnostics of brucellosis in humans and animals is mainly serologic, which may be performed by means of a number of methods [10, 15, 25, 27, 28, 29, 30, 31]. Classical diagnostics has been known since the end of the 19<sup>th</sup> century: agglutination

and its modifications, and complement fixation test are still routinely applied, improved and supplemented by new tests, and remain a basis for laboratory diagnosing of brucellosis in humans and animals [25, 31]. A tremendous experience collected worldwide for a century in serologic diagnostics of brucellosis allowed, together with the recognition of a number of pathomechanisms of this disease in humans and animals, a precise determination of clinical correlations and serologic response; thus, a precise diagnostics in various periods and forms of brucellosis and at various states of body reactivity [32, 33]. Classical tests technically optimized in many modifications; however, unchangeable in their essence, i.e. Wright agglutination reaction – with its valuable modification in the form of 2-mercaptoethanol (2-ME) test and complement fixation test (CFT) have served for decades (and still do), mainly for the detection of new cases of brucellosis. In addition, in the past, both in Poland and worldwide, other tests were performed, such as: opsonophagocytic reaction, radioimmunological tests, indirect immunofluorescence reaction, or passive haemagglutination reaction [25, 34, 35]. Both agglutinating antibodies and complement-fixing antibodies occur quickly after being infected (according to various researchers in the first days, and even hours of infection) and maintain themselves individually. Coomb's test (AGT) detecting specific incomplete antibodies which maintain themselves for years, and sometimes the only antibodies detectable, in chronic brucellosis has become a valuable and even indispensable supplementation of the classical tests.

**Agglutination reaction (Wright reaction)** with blood serum (AR) is applied in routine brucellosis diagnostics in humans. It may be carried out using tubes or plates [36].

With the use of this reaction anti-*Brucella* agglutinins are detected. This reaction consists in the binding of *Brucella* to specific antibodies which are present in the sera examined. This results in the decrease in electric charge and change in the physical and chemical structure of bacterial cells. Their hydrophilic character changes into hydrophobic. This leads to the formation of clumps, which in the tube agglutination fall to the bottom of the test tube in the form of sediment [37].

Agglutinating antibodies form mainly immunoglobulins of the IgM class. The time of their persistence in the body varies. They occur as early as in the first stage of the disease, most frequently 6–7 days after infection. In acute and sub-acute forms of brucellosis in humans agglutination reaction gives positive results with high titres.

*Bilecki* emphasizes that the agglutination reaction may be positive in the course of other infectious diseases, such as tularemia, exanthematous typhus, tuberculosis, or in individuals vaccinated against cholera or typhoid fever. In addition, serologic cross-reactions may occur between *Yersinia enterocolitica* 03 and 09, and classical species of the genus *Brucella* [25]. Negative results in the agglutination reaction may occur:

- in newly-infected individuals (when anti-*Brucella* agglutinins have not yet been produced);
- in individuals with chronic brucellosis (in whom the level of agglutinins reached zero value);
- in individuals with broken immunity.

**Complement fixation test (CFT)** – apart from agglutination reaction (AR) – is the second diagnostic test used in the

diagnosis of brucellosis in humans [36]. This test is sensitive and specific.

This is the method for detection of the level of antibodies of the IgG class, which occur approximately on day 20 of the disease. High titres are observed during the first and second years of the disease, while several years after infection the results of studies may be seronegative.

## PRINCIPLE OF THE METHOD

This is a two-phase reaction with participation of 5 components which create a bacteriolytic system (test system, Phase I): antibody + antigen, and haemolytic system (indicator system, Phase II): complement + hemolysin + blood cells. Individual components are applied in equal volume and strictly specified concentration, established by titre testing. The test consists in the fixation of the complement by immune specific complexes antigen-antibody in the test or indicator system. The process of complement fixation in the test system, leading to antigen dilution (bacteriolysis) is not perceived after the termination of Phase I, only in Phase II it is manifested by haemolysis inhibition (positive reaction). If the serum tested does not contain antibodies specific for a given antigen (anti-*Brucella*), the complement unfixed in Phase I causes in Phase II easily observable haemolysis of blood cells (negative reaction).

**Coombs antiglobulin reaction (AGT)** – is the test of very big diagnostic value. This reaction is especially valuable in retrospective studies, allows the detection of incomplete antibodies in the cases of chronic brucellosis [38].

The titres of incomplete antibodies preserve themselves for the longest time and are considerably higher than the titres of complete antibodies.

Coombs antiglobulin reaction is performed with sera which react negatively in Wright's agglutination reaction.

**Coagglutination test (COAT)** – is the subsequent reaction used in the diagnostics of brucellosis. *Staphylococcus aureus* possesses a surface antigen – protein A, which has an extraordinary property of reacting with gamma globulins of humans and various species of animals. This property consists in combining protein A with the Fc portion of gamma globulin, while the Fab region responsible for specific antibody activity remains free and capable of binding to the antigen.

In the coagglutination test, there occurs a type of reaction in which Cowan I protein A capability to bind with Fc portion of gamma globulin is used to detect incomplete anti-*Brucella* antibodies.

A great diagnostic value of this reaction is that it allows the diagnosis of the cases of chronic brucellosis by detecting incomplete antibodies [39]. The starting point for the performance of the coagglutination test is the classical agglutination reaction (AR) performed in dilutions of the serum examined from 1/25 – 1/200.

The coagglutination test is performed with sera which react negatively in the Wright's agglutination reaction.

## 2-mercaptoethanol agglutination test ('reduction' reaction)

In the serologic diagnostics of brucellosis, increasingly more emphasis is being placed currently on the use of additional

qualitative reactions, which enable the differentiation of antibodies of the IgM and IgG classes. The majority of these reactions are based on the principle of reducing agglutination titre by inactivation or selective removal of the antibodies of IgM class from the investigated serum. Inactivation of the IgM may be obtained by their reduction with the use of 2-mercaptoethanol (2-ME) or cysteine hydrochloride, by decreasing the pH of the reaction environment or elevation of the temperature of reaction incubation. According to the technique of IgM inactivation, the following qualitative reactions are applied: 2-ME – Mercaptoethanol Test, and Heat Inactivation Test, agglutination with acid antigen (Card Test or Rose Bengal Test), and Rivanol Test. From the above-mentioned tests, the reaction with 2-mercaptoethanol (2-ME) is increasingly more often used in both human and veterinary medicine. The mechanism of reducing effect of 2-mercaptoethanol consists in 'breaking' the –S–S– bindings. This results in the loss of biological activity by the IgM, and thus the loss of its agglutination capabilities. The reaction with 2-mercaptoethanol makes it impossible to indicate whether and in what amount the agglutinins of the IgG class occur in the examined serum. The degree of reduction of the level of agglutinins under the effect of 2-mercaptoethanol is specified by parallel tests using agglutination reaction and the reaction with 2-mercaptoethanol. The total disappearance of agglutination reaction evidences that agglutinins active in agglutination reaction belong to the IgM class, and to the contrary, the lack of the effect of reducing the titre after 2-ME reduction is evidence that agglutinins present in the investigated serum belong to the IgG class. In turn, a partial reduction in agglutination reaction under the effect of 2-ME evidences that the agglutinins present in serum belong partially to the IgM, and partially to the IgG classes, the higher the degree of agglutination reaction the higher the IgM/IgG antibodies ratio [40,41].

**Burnet's skin allergy test** – is especially useful in cases suspected of brucellosis infection, when other serologic reactions are unclear. It is a sensitive and specific reaction. Positive Burnet's reaction evidences the allergic re-tuning of the body as a result of infection and may preserve itself long after being cured. This reaction consists in injecting intradermally, on the inner side of the forearm, of 0.05–0.1 ml of diagnostic Brucellin. The result is read after 24 and 48 hours. Local reaction may occur in the form of redness and infiltration of various sizes, often a blister, or even necrotic changes in the centre of the change. General reaction may be manifested by sub-febrile or febrile states, with an aggravation of general and focal symptoms of brucellosis [10]. Initially, for the performance of Burnet's reaction, Brucellin PS was applied obtained by the freezing and unfreezing of *Brucella* culture multiple times at a dose of 0.02–0.05 ml [42]. However, this was replaced by Brucellin PD, milder in effect, consisting of *Brucella* cells disrupted by ultrasounds. Brucellin PD was used at a dose of 0.05–0.1 ml. In the diagnostics of brucellosis it should be remembered not to precede with serologic tests by the performance of Burnet's test, because an introduction of Brucellin (PS and PD) induces positive serologic reactions (agglutination, complement fixation), and such a state lasts for 8–10 weeks.

In recent years, methods of molecular biology have been used increasingly often in the diagnostics of brucellosis, especially the PCR. These methods may be used on 3 levels

of diagnostic tests. The first level confirms that the genetic material examined belongs to the germs *Brucella*, therefore if it is genus specific; the second level allows the determination of affiliation to a species or possibly *Brucella* biotype, while the third level enables even more precise determination of characteristics of the strain isolated, i.e. its typing. Due to this, its affinity may be easily determined to the strains isolated to date, as well as the origin and source of infection [15].

## CLINICAL FORMS AND SYMPTOMS

The form of the clinical course of brucellosis in humans is conditioned by 2 groups of causative agents, which may generally be specified as co-dependence on two basic elements:

1. the above-mentioned pathogenicity of the germ, massiveness and route of infection (e.g. very severe laboratory-acquired inhalation infections);

2. effectiveness of immune mechanisms of macroorganism: *brucellosis is a classic example of the decisive role of the individual factor in reaction to contact with the germ* [43].

Both groups of factors are very large and still insufficiently recognized. Thus, the final image of the disease is shaped by the interaction between these 2 phenomena over time [10, 25,43]. Based on the course of the disease, the following forms of brucellosis are distinguished:

1. acute brucellosis, which is characterized by: weakness, undulant fever, headaches, pain involving muscles and joints (60% of cases – pain in the lumbar region of the spine), hot flushes, testicular pain in men, fine red rash (up to 5% of cases), enlarged liver and spleen (approximately 50–60% of cases), symptoms on the part of the gastrointestinal tract: stomach ache, diarrhea, nausea, vomiting, constipation, lack of appetite; the acute phase may end in death, curing, transition into a sub-acute or chronic form;

2. sub-acute brucellosis, in which there occur all or the majority of the symptoms typical of the acute course; however, more weakly expressed;

3. chronic brucellosis:

a) primary

b) secondary

– chronic brucellosis may be both:

> seropositive, and

> seronegative (detected by Burnet's reaction, PCR or even the isolation of *Brucella* rods from human autopsy material), in which there occur: damage to the osteoarticular system of a degenerative character, enlargement of damage to the liver, non-specific neurological symptoms;

4. sub-clinical and asymptomatic brucellosis;

5. some researchers have also introduced the terms 'metabrucellosis' and brucellosis allergy'.

The image of brucellosis as a human disease is much varied and non-specific, one of the richest in pathology, with literally all systems and organs affected [5], usually creating great diagnostic difficulties. Therefore, the suspicion of brucellosis must be supported by laboratory diagnostics [2, 27]. The cultivation of *Brucella spp.* from a patient is difficult and dangerous, and is usually successful only in acute brucellosis (exceptionally in chronic form of the disease). Many serologic tests show specific dynamics in the course of the disease,

which is sometimes difficult to interpret. Similar difficulties may arise from – on the other hand very valuable – delayed hypersensitivity allergic tests, such as Burnet's dermal reaction (which are rarely used today), while the application of the PCR method on a wider scale still remains a future issue and may turn out to be useful only in some cases [15, 28, 44]. Difficulties with diagnosing brucellosis in humans, also in Poland, are enhanced by: ongoing pathomorphosis of the clinical course of the disease, and decreasing experience of both medical and veterinary physicians with respect to its clinical image. Especially the transition of the disease into the chronic form, with a many-year course, changeable concerning the appearance of the symptoms during its course [5, 9], consisting of somehow alternating periods of remissions and aggravations which cannot be foreseen, neither with respect to the time of their occurrence nor the type of clinical manifestation on the part of many affected organs and systems, has created a tremendous public health problem in many countries worldwide, including Poland. The words of Professor *Zdzisław Dziubek*, an outstanding expert in brucellosis, successor and continuator of the work by *Bertold Kassur*, are still relevant today: *'Among patients with brucellosis there is still a conviction, which by the way is in a way right, that brucellosis is incurable. Certainly the treatment of the chronic forms is not successful, in some cases it is not possible to repair the damage caused by the long-term process; however, to a great degree it is possible to prevent further harm, and sometimes to achieve a clear improvement of the damage which has already been done. Nevertheless, the precondition to achieve such effects is systematic treatment received in a specialist centre (...). Despite Poland being announced as a country free from brucellosis, this disease in humans for many years will still remain a serious problem among groups occupationally exposed to infection with this disease'* [45].

For several dozen years in Poland, brucellosis was one of the main veterinary problems as a cattle disease and one of the most frequent and most dangerous zoonoses [25, 46]. Undoubtedly, it occurred for centuries, and in the beginning of the 20<sup>th</sup> century was found to be a veterinary-epizootic problem. The number of the cases diagnosed in humans increased as early as during the period between the 1<sup>st</sup> and 2<sup>nd</sup> World Wars [47]. The mass occurrence of brucellosis in cattle – and secondarily in humans – has become a challenge for both medicines – veterinary and human, directly after the liberation of Poland in the years 1944–1945. The Lublin-Puławy Centre has become the main centre for prophylaxis, diagnostics, treatment and control of brucellosis in humans and animals. With respect to humans, the leading role was taken over, due to the visionary by *Parnas* and the *Tuszkiewicz*, by the Witold Chodźko Institute of Rural Health (IMW) in Lublin (present name), which continues studies of brucellosis until today. Brucellosis in humans has been continually registered in Poland since 1945 [48]. Since 1956, it has been considered as an occupational disease (until 2008 as a selected nosologic unit [49], and since 2009 under the common name 'infectious and parasitic diseases' [50], which have to be reported and registered.

## SUMMARY

The image of brucellosis as a human disease is very varied and non-specific, one of the richest in pathology, with literally all the organs and systems affected, creating great diagnostic difficulties. The suspicion of brucellosis, apart from epidemiological history taking and clinical symptoms, must therefore be supported by laboratory diagnostics.

The current epidemiological situation of brucellosis, beneficial for Poland as a country free from native brucellosis in humans, does not, however, allow us to forget about the still occurring (although on a trace level) cases of brucellosis in cattle, the circulation of the germ in the environment of wild and even domestic animals, cases of imported brucellosis in humans, as well as about still existing potential for patients with chronic brucellosis acquired years ago, but still newly diagnosed.

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