TULAREMIA – SERIOUS ZOONOTIC DISEASE

Marcin Weiner[1], Maria Kubajka[2]

[1]Pope John Paul II State School of Higher Education in Biała Podlaska
[2]National Veterinary Research Institute in Puławy

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Summary: Tularemia is an acute, infectious zoonotic disease caused by a smal. aerobic, intracellular, gram-negative bacillus *Francisella tularensis*. Tularemia was firstly described towards the end of nineteenth century in Japan, however, the name *Francisella* comes from Edward Francis, an American researcher who in 1911 detected this bacterium in squirrels in Tulare County, California. In Poland tularemia in humans was recognized for the first time in 1949. In the years 1949 to 2009, over 600 tularemia cases were recorded in Poland, with one fatality in 1983. Initial work on the use of *F. tularensis* as a biological weapon was carried out in the 30s of the twentieth century simultaneously in the United States, Soviet Union and Japan. The natural reservoirs of the micro-organism are rodents and lagomorphs, which can be a source of infection for other animals and humans. Human infection occurs through direct contact with sick animal. inhalation of dust contaminated with feces of sick animals and it takes place mainly in the farms involved in the animal production, to a lesser extent as a result of contaminated food and water.

Keywords: *Francisella tularensis*, tularemia, bioterrorism, zoonosis

Introduction

Tularemia is a zoonotic infectious disease, also called the "plague of rodents", "wild hare disease" or "rabbit fever" (Kłapeć, Cholewa 2011). An etiological agent of this zoonosis is bacterium *Francisella tularensis*, which was isolated for the first time during an epidemic of tularemia in squirrels in Tulare County, California in 1912 (Hansen et al. 2011, Oyston et al. 2008). The name of the bacterium comes from the name of the researcher, Dr. Edward Francis, dealing with these pathogens (McCoy and Chapin 1912). Although the micro-organism is pathogenic to 190 species of "animals", clinical symptoms occur mainly in lagomorphs and rodents (Glinski and Kostro 2005, Reed et al. 2014). The natural reservoirs are murine, muskrats, water rats, ground squirrels, voles and rabbits (Rastawicki and Jagielski 2005, Osiak et al. 2006). The given zoonosis is described as a disease with an acute course, but in many cases it can be mild or asymptomatic (Rastawicki et al. 2005). Most often the source of an infection is an arthropod, as well as direct contact with sick animal or biological material derived from infected animals (meat, water, contaminated dust) (Mierzyńska et al. 2002, Moniuszko et al. 2010). Tularemia in humans may take different forms depending on the route of entry into the body. This may be a direct contact through the skin and / or mucous membranes, aerogenic route as well as by mouth. Tularemia in humans may take various forms, depending on the route of entry, virulence, and the infectious dose. Depending on the route of entry, virulence and infectious dose different forms of this disease can be distinguished.

Etiology

Etiological agent of tularemia is small (0.2-0.7 μm), gram-negative, aerobic, non-motile and not producing endospores, granulomatous bacterium *Francisella tularensis* (Ellis et al. 2002). Tularemia grows under aerobic conditions on alkaline substrate (pH 7.2) at 37±2°C. The growth occurs after 2 - 5 days in a form of smal. mucilaginous and transparent colonies.

Taxonomic position of *F. tularensis* underwent frequent changes (Rastawicki and Jagielski 2005). According to the latest Bergey's taxonomy, the pathogen belongs to *Francisellaceae* family, genus *Francisella*, which includes two species of *Francisella*: *tularensis* and *philomiragia* (Brenner 2005). *F. tularensis* consists of four subspecies:

Address for correspondence: Marcin Weiner, Pope John Paul II State School of Higher Education in Biała Podlaska, Sidorska 95/97 Street, 21-500 Biała Podlaska, e-mail: m.weiner@dydaktyka.pswbp.pl, phone: (83) 344 99 18

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1. *Francisella tularensis* subspecies *tularensis* (formerly type A or subspecies Nearctic),
2. *Francisella tularensis* subsp. *holarctica* (formerly type B or subspecies holarctica),
   2.1.biovar I erythromycin sensitive,
   2.2.biovar II erythromycin resistant,
   2.3.biovar japonica,
3. *Francisella tularensis* subsp. *mediasiatica*,
4. *Francisella tularensis* subsp. *novicida*.

Those subspecies have been classified primarily on the basis of the genetic code, virulence, ability to produce acid from glycerol and citrulline ureidase activity.

F. tularensis subsp. *tularensis* is found mainly in North America and is highly virulent for humans and rabbits. This subspecies is responsible for approximately 70% of cases of *Francisella* sickness. The infectious dose is <10 CFU (*colony forming unit*) and can lead to life-threatening diseases, in particular when the respiratory tract is being infected. *F. tularensis* subsp. *holarctica* is more common and differs from *F. tularensis* in terms of biochemical properties and reduced virulence in humans and animals.

F. holarctica is primarily isolated from aquatic animals such as beavers and muskrats in the northern areas of North America, and is a major cause of tularemia in hares and small rodents in northern areas of Europe and Asia (Osiak et al. 2006, Pechous et al. 2009). *F. mediasiatica* has a similar virulence to the subspecies *F. holarctica* but its geographical distribution is limited to Central Asia and the former Republic of Soviet Union (Pechous et al. 2009).

Tularemia bacillus is sensitive to high heat, sunlight and UV radiation as well as to majority of the commercial disinfectants (Rastawicki and Jagielski 2005). In the environment with favorable conditions it can survive for up to several months (Tab. 1).

This microorganism is listed in the 3rd place after the Anthrax bacillus (*Bacillus anthracis*) and botulinum toxin (a neurotoxin produced by the bacterium *Clostridium botulinum*) as a microbiological factor applicable to bioterrorism (Glinski and Kostro 2005, Reed et al 2014).

Table 1. *Francisella tularensis* survival in different environments

Potential source of infection	**Survival**
Dried skins	~ 45 days
Dry hay and straw, pasturage	~ 120 days
Hare carcasses	~133 days
Hemolymph of ticks and insects	~240 days
Soil, silt	Few weeks
Water bodies, corpses of dead animals	~3 months
Frozen meat (e.g. rabbit)	Several years

Source and route of infection

The main reservoirs of the germ are arthropods carrying bacteria and infected organism itself. The natural reservoirs are rabbits, hares, squirrels, nutria, mice, rats, however there are cases to eradicate the infection in cattle, horses, sheep, goats, or even pigs and dogs. Humans can be infected by mosquitoes, ticks, flies, or through the direct contact with contaminated environment or the inhalation of contaminated dust. There are cases of falling ill after the contact with material coming from sick animal. or after eating meat from rabbit or hare. Natural circulation of infection exists because of the circulation of the germ in the environment: rodent (donor) - arthropod (carrier) - rodent (recipient). During this stage new epidemics arise (Kłapeć, Cholewa 2011, Oyston 2008).

Occurrence

The disease is widespread. The natural outbreaks of tularemia occurred in the USA, Mexico, Canada, Yugoslavia, Spain, Czech Republic, Slovakia, Scandinavia, Turkey, the former Soviet Union, Japan and many Asian countries. In the United States in the years 1981-1987 the cases of tularemia were reported in all states. In Europe, the first laboratory confirmed cases were registered in the USSR in 1926. In later years, great epidemic waves were observed, during which tularemia moved to the west and south of the continent. The first wave of epidemic reached Scandinavia (illnesses were reported in Norway in 1929 and in Sweden in 1931) and Central Europe - Moravia

(Austria and the Czech Republic in the years 1935-1937). The second wave occurred during World War II in the northern part of the Soviet Union. In the old endemic hotbed new epidemics broke out. This wave was characterized by a high expansiveness, it passed to remote areas previously free of tularemia. In this way, in the mid-forties, it reached the mouth of the river Neman and along the Baltic coast, to the west Europe (epidemics in East and West Germany, Belgium, France). Hereafter, in some parts of Europe, the germ created reservoirs among the native fauna (persistent infection of rodents and arthropods) - creating the so-called natural hotbed. In these areas the isolated cases of human illnesses were observed and cyclical epidemics were breaking out. Maximum increase of cases of tularemia in the world was recorded in the years 1930-1950. New cases of illnesses and epidemics, the source of which are not only hares, appeared in Turkey (Akalin et al. 2009, Gurcan 2007), Spain in 1997 (Anda et al. 2001), Georgia in 2006 (Chitadze et al. 2009) and Germany in 2005 (Hauri et al. 2010). More and more often the source of disease is water (spring water, water from recreation areas) and the food, in Italy in 1982 (Greco et al. 1998) and Bulgaria (Christova et al. 2004). In 2000, Sweden (Elliason et al. 2002) reported an outbreak of tularemia in areas previously not considered endemic. 270 people fell ill. Epidemiological analysis showed that the disease was caused by a mosquito bite, passively transferring germs. Data from the literature shows that in Sweden until 2000, 6,000 people died from tularemia (Elliason et al. 2002). In Poland, the endemic hotbeds of tularemia are present mainly in the north of the country (near Bialystok, Gdansk, Bydgoszcz, Szczecin) and near Poznan. In Poland tularemia in humans was diagnosed for the first time in 1949 in Lodz, where the source of infection was probably the skin of a hare. The first major epidemic outbreak was detected in the Olsztyn province in 1950 and the likely cause of the infection was a hare. The cause of the next cases were laboratory animals: in 1950, 4 employees of PZH (National Institute of Hygiene) were infected and fell ill, and following outbreak occurred in the Szczecin province in the years 1952-1953, where 70 cases of tularemia were recorded. Rabbit was considered to be the source of infection in almost all outbreaks (Malottke and Dominowska 1973, Skrodzki et al. 1954). Another outbreak of tularemia was registered in Warsaw, in a company being in the venison trade, where 18 employees fell sick. For that reason an approximate test was being carried out on the employees working in this type of enterprises in 10 provinces. A total of 526 persons were examined. Positive serological and allergic results were obtained in 10 cases, and 5 people were having symptomatic disease (Kicińska et al. 1954). Pacewicz and other contributors, in 1999 carried out tests on 716 forestry workers from the regions the north-eastern Poland for antibodies against *F. tularensis*. Only one employee had positive results. The tested person showed no signs of disease neither in the time of the test nor in the past (Pacewicz et al. 2004). In 2002 the same authors carried out another test on the 55 patients with enlarged lymph nodes, who have been admitted to clinic in order to establish a diagnosis - the results were negative. They have tested as well a group of 765 forestry workers from the same regions of Poland. 20 patients had positive results, but none of these persons showed symptoms suggesting of having tularemia in the past. The tests carried on 480 forestry workers in selected regions of Poland (province.: Podlasie, Warmia-Masuria, Kielce, Opole) in 42 cases (8.8%) showed the presence of antibodies for the antigens of *F. tularensis* (Rastawicki and Jagielski 2005). In the years 1949-2009, 614 people fell sick on tularemia; one fatal case was recorded in 1983.

Pathogenesis

The infectious dose of *F. tularensis* for humans depends on the route of infection, and it ranges from 10 to 50 CFU when the pathogen was injected intradermally or by inhalation and 10 CFU after intake (Sjostedt 2007). *F. tularensis* have the ability to intensively multiply within macrophages. The virulent ability of the germ interferes with the resistance of the host organism, resulting in bacterial cell division in the cytoplasm (Carvalho et al. 2014). It can trigger off a cell death by apoptosis, releasing the bacteria to infect new cells (Glinski and Kostro 2003). Bacillus *F. tularensis* invade the lymphatic vessels using macrophages and can cause inflammation of the lymph nodes. When the body's resistance is broken bacteremia and then sepsis are being developed. After further multiplication they spread throughout the body by circulatory system occupying much of the internal organs and reaching to the lungs, spleen, liver and kidneys. At a later stage, an inflammatory condition develops resulting in local necrosis with infiltration. In the final stage, the secretory changes develop, which takes form of caseating granulomas characteristic of tuberculosis and sarcoidosis (Hansen et al. 2011, Moniuszko et al. 2010, Osiak et al. 2006). The organism fights the microorganism by creating multiple defense mechanisms. In the early stages of the infection PMNs leukocytes (polymorphonuclear leukocytes) destroys bacteria. TNF factor (tumor necrosis factor) and interferon gamma (IFN-γ), stops the infection process. Lipid rich capsule protects pathogen against complement lysis and as a result reticuloendothelial system is being populated by the microorganisms. Within 2 days of infection, the body's cellular immunity is dependent on neutrophilia: interleukin 10, interleukin 12, IFN - γ and TNF-α, but when the infection progresses, T-cells have an important role in combating infection (Kłapeć i Cholewa 2011 Moniuszko et al. 2010). ACP protein, which can be found in F. tularensis, demonstrates

acid phosphatase activity. Low pH is essential to the bacteria in phagosome macrophages to absorb iron and to multiply. Acidic environment result in the release of iron from transferrin host and may cause additional virulence factors. In the moment when the element is no longer available *F. tularensis* dies. The effects between macrophages and bacteria depend on the types of macrophages. Activation of peritoneal macrophages leads to the production of nitric oxide - NO, and alveolar macrophages (pulmonary) are activated by interferon - γ. The initial stage of bacterial cell multiplication within macrophages runs at a slow pace, but after 12 hours, it accelerates. In fact, it causes them to die off, and the released *F. tularensis* may take up subsequent cells of the host (Osiak et al. 2006). LPS (Lipopolysaccharide) of *F. tularensis* plays a significant role in the development of bacteria in macrophages. Protein 23-kDa fulfills important functions in intracellular multiplication and hinders post inflammatory cytokine actions.

Epidemiology

Tularaemia occurs in a number of clinical forms. Its symptoms can be a bit different depending on what way the bacteria entered the organism. The illness can be undergone in various forms – from acute to mild or even asymptomatic. The beginning of the illness is sudden – 38-40 °C fever, headache, throat ache and pain of muscles. Sometimes diarrhoea, nausea and vomiting occur. As the illness progresses, the organism gets weakened and loss of body weight occurs. Incubation period takes around 3 to 5 days, but there occur extreme cases, in which symptoms appear very fast (during one day) or relatively late (even after twenty days from the infection).

In Europe 90% of cases of tularaemia are of the ulcerating-nodal form, which occurs usually after contact with a sick animal or after getting bitten by arthropods. After 3-5 days from exposition, in the place of bacterial penetration there develops a primary lesion in the form of erythematous pellet, which then becomes ulcer and heals fast. After a short incubation period (3-6 days), there appear flu-like symptoms in the infected person and local lymph nodes enlarge – which most often is the only reason why patients come to the doctor. Through the lymphatic system it comes to spreading of the bacteria to the internal organs, including lungs, liver, spleen and kidneys (Kłapeć, Cholewa 2011).

In around 5% of cases tularaemia is diagnosed in a tonsillitis form, which occurs after consumption of infected water or food. It courses with exudative inflammation of oral cavity, throat and/or tonsils and jugular lymphadenopathy. This can suggest streptococcal tonsillitis and in these cases the treatment is performed using potassium penicillin G, for which the *F. tularensis* bacteria are immune. This form can be hard to distinguish from ulcerating-nodal form if the patient was bitten by arthropod in the head or neck. Then the lymphatic nodes also get enlarged and the primary lesion can be unapparent (Rastawicki, Jagielski 2005).

Among other forms of tularaemia, the forms which occur much less frequently are listed below:

- pulmonary – caused by the penetration of microorganisms through respiratory tract or occurs as a complication of other forms of tularaemia. The symptoms are nonspecific: pains in the chest, dry couch, high fever. The most characteristic symptom is the enlargement of axillary lymphatic nodes. In the X-ray of the chest vesicular concentrations spanning over the lobe or segment of the lungs, granulomatous changes or abscesses can be visible. There can also occur the enlargement of the cavities and the exudation in pleura.
- ocular-nodal – occurring rarely. Courses with ulcerative conjunctivitis. The infection takes place in effect of rubbing the eyes with infected fingers.
- gastric-intestinal – occurs after consumption of infected water or food. Courses with mild diarrhoea or in severe form – with ulceration of intestines.
- typhoid – a historical name, very controversial nowadays. Concerns the cases of tularaemia coursing without primary lesions – primary lesions of skin, nodes, eyes, oral cavity and lungs. The sudden beginning, with high fever and muscle aches, is characteristic. In some cases there occur diarrhoea, dry couch and pain in the chest. The most common complications are rhabdomyolisis, inflammation of the liver, kidneys and joints. This form courses with high mortality rate reaching 50% of cases. The way of infection is unknown.

Diagnosis

Identification of *F. tularensis* is hard and should take place in specialist laboratories of BSL3 class. The variety of laboratory methods of tularaemia analysis allows for a fast and precise diagnosis of the illness from the moment of infection to the recovery period, and even after death (Kłapeć, Cholewa 2011). In humans each material intended for identification should be delivered before the start of antibiotic therapy. Samples can include blood, serum, specimen of lymphatic nodes, saliva, material from digestive tract or respiratory tract, scrapings from places changed by illness, urine. If there is a suspicion of the occurrence of *F. tularensis* in animal. sample of serum, drawn

at least 14 days after the occurrence of symptoms, should be given to laboratory for diagnosis. In the case of dead specimen, [following parts of the body] the following can be analysed: lymphatic nodes, bone marrow, organs (lung, liver, spleen). In the case of epidemic outbreak environmental samples, like water, soil, rodent faeces as well as blood-sucking insects, are sent for analysis (Cavalho et al. 2014).

The methods of laboratory diagnosis of *F. tularensis* infection: microscopic, serological, bacteriological as well as molecular methods, are described in Table 2.

Table 2. Methods of laboratory diagnosis of *F. tularensis* infection

Type of analysis	**Diagnostic method/ Nutrient**	**Description**
Microscopic formulations	Gram Method or Giems Method colouring	In coloured Gram formulations, *F.tularensis* is coloured negatively in red-pink (taking the colour of the dye). Giems Method: formulation is coloured in pink – blue, taking form of a ball or a rod of various length
Histological formulations	Direct and indirect immunofluorencence reaction, Immunochromatography reaction, Immunoenzymatic reaction, Precipitation in capillaries reaction	Extremely sensitive and fast methods, which allow detecting the microbe already on the level below 10^3CFU/ml.
Serological analyses	Test tube agglutination reaction, Microagglutination reaction, ELISA reaction, Latex reaction	Serological methods allow detecting inherent antibodies for *F. tularensis* antigens after approximately 14 days from the occurrence of illness symptoms.
Cell cultures	Agar with the addition of blood, cysteine and glucose, Chocolate agar (cysteine agar enriched with 9% addition of ram erythrocytes) Cysteine Heart Agar with the addition of haemoglobin (CHA), Thayer – Martin Agar with supplement Mueller – Hinton bouillon with the addition of 0,025% iron pyrophosphate	*F. tularensis* colonies are round, smooth, a bit mucous, have 2 – 4mm diameter and greenish-white colour. On flat bases, the growth is observed after 18h, in the temperature of 37°C (some strains grow from 3 to 6 or even 10 days). Sometimes the temperature of 28°C is used, which is necessary in detailed identification of *F. tularensis* from *Yersinia pestis, F.tularensis* subsp. *novicida , F.philomiragia.*
Bioassay	Biological analyses are infection of mice, guinea pigs or rabbits with the material containing living, malignant *F. tularensis* germs. Infection is done by various methods: subcutaneously, intramuscularly, intravenously, through respiratory tract, through digestive tract, through instillation of the infected material to the conjunctival sac or nose.	Deaths occur from 2 to 10 days. [There are] haemorrhagic enlargements of lymphatic nodes, liver, spleen, multiple necrotic foci.
Molecular biology methods	Polymerase Chain Reaction (PCR) LR-REP-PCR and ERIC- PCR.	There is a search for fragments of genes coding protein of outer membrane. It allows distinguishing *F. tularensis* on the subspecies level.

Tularaemia as a biological weapon

F. tularensis is a number 3 pathogen, after bacillus of anthrax and botulinum toxin, used as a potential biological weapon in bioterrorism on account of its biological properties (Mierzyńska, Hermanowska-Szpakowicz 2002). American Centers for Disease Control and Prevention (CDC) classified *F. tularensis* as a bioterrorist mean, because it is highly infectious, pathogenic, spreads easily, influences public health and probability of mortality rate(Chomiczewski 2003).

The multitude of ways of bacterial transmission makes counteraction, and also finding the source of infection, harder. In case of bioterrorist attack, *F. tularensis* can be spread by wind over long distances. Germs can also infect carriers (e.g. lice, fleas, mice, rats), from which the pathogenic microbes can move to people directly or to water and food (Chomiczewski 2003). First researches using *F. tularensis* were conducted already before World War II in

such countries as USSR, USA and Japan. It is supposed that these bacteria were used by Russians in the years 1942 – 1943 during the fights over Stalingrad. The proof for it were massive pneumonias among Russian and German soldiers and civilian population. In the years 1932 – 1945 Japan conducted intensive researches of biological weapons development in Manchuria. After the World War II USA and USSR still conducted researches at offensive use of this pathogen. Americans conducted the works until the end of the sixties, developing a technique of using tularaemia rods in the form of aerosol. During the Soviet programme, conducted in the nineties, a strain immune to vaccines and antibiotics was developed. In 1970 WHO experts made a simulation which suggested that spraying 50 kg of the aerosol suspension of tularaemia rods from the plane two kilometres over the area with 500,000 residents would cause 30,000 fatalities and 125,000 serious illnesses (Chomiczewski 2003).

Treatment

Quick diagnosis of tularaemia and adequate treatment on the areas where this illness occurs relatively rarely is hard to achieve, because the symptoms of tularaemia are little specific and usually give rise to suspicion of other infectious diseases. The diagnosis of the illness relies mostly on the data from [medical] examination (Rastawicki i Jagielski 2005).

In most cases correct antibiotic therapy leads to full healing. In the treatment of this illness streptomycin is given to the diseased. The medicine is given in the quantity of 1g intramuscularly, two times a day over a period of 10 days. Gentamycin is an alternative and can be given intravenously in the quantity of 5 mg/kg, once a day over a period of 10 days. In case of an epidemic, relapses or immunity for the early treatment [these medicines] can be given:

- Doxycycline: 100 mg orally, two times a day, over a period of two weeks,
- Ciprofloxacin: 500 mg orally, two times a day, over a period of two weeks,

For children the same antibiotics are used (also in case of epidemic) in reduced doses:

- Streptomycin 15 mg/kg intramuscularly, two times a day,
- Gentamycin: 2,5 mg/kg intramuscularly or intravenously, three times a day,
- Doxycycline: for children weighing over 45 kg, 100 mg orally, two times a day, over a period of two weeks,

For children weighing below 45 kg, 2,2 mg/kg orally, two times a day, over a period of two weeks,

- Ciprofloxacin: 15 mg/kg orally, two times a day, over a period of two weeks (Osiak et al. 2006; Dennis et al. 2001).

Inherent prophylaxis

First attempts to create a vaccine against tularaemia did not give demanded results, because of very low immunity in people and animals for it. Next researches on acute, attenuated vaccine were conducted already before the World War II in onetime Soviet Union. In the beginning of 1940 new strain of F. tularensis, marked as 15, was isolated. [Strain] marked as 155 was another strain, obtained in the Gamaleja Institute in Moscow. Thanks to these strains the acute vaccine that was mass produced came into being. This vaccine was in turn given to the US Army Medical Research Institute of Infectious Diseases where, during another tests, acute vaccine strain LVS (Live Vaccine Strain) was obtained. Vaccine from LVS can be given the erogenous, oral, as well as subcutaneous way, which proved to be best. It does not give full protection against the inhalatory form of tularaemia, it just has a slight influence of making the course of the illness milder. Lately a mutated, attenuated strain Schu S4 has been discovered, which gives a much better protection against inhalatory infection (Kłapeć, Cholewa 2011, Osiak et al. 2006, Pechous 2009). Researches on creating a vaccine containing antigens capable of inducing humoral and cellular immunity are conducted. The only antigen inducing immunological reaction against *F. tularensis* is LPS (Lipopolysaccharide*)*. At this moment there is no licensed vaccine against tularaemia on the market. The only available vaccine is attenuated LVS, which is available only to persons of high-risk group and the availability of which is limited.

Administrative proceedings

Tularaemia is in the specification of infectious diseases and human infections covered by The Act on the Infectious Diseases and Infections of 31 January 2013 (*Ustawa o chorobach zakaźnych i zakażeniach z 31 stycznia 2013 r.*), and in view of the danger for human health the animals with tularaemia are recommended to be eliminated on the basis of The Act of 11 March 2004 on the Protection of Animal Health and Combating Infectious Diseases of Animals (*Ustawa z 11 marca 2004r. o ochronie zdrowia zwierząt oraz zwalczaniu chorób zakaźnych zwierząt*). Combating tularaemia relies on neutralizing the source of infection and cutting out the paths of spreading the disease. Preventing the illness relies on avoiding situations connected with the risk of contact with infected

material and on thermal or chemical preservation of carcasses of dead animal. eliminating of field rodents and complying with hygiene norms. The best method is not residing on endemic territories of occurrence of tularaemia, and when it is impossible, using appropriate prevention means. While residing on forest territories and meadows (work, tourism) the precautions which protect against biting by a tick should be kept. Extreme caution should be exercised while skinning of hares or rabbits (Magdzik 1986).

Despite the fact that in Poland tularaemia is so far not a serious problem, the occurrence of this illness in neighbouring countries and the rising number of infections both in neighbouring countries and in Poland cause that it [(tularaemia)] is to be taken into account during the microbiological diagnosis of infections. In conjunction with the geopolitical situation it should also be considered as a potential biological weapon.

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