

## THE IMPORTANCE OF TRANSMISSION TICK-BORNE ENCEPHALITIS THROUGH MILK OF INFECTED ANIMALS

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### Abstract

Tick-borne encephalitis (TBE) is a viral zoonotic disease of humans, goats, ewes, cows. Infection is found in nature in many wild and domestic animals, including dogs. The most important reservoirs of viruses in nature are small rodents. Approximately 5,000 - 12,000 cases of TBE are diagnosed in Europe each year. People are usually infected over ticks (*Ixodes ricinus*, *Ixodes persulcatus*) and infections are usually manifested by symptoms like: flu, fever, headache, encephalitis, ataxia, convulsions, tremor, paresis, paralysis and death. Paralysis occurs in 6% of cases and mortality is 1 - 2% of the Western subtype tick-born encephalitis virus (TBEV).

In recent times, the number of epidemics of this disease caused by the consumption of raw milk of infected animals increases. TBE spreads through raw milk almost every year in endemic countries. These epidemics point to the importance of the food pathway in the spread of TBEV. Raw milk often consumed people on farms because of better taste and simple preparation, or in order to prevent and treatment certain diseases. TBEV belongs to the genus *Flavivirus*, the family of *Flaviviridae*. It is enveloped RNA virus, so it is relatively sensitive on temperature and detergents. However, virus remains infectious in gastric acid (pH 1.49 - 1.80) up to two hours. During retention in the digestive tract, they do not cause the symptoms of the disease, but after reaching to other organs (encephalitis). The mechanism of infection development with TBEV is the same as food-borne infections induced by enteroviruses. Ruminants are infected during grazing by the bite of ticks; goats are particularly sensitive because of way of nutrition (grass and bushes). Goats, ewes and cows infected with TBEV virus do not show clinical signs of disease, but excrete viruses through milk. The amount of virus that is secreted by milk greatly varies between individual animals. The infectivity of the virus remains preserved in various milk products such as

yogurt, cheese, butter. In samples with small number of viruses, the virus loses infectivity after treatment at 65°C for five minutes, whereas in milk with higher number of viruses, the virus remains infectious after 30 minutes on this temperature. Treatment at 100 °C three minutes destroys the infectious virus.

This indicates that in order to reduce the risk of infection by TBEV before consumption milk should be pasteurized - boiled. If consumers insist on raw milk consumption, goat vaccination should be carried out in endemic areas. In immunized goats, not established presence of TBEV in milk, which means that immunization prevents viral infection and viremia. It is not yet fully known how long the immune system lasts in goats and whether the natural immunity lasts lifetime. There is a need to work on tick-borne encephalitis diagnosis in animals and humans in countries where no tests have been conducted and in which a ticks that convey the disease are present. One of the countries for which there is no information on the spread of tick-borne encephalitis is also Montenegro.

**Key words:** Tick-borne encephalitis, Zoonosis, Virus, Milk, Goats, Montenegro.

### 1. Introduction

Tick-borne encephalitis (TBE) is a viral zoonotic disease of: humans, goats, ewes, and cows. Infection is found in nature in many wild and domestic animals, including dogs. About one hundred animals (mammals, birds, reptiles) can be infected with tick-borne encephalitis virus (TBEV). TBE occurs in many countries of Europe, the former Soviet Union and Asia [1]. Approximately 5,000-12,000 cases of TBE are diagnosed in Europe each year. Tick-borne encephalitis is endemic

in 27 European countries [2]. Countries of very high risk (incidence over 10/100,000) are: Russia, Estonia, Latvia, Lithuania, Czech Republic and Slovenia. Slovenia is one of the countries with the highest incidence [3]. Germany, Poland, Switzerland, Finland, Slovakia and Hungary also have a high incidence of tick-borne encephalitis [4, 5]. In the period from 1990 to 2009, nearly 170,000 clinically ill people were registered in Europe and Russia [6]. Austria is the only country with a progressive reduction of the spread since 1981, because of vaccination [7]. People are usually infected over ticks (*Ixodes ricinus*, *Ixodes persulcatus*). In recent times, the number of epidemics of this disease caused by the consumption of raw milk of infected animals increases. The tick-borne encephalitis virus is a major cause of human disease which is being studied also from the aspect of food safety [8].

## 2. Epidemiology of tick-borne encephalitis

People are usually infected over tick bite (*Ixodes ricinus*, *Ixodes persulcatus*) and infections are usually manifested by symptoms like: flu, fever, headache, encephalitis, ataxia, convulsions, tremor, paresis, paralysis and death. Paralysis occurs in 6% of cases and mortality is 1 - 2% of the Western subtype tick-borne encephalitis virus [9]. The incubation period lasts 4 - 28 days, most often 7 - 10 days. In cases of infection through unpasteurized milk and milk products, the incubation lasts shorter, 3 - 4 days [10].

The most important reservoirs of viruses in nature are small rodents [10, 11]). Of these, the TBE virus is transmitted to humans and other animals by excretion in the viremia stage or through ticks [7]. The tick-borne encephalitis virus is maintained in nature thanks to permanently infected vectors and reservoirs between which, during feeding of the ticks, virus can be transmitted in both directions [12]. Animals such as: foxes, rabbits, deer, wild boars, sheep, cattle, and dogs do not cause ticks infection, because the viral titer after the infection of these animals is small [7]. However, according to some authors wild animals (roe deer, red deer, wild boar, rabbit) are important reservoirs of tick-borne encephalitis virus [13, 14].

TBEV belongs to the genus *Flavivirus*, the family of *Flaviviridae*. It is enveloped single-chain RNA virus, so it is relatively sensitive on temperature and detergents. However, virus remains infectious in gastric acid (pH 1.49 - 1.8) up to two hours. Three subtypes of tick-borne encephalitis virus were identified: European (Western), Far-Eastern and Siberian subtype tick-borne encephalitis virus [10, 15]. The most severe form of disease causes Far-Eastern subtype which cause severe febrile illness associated with encephalitis and a fatality rate up to 35% [7].

Virus multiplication takes place in the cytoplasm of infected cells. After binding to the cellular receptor, virus enters in cells by endocytosis. It has been shown that at least 16 types of ticks can transmit a tick-borne virus, but two types of viral species are most important: *Ixodes ricinus* - European subtype vector and *Ixodes persulcatus* - a vector of Far-Eastern and Siberian virus subtypes. In the population of ticks virus is transmitted transovarially and transstadially and they are also the reservoirs of infections. In the period 1993 - 2013 a total of 777 patients with tick-borne encephalitis were registered in Croatia [8]. There is no data on the spread of tick-borne encephalitis in Serbia, because no routine diagnostics is performed. However, the authors emphasize the importance of testing the spread of this zoonosis, because the results of the preliminary serum testing in Vojvodina were positive and because of reported cases in neighboring countries such as Hungary and Croatia [10]. The spread of this disease also was not observed in Montenegro; bearing in mind the presence of vectors and potential reservoirs of the disease, also occurrence of this disease in the surrounding countries, these investigations should be done in the future.

### 2.1 Transmission of tick-borne encephalitis to humans through milk

In recent times, the number of epidemics of this disease caused by the consumption of raw milk of infected animals increases. Raw milk often consumed people on farms because of better taste and simple preparation, or in order to prevent and treatment certain diseases [16, 17]. About 1% of all TBEV infections are caused by the consumption of unpasteurized milk or dairy products from infected animals, in particular goats. Epidemics caused by food are most common in Eastern Europe and the Baltic countries than in Central Europe [18]. TBE spreads through raw milk almost every year in endemic countries. Epidemics resulting after consumption of milk or dairy products of infected animals are recorded in a large number of countries, including Slovenia and Hungary [3, 19]. The first epidemic caused by the consumption of raw milk was recorded in 1951 in Roznova, Slovakia, when more than 600 people were infected and 271 people were hospitalized. First epidemic caused by consumption of fresh sheep cheese recorded in Western Slovakia 1974 [20]. In Hungary, in 2007, 30 people were ill after consumption of raw milk of diseased goats [21]. Epidemic caused by raw milk and cheese in Croatia was first recorded 2015 [22]. This way of transfer was also established in: Albania, Hungary, Latvia, Lithuania, Poland, Russia and Slovakia, but not in Western Europe. The transmission of TBEV through unpasteurized goat's milk was found in humans and domestic pigs in the Alps above 1,500 m above sea level. No clinical signs of disease have been identified in pigs [23]. These epidemics point to the

importance of the food pathway in the TBEV spread. An increasing number of small producers who sell unpasteurized milk and dairy products increase the risk of transmission of the virus [6].

During retention in the digestive tract, virus not cause the symptoms of the disease, only after reaching to other organs (encephalitis). The mechanism of infection development with TBEV is the same as food-borne infections induced by enteroviruses. Ruminants are infected during grazing by the bite of ticks; goats are particularly sensitive because of way of nutrition (grass and bushes). Goats, ewes and cows infected with TBEV virus do not show clinical signs of disease, but excrete viruses through milk. The amount of virus that is secreted by milk greatly varies between individual animals.

The infectivity of the TBE virus remains preserved in various milk products such as yogurt, cheese, butter. In samples with small number of viruses, the virus loses infectivity after treatment at 65 °C for five minutes, whereas in milk with higher number of viruses, the virus remains infectious after 30 minutes on this temperature [19]. Treatment at 100 °C three minutes destroys the infectious virus. The virus is infected in milk at 4 °C for two weeks and in butter for 60 days. By heating milk, the virus titer is reduced, but only pasteurization inactivates the virus [24]. The tick-borne encephalitis virus is a major cause of human disease which is being studied also from the aspect of food safety [8].

## 2.2 Possibilities of preventing transmission of tick-borne encephalitis to humans through milk

In order to reduce the risk of infection by TBEV before consumption milk should be pasteurized - boiled. If consumers insist on raw milk consumption, goat vaccination should be carried out in endemic areas. Non-sterilized milk of infected domestic animals, and in particular goat, can be a significant source of human infection by tick-borne encephalitis virus. Considering the trend of consuming raw foods, greater attention should be devoted to educating the population - potential consumers of raw goat's milk on the risks of such consumption. Intensified co-operation between human and veterinary medical experts is a key element of successful control of tick-borne encephalitis. In immunized goats TBEV presence in milk it is not established, which means that immunization prevents viral infection and viremia. It is not yet fully known how long the immune system lasts in goats and whether the natural immunity lasts for a lifetime. After a primary vaccination, re-vaccination is required every three to five years [5].

## 3. Conclusions

- The tick-borne encephalitis virus is a major cause of human disease which is being studied also from the aspect of food safety. Epidemics caused by the consumption of raw milk of infected animals point to the importance of the food pathway in the spread of TBEV.
- In order to reduce the risk of infection by TBEV before consumption milk should be pasteurized - boiled. If consumers insist on raw milk consumption, goat vaccination should be carried out in endemic areas.
- There is a need to work on tick-borne encephalitis diagnosis in animals and humans in countries where no tests have been conducted and in which ticks that convey the disease are present. One of the countries for which there is no information on the spread of tick-borne encephalitis is Montenegro.

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