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Risk assessment of the alimentary transmission of tick-borne encephalitis viruses from goats to humans by milk and milk products in Swiss alpine regions

Risikobewertung der alimentären Übertragung von Frühsommer-Meningoenzephalitis-Viren von der Ziege auf den Menschen durch Milch und Milchprodukte in den Schweizer Alpenregionen

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Summary

Viral zoonosis tick-borne encephalitis (TBE) is usually transmitted to humans by the bite of an infected tick. Another possible way to become infected with the viral pathogen is through the consumption of raw milk and raw milk products. Based on the seroprevalence of antibodies against TBE viruses in goats in the Valais canton in a recently published study, a risk assessment for the viral contamination of goat milk was performed for this area. The probability of virus-contaminated milk was calculated to range between 0.0012% and 0.024% of household milk.

Keywords: Tick-borne encephalitis, alimentary transmission, raw goat milk, raw goat milk products, risk assessment, Switzerland

Introduction

Tick-borne encephalitis (TBE) cases have been on the rise in Switzerland for several years (FOPH, 2019a). Although this viral infectious disease is usually transmitted to humans by tick bites, in recent years cases transmitted by the consumption of raw goat milk and raw goat milk products have been reported in German-speaking countries (Holzmann et al., 2009; Brockmann et al., 2018).

This article conducts a risk assessment for milk contaminated with tick-borne encephalitis virus (TBEV) in the alpine regions of the Valais canton. The calculations are based on current data from the literature and a study conducted in the Valais canton on the seroprevalence of TBEV-specific antibodies in goats (Rieille et al., 2017).

TBE virus transmission and pathogenesis

TBEV, a member of the *Flavivirus* genus, is an enveloped virus with a single-stranded positive sense RNA genome (Heinz, 1999). Three main antigenic subtypes of TBEV are recognized: the Far Eastern subtype, the Siberian subtype, and the European subtype. In addition, two new subtypes (Baikalian and Himalayan) have recently been proposed (Demina et al., 2010; Kozlova et al., 2018; Dai et al., 2018; Ruˇzek et al., 2019). So far, only one, the European subtype, has been detected in Europe since the 1950s (Brockmann et al., 2018).

TBE is the most common viral zoonosis transmitted to humans by ticks in Central and Eastern Europe and Asia (Süss, 2011). In Central Europe, the castor bean tick, *Ixodes ricinus,* is the most common tick species and the main vector of various viral and bacterial zoonotic pathogens, including TBEV. *I. ricinus* develops through three developmental stages: larvae, nymphs, and adult male or female ticks. Ticks usually get infected with TBEV during the first two developmental stages. Larvae and nymphs feed mainly on small mammals, such as certain mouse populations, hedgehogs, and moles. For these animals, TBEV is apathogenic (FLI, 2019). During the viremic phase, animal hosts can pass the virus on to engorged ticks, thereby maintaining the circulation of the pathogen in the natural environment. Within ticks, the persistence of the virus occurs through viremic, transstadial and transovarial transmission (Gritsun et al., 2003).

The symptomatic disease is typically biphasic when caused by European subtype viruses, including a viremic stage with flu-like symptoms starting about eight days (4–28 days) after the tick bite, an asymptomatic interval of about one week (ranging between 1–33 days), and a second stage with neurological manifestations ranging from mild meningitis to severe encephalitis with or without myelitis and spinal paralysis (Kaiser, 2010; Hudopisk et al., 2013; Bogovic and Strle, 2015).

In contrast, the foodborne disease historically called biphasic milk fever begins after a shorter incubation period of 2–4 days. In about 50% of the cases, patients showed the monophasic form of the disease, manifesting intracranial hypertension, severe headaches, nausea, vomiting, weakness, loss of appetite, dizziness, drowsiness, gastrointestinal problems, epistaxis, pharyngitis, laryngitis, photophobia, and an elevated body temperature. The other 50% of the patients developed a severe biphasic disease. In the first phase, which lasted about seven days, visual disturbances, blurred vision or diplopia, and a temperature of up to 40.0°C occurred. The second phase began after about eight days of remission, and signs of meningeal irritation and/or encephalitis occurred. In most cases, the disease course was benign in the second phase. In mild cases, it lasted 3–4 days and in more severe cases 14–21 days (Dorko et al., 2018). However, encephalitic symptoms such as extrapyramidal, vegetative, and nuclear lesions persisted longer, in some cases longer than five months (Růžek et al., 2010).

TBE distribution and alimentary transmission of TBEV

TBE is widespread in Eurasia, with approximately 3,000 disease cases per year in Europe (increasingly in Eastern Europe) and 11,000 per year in Russia (Gäumann et al., 2010). In Switzerland, this viral zoonosis has been observed since 1984 (FOPH, 2019b). The number of reported TBE cases has been increasing since then, with annual case numbers showing pronounced fluctuations (Schuler et al., 2014). *I. ricinus* ticks are present throughout Switzerland at altitudes of up to 2,000 meters above sea level. TBEV-infected ticks are found in so-called natural foci that vary considerably in size from a few square meters to several square kilometers (Zeman, 1997; Süss, 2003). Tick activity is highest from March to November. As a consequence, the number of TBE cases undulates in warmer months (FOPH, 2019c). The areas with natural foci of TBEV-infected ticks have expanded in recent decades, spreading from the north-east to the south and west of the country (FOPH, 2019b). Only the cantons of Geneva and Ticino have so far been largely spared. Therefore, the whole of Switzerland, with the exception of these two cantons, is considered a TBE risk area (FOPH, 2019a). Nevertheless, virus-positive ticks and TBEV infections in goats have already been detected in the Ticino canton (Casati Pagani et al., 2019).

In goat herds, the number of infected animals in a given area is highly variable. It is not rare for a herd to contain only one animal with TBEV-specific antibodies (Rieille et al., 2017) or to detect TBEV by polymerase chain reaction in a bulk tank milk coming from the milk of only one animal (Veterinary and Food Institute in Dolny Kubin, 2019).

Goats excrete the infectious virus via milk in the viremic phase which lasts a few days (Van Tongeren, 1955; Veterinary and Food Institute in Dolny Kubin, 2019). TBEV can be detected in goat milk 2–6 days after the animal is infected (Balogh et al., 2012). The viremic phase is limited to a few days, and the infected animal usually doesn't show clinical symptoms. However, following seroconversion, TBEV-specific antibodies can be detected for several months to years afterward (Klaus et al., 2014).

In recent years, eight human cases of alimentary-transmitted TBE have been documented in German-speaking countries. In both cases, cheese made from raw goat milk had been consumed, and the patients had not been vaccinated against TBE (Holzmann et al., 2009; Brockmann et al., 2018). The latest case of alimentary-transmitted TBE in Europe was observed in France in 2020 in the Auvergne-Rhône-Alpes region, not far from the Swiss border. A total of 37 persons were examined who showed typical symptoms after the consumption of raw goat milk cheese. For the majority of those examined, the alimentary transmission of TBEV was scientifically proven (ARS de Auvergne-Rhône-Alpes, 2020).

Infectious TBEV can be inactivated by thermal treatment of the milk (Saier et al., 2015). However, the complete biological inactivation by pasteurization has not reliably proven since inactivation is related to how highly concentrated the virus is. It is assumed that pasteurization reduces the titer by 3-log levels; whether viruses capable of repro-

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duction are still present in the substrate has not been tested (Kaiser, 2010).

For raw milk processing, therefore, sufficient thermal treatment by pasteurization, ultra-high heating, or boiling is recommended (BfR, 2016).

Prevalence studies of TBEV in ticks and small ruminants

An average virus prevalence of 0.46% in ticks throughout Switzerland is relatively low compared to other tick-borne pathogens (Gäumann et al., 2010). Based on theoretical models, this can be explained by the short viremic phase of host organisms infectious to ticks (Randolph et al., 1996; Hartemink et al., 2008; Harrison and Bennett, 2012).

A study documented seroprevalence rates of 0–43% between single herds in districts in the German federal states Baden-Württemberg, Bavaria, and Thuringia in sheep and goat sera. Sera were examined by using an enzyme-linked immunosorbent assay (ELISA) for TBEV-specific immunoglobulins M and G. These considerable differences confirmed the known patchy pattern of TBEV foci (Klaus et al., 2012).

In Switzerland, two prevalence studies with goats as sentinels have been carried out so far. In the Valais canton, a seroprevalence of 4.25% was found in a study analyzing more than 4,000 goat sera (Rieille et al., 2017). In addition to the known risk areas, two unknown TBEV foci were found in this study. This example illustrates that goats and sheep are suitable as TBE sentinels for the detection of new risk areas.

In a second study, ticks, goat herds, and goat owners in Ticino were investigated (Casati Pagani et al., 2019). Since in this region no TBE diseases have been documented in humans to date, the area is classified as non-endemic. However, a seroprevalence of 14.6% in goats was shown, and the virus was detected in ticks with a prevalence of 0.35%. No antibodies against TBEV were detected in any of the goat owners, some of whom regularly consumed raw milk from their own animals. This study showed that the pathogen is already circulating between ticks and host organisms even in the non-endemic Ticino area.

Calculation Parameters

Based on the available literature, the probability of raw goat milk being contaminated with TBEV can be calculated (Table 1). Taking into account the seroprevalence of 4.25% in goats in the alpine region of the Valais canton between October 2011 and March 2012 (Rieille et al., 2017) and an average lactation period of 240 days (Ringdorfer, 2009), the probability of milking one infected animal during a viremic phase of seven days can be calculated.

This calculation takes into account that a seropositive adult goat of 3.4 years (95% confidence limits [CL]: 2.8–4.1 years) has spent two lactation periods in the field. However, the animal would already have been on the pasture during its first 12–15 months before reaching its lactation age and could have been infected with the virus. This young goat age reduces the probability of being milked during a viremic phase. Therefore, a third period with the length of a further lactation period on the field was added to the two lactation periods of 240 days each. Moreover, considering the 95% confidence limit with a maximum value of 4.1 years (Rielle et al. 2017), a fourth lactation period on the field could be added. Thus, the goat's critical period increased to a total of 720–960 days during which it could only excrete the virus via milk for seven days.

The 2018 Swiss milk statistics show that the majority of the total annual quantity of goat milk is marketed milk (SBV et al., 2019). As goat milk for commercial distribution usually undergoes thermal treatment before processing or consumption, contamination of TBEV-infected drinking milk and milk products is unlikely. This has therefore not been taken into account for the risk assessment.

Feed milk was also excluded from the calculation as it is fed as raw milk to young animals that have not yet undergone a lactation phase and therefore do not represent a potential source of contaminated milk.

About 4% of the total annual production of goat milk is used as household milk. The milk is processed on the farm, is mainly used for the farm's own consumption, and is not intended for sale. As household milk is not subject to any external control and therefore represents a potential risk of virus transmission in the case of further processing as raw milk, it was taken into account in the risk assessment. The proportion of household milk consumed as raw milk or cheese made from raw milk is unknown.

Calculations and Results

The probabilities were calculated by using the parameters shown in Table 1 as follows:

For the calculation of the probabilities, the average milk yield of the animals is taken into account (SBV et al., 2019). The assumed viremia duration of an infected goat *(a)* was divided by the duration of a critical period of 720 or 960 days, respectively, which in turn is the product of the number of periods in the field *(b)* and the average duration of a lactation period *(c)*. Multiplication with the experimentally proven seroprevalence in the Valais canton *(d)* leads to a probability range of 0.031–0.041% for a goat to be milked during the viremic phase.

These probabilities, multiplied by the percentage of household milk *(f)* of the total annual amount of goat milk *(e)*, gives a probability range of 0.0012–0.0016% for household milk to be contaminated by TBEV.

TABLE 1: *Values taken into account for the risk assessment.*

Variable	Description	Value	Unit	Reference
a	Assumed length of the viremic phase	7	d	
b	Number of periods in the field (based on the average age (= 3.4 years; 95% confidence limits [CL]: 2.8–4.1 years) of a seropositive goat in the Valais canton	3–4	–	Rieille et al., 2017
c	Average duration of goat lactation	240	d	Ringdorfer, 2009
d	Seroprevalence in the Valais canton	4.25	%	Rieille et al., 2017
e *f*	Annual production of dairy goat milk (Switzerland, 2018) Household milk Feed milk Marketed milk	23.3 0.9 7.4 15.0	10^3 t	SBV et al., 2019
g	Number of goat sera analyzed in the Valais canton	4.114	–	Rieille et al., 2017
h	Number of herds examined in the Valais canton	277	–	Rieille et al., 2017

In a study by Rieille et al. (2017), 4,114 goat sera *(g)* from 277 herds *(h)* were analyzed, leading to an average herd size of 14.9 animals. Furthermore, the hypothetically worst-case scenario, in which during 3–4 lactation periods all goats within an average herd would undergo TBEV infection, was calculated. The calculated probabilities of 0.0012–0.0016% were multiplied by an average herd size of 14.9 animals. This leads to the probability of contaminated household milk to be 0.017–0.024%.

Discussion

The calculated probabilities are average values since the TBEV foci are distributed in a very patchy manner. Thus, the virus prevalence in ticks and the seroprevalence in goats fluctuate considerably (Zeman, 1997; Süss, 2003). Furthermore, the classification as risk area refers to human TBE infections and does not reflect the geographical distribution of the virus in ticks or host animals.

Within the last 10 years (2009–2018), 3,514 TBE cases have been documented in Germany and 908 in Austria (RKI, 2019; Zentrum für Virologie der Medizinischen Universität Wien, 2019). The fact that during this period only eight people in both countries have been proven to be infected with TBEV via the alimentary route (Holzmann et al., 2009; Brockmann et al., 2018) illustrates the low risk of infection after the consumption of raw goat milk and raw goat milk products. The calculated very low probability of raw goat milk in carrying the infectious virus in the selected alpine area in Switzerland supports the low probability of acquiring the disease by the alimentary route.

Nevertheless, sufficient thermal treatment must always be ensured when processing goat milk. Since household milk is processed directly on the farm for self-consumption, it is not subject to external controls and represents the highest risk for the alimentary transmission of TBEV. This recommendation is therefore aimed at small goat farms as well as raw goat milk processors in particular.

To date, the seroprevalence of TBEV-specific antibodies in goats has been determined in two Swiss cantons (Rieille et al., 2017; Casati Pagani et al., 2019). Since the areas that have been investigated are limited to the alpine regions of the Valais and Ticino cantons, it is not possible to expand our risk assessment to the whole of Switzerland based on the available data. Nevertheless, these studies have shown that small ruminants are suitable as sentinels for TBEV prevalence studies. To follow TBEV's spread in Switzerland closely and identify new foci of the virus at an early stage, further prevalence studies with goats as sentinels should be carried out on a Swiss-wide scale. However, the relatively low prevalence in the host organisms requires many animals to be investigated and is therefore costly.

Conclusion

In this risk assessment based on the seroprevalence of antibodies against TBEV in goats in the Valais canton, the average probability of the viral contamination of goat milk for this area was calculated to be 0.0012–0.0016% for milk used as household milk. As a worst-case scenario, assuming a seroprevalence of 100% within an individual herd leads to a maximum probability of 0.024%.

Conflict of interest

The authors declare that they have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the contents or opinions presented in the above manuscript.

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