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Berl Münch Tierärztl Wochenschr 123, 441–445 (2010)
DOI 10.2376/0005-9366-123-441

ISSN 0005-9366

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Eingegangen: 30.04.2010
Angenommen: 09.09.2010

Online first: 21.10.2010

http://vetline.de/zeitschriften/bmtw/open_access.htm

Short communication/Kurzbericht

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Can goats be used as sentinels for Tick-borne encephalitis (TBE) in non-endemic areas? Experimental studies and epizootiological observations

Sind Ziegen als Sentinels für Frühsommer-Meningoenzephalitis (FSME) in einem FSME-Nicht-Risiko-Gebiet geeignet? Experimentelle Untersuchungen und epizootiologische Beobachtungen

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Summary

A goat flock grazing year-round on a meadow in a "TBE non-risk area" in Thuringia, Germany, with a history of only isolated human TBE cases was examined repeatedly for TBE virus-(TBEV)-specific antibodies and TBEV RNA between October 2008 and December 2009. Surprisingly, TBEV specific antibodies were detected in one goat, which had never left this area. To compare the results of a natural contact to TBEV with a defined contact to TBEV, two goats were immunized experimentally. Both animals developed TBEV-specific antibodies, one goat however in a delayed and reduced manner. In addition, 177 ticks were collected from the meadow in May and June 2009, and were examined by real-time RT-PCR. However, no TBEV RNA could be detected. The results suggest that goats can be used as TBEV sentinels in defined areas. To verify this observation further investigations with a large number of animals are recommended.

Keywords: *Ixodes ricinus*, Tick-borne encephalitis virus, TBEV, serum neutralization test, RT-PCR, ELISA

Zusammenfassung

Es wird über Untersuchungen auf FSME-Virus-spezifische Antikörper sowie FSME Virus RNA in einer Ziegenherde berichtet, die sich über das gesamte Jahr auf einer Weide in einem FSME-Nicht-Risiko-Gebiet in Thüringen befand, in dem in der Vergangenheit äußerst selten einzelne humane FSME-Fälle auftraten. Die Untersuchungen wurden wiederholt zwischen Oktober 2008 und Dezember 2009 durchgeführt. Erstaunlicherweise wies dabei eine Ziege, welche diese Weide niemals verlassen hatte, spezifische FSME-Virus-Antikörpertiter auf. Um diese Ergebnisse eines Kontakts zu FSME-Viren via Zeckenstich mit einem definierten Kontakt zu FSME-Virus-Antigen vergleichen zu können, wurden zwei Ziegen experimentell immunisiert. Beide Tiere entwickelten FSME-Virus-Antikörpertiter, allerdings ein Tier zeitverzögert und erst nach mehrfachem Antigenkontakt. Außerdem wurden auf der Weide im Mai und Juni 2009 177 Zecken gesammelt. In keiner der Zecken wurde jedoch FSME-Virus RNA gefunden. Nach diesem Ergebnis könnten Ziegen als Sentinels für die Bewertung des FSME-Risikos in einer Region geeignet sein, was in weiteren Untersuchungen an einer großen Tierzahl verifiziert werden sollte.

Schlüsselwörter: *Ixodes ricinus*, Frühsommer-Meningoenzephalitis, FSME, Serum-Neutralisationstest, RT-PCR, ELISA

Introduction

In Europe, TBE is the most important human virus disease transmitted by ticks and a classical viral zoonosis. In veterinary medicine, clinical cases of TBE with neurological symptoms are seldom, but have been known in dogs for more than 30 years (Leschnik et al., 2002). Rarely, TBE has been described in horses (Waldvogel et al., 1981; Grabner, 1993) and monkeys (Süss et al, 2007; 2008).

In contrast to *Borrelia burgdorferi* s.l., that is ubiquitously endemic in areas in Germany where *Ixodes ricinus* occurs, TBEV circulates between vector ticks and competent hosts in so-called natural foci or in risk areas, whose geographic extension as a rule is strictly limited and can be very small (Kupča et al, 2010). The reason for the patchwork-like distribution of TBEV is not quite clear. Korenberg (2009) summarizes numerous possible factors which influence the manifestation of a natural TBE focus, ranging from virus prevalence, vector occurrence and host activity to climate changes. Also socio-economic factors may lead to a more intensive contact of hosts to TBEV by agricultural land use or leisure activities. As there is a multitude of factors which influence TBEV prevalence in ticks and TBE incidence in humans it is recommended to consider all available information on the local situation to define a TBE risk in a specific area (Süss et al., 2010). Besides the incidence of autochthonous human cases as the official statistical tool to define TBE risk areas in Germany (Robert-Koch-Institute, 2007), the TBEV prevalence in ticks can provide additional information. However, a description of the epidemiological situation based on the collection and analysis of ticks is time-consuming and expensive. In TBE risk areas in Germany e. g. Süss et al. (2004) found TBEV prevalences in ticks of 0 to 4.8% in adults, and 0 to 3.4% in nymphs in eight locations in Baden-Württemberg, and of 0 to 5.3% in adults, and 0 to 1.4% in nymphs in five locations in Bavaria. In total, 21 710 field collected *I. ricinus* were investigated (18 340 nymphs, and 3350 adults) over a six-year period from 1997 to 2002. Because of the low prevalence of TBEV in ticks and based on the experiences with cow sera and milk (Leutloff et al., 2006) our initial aim was to collect sera from grazing animals (goats, sheep), which could serve as specific sentinels for characterization of TBE risk areas by means of sero-surveillance tests. These investigations are in progress.

In this report we describe our results obtained from a goat flock in a TBE non-risk area, district Suhl (50°36′41″N, 10°41′31″E), Thuringia over one year (Fig. 1A). This district was defined as a TBE non-risk area based on the incidence of autochthonous human cases according to the guidelines of the Robert Koch-Institute (Robert-Koch-Institute, 2007). An administrative district is defined as a TBE risk area when the number of reported (registered) TBE cases in the years 2002–2006, 2003–2007 or 2004–2008 in the district or in the district region (this is the given district plus all the neighbouring districts) is significantly ($p < 0.05$) higher than the number of cases to be expected at an incidence of one case per 100 000 inhabitants.

Based on this definition which associates the number of reported cases (incidence) within an administrative district with a certain risk of disease for unvaccinated persons in this area, 136 of the 440 German districts are currently considered as risk areas, as opposed to 63 in 1998. The district Suhl is still classified as non-risk area up to now (Robert-Koch-Institute, 2010) with only one TBE case in 2003, 2006 and 2008. The southern and eastern districts of Thuringia are TBE risk areas, with an only small number of human TBE cases over the past few years. In the non-risk areas only isolated autochthonous cases are observed (Fig. 1A).

Material and Methods

The number of evaluated methods for veterinary use is small. The reason might be the rareness of TBE cases in animals. A commercially available kit is the Immunozym FSME IgG all species kit (Progen GmbH, Heidelberg, DE). It is recommended for antibody detection in animals, but for research purposes only. Another kit to determine TBE-specific antibodies is the Immunozym FSME IgM kit (Progen GmbH, Heidelberg, DE). It is recommended for human serum, plasma and liquor samples in routine diagnosis. Both kits are two-step ELISAs. For antibody detection in the goat samples, the IMMUNOZYM FSME IgM kit (Progen GmbH, Heidelberg, DE) was used according to the manufacturer's instructions but without the human-specific blocking step for IgG to determine the whole Ig-fraction (Müller, 1997). Laboratory tests with defined goat sera revealed that this kit was more sensitive for TBEV antibodies in animals than the all species kit. Less than 5 units/l were defined to be negative, 5–7 units/l borderline, and a score of 8 and more units/l was defined to be positive (8–14 +, > 14–50 ++ and > 50 +++). This was defined by testing and evaluating a large number of dog sera and should be verified by creating a species-specific cut off for veterinary use (Müller, 1997; Klaus et al., 2010b).

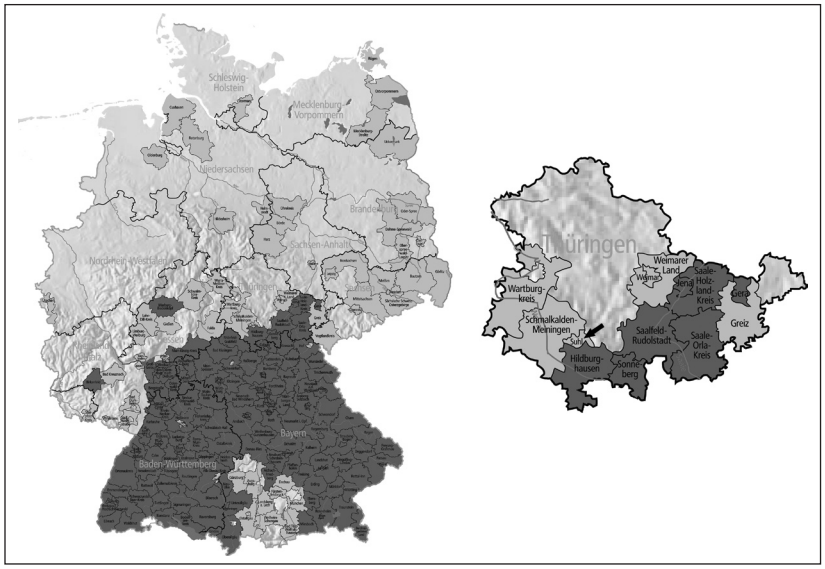

FIGURE 1A: *Sera and tick collection in Suhl, Thuringia, in 2009 (black arrow). Dark grey coloured districts: TBE risk areas, middle grey coloured districts: Non TBE risk area, but single autochthonous human TBE cases occurred, light grey coloured: no TBE risk areas and no single TBE cases observed. For map see http://www.zecken.de/index.php? id = 500.*

TABLE 1A: *Investigated goats (Suhl)*

Date of investigation	No. of goats tested	TBEV antibody positive*	ELISA (U/l) (serum)	SNT (serum)	ELISA (U/l) (milk)	SNT (milk)
15.10.2008	7	1	31,18	80	not tested	not tested
18.02.2009	2	1	26,27	40	not tested	not tested
20.05.2009	5	1	41,95	20	15,25	10
15.06.2009	4	1	25,57	60	8,12	7,5
11.12.2009	3	1	20,95	40	not tested	not tested

* TBEV antibodies in serum and milk were always detected in the same goat.

In order to avoid false positive results, all positive ELISA results of field collected samples were confirmed by the serum neutralization test (SNT) as a gold standard according to Holzmann et al. (1996), but in a modified version. For the SNT, the low pathogenic strain Langat was used with about 100 TCID50/well. The virus titre used was confirmed by back-titrations. Serum samples were titrated in duplicates starting at a dilution of 1:5 in MEM Earle's medium, and a BHK-21 cell suspension was added to the virus-serum-sample and incubated at 37°C for 4 days. Subsequently, virus replication in the wells was detected by immunofluorescence analysis using a TBEV specific rabbit-antiserum. Titres were expressed as the reciprocal of dilutions that caused 50% neutralization (ND50).

In order to compare the results of a natural contact to TBEV with a defined infection, two goats were immunized experimentally four times at the Friedrich-Loeffler-Institute Jena with a vaccine prepared for human medicine (FSME-IMMUN Erwachsene, Baxter Deutschland GmbH, Unterschleißheim, DE). The goats were bought from a flock in Greußen, district Sömmerda in Thuringia in January 2009, the district is a TBE non-risk area up to now and had no TBEV antibodies before immunization. One dose of 0.5 ml was injected subcutaneously per goat and immunization, this is 2.4 µg inactivated virus antigen, which is also used regularly for adult humans. Over a period of 15 weeks sera were collected and examined for TBEV-specific antibodies by ELISA.

Collected ticks (127 nymphs, 26 female, 15 male, 9 engorged, but not differentiated ticks, no larvae) were ground up in a mixer mill with 3 stainless steel beads of 3 mm (Retsch GmbH, Haan, DE) and 400 µl medium (MEM Earle, Biochrom AG, Berlin, DE) to allow the isolation of a potential TBEV strain. Aliquots of this suspension were pooled (50 µl from 10 male or female adults or 10 nymphs), and 140 µl of each pool was used for RNA extraction according to the manufacturer's instructions using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, DE). For detection of TBEV-specific RNA, two quantitative real-time RT-PCR protocols were used, one according to Schwaiger and Cassinotti (2003), modified by Klaus et al. (2010a), and a second protocol according to Klaus et al. (2010b). This second real-time RT-PCR assay was developed for increasing the diagnostic sensitivity and the safety of the TBEV genome detection in field samples and is located in the 5` non translated region of the TBEV genome. The main task was the creation of an independent TBEV assay with a similar performance as the well-established and highly sensitive TBEV assay published by Schwaiger and Cassinotti (2003).

Although the period of viraemia is very short and it is very unlikely to find TBEV in sera, all serum samples were tested for TBEV RNA. The RNA extraction was done by using the QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, DE) according to the manufacturer's instructions and the same real-time RT-PCR protocols as for the ticks were used.

Results and Discussion

In the district Suhl – a TBE non-risk area with only 3 human TBE cases, each in 2003, 2006 and 2008 – TBEV specific antibodies were detected in one goat (born in 2005) during routine examination of a small goat flock (7 goats). The flock was kept under very natural conditions in an open pen, and from some of the goats fresh milk was obtained and raw milk cheese was produced by the flock owner for personal use only. The flock owner never felt ill in 2008. He had a TBEV antibody titre (ELISA: 31,2 U/I, SNT: 120), but was immunized against TBEV in spring 2008. He could not remember a tick bite, which is, however, very often observed in patients with a clinical course of TBE, and he could not surely exclude a tick bite in the past. Therefore, it is unfortunately impossible to tell whether this titre is the result of immunization, of seroconversion after alimentary TBE or of an infection via tick bite.

The surprising result of detecting TBEV specific antibodies in a goat in a TBEV non-endemic area was the reason for retesting the goat in February 2009, the whole flock in May and June, and 3 goats again in December 2009. Blood samples and some milk samples were collected (Tab. 1A). All goats were healthy and no clinical signs of illness were seen over the whole period of investigation. In addition to the blood and milk samples of May and June 2009, 177 ticks were collected by flagging on the meadows where the goats are kept year-round (Tab. 1B).

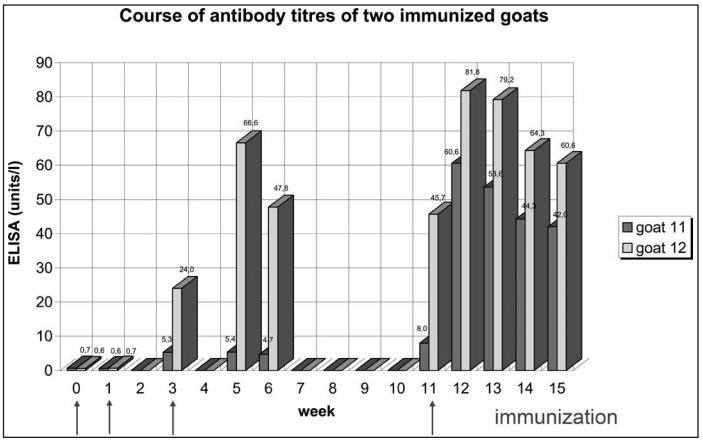

FIGURE 1B: *Course of TBEV-ELISA-antibody titres of two immunized goats. Blood samples were collected before immunization (0) and 1, 3, 5, 6, 11, 12, 13, 14 and 15 weeks after the first immunization (Black arrow: immunization).*

TABLE 1B: *Ticks collected in May and June 2009*

	May	June	total
female	12	14	**26**
male	10	5	**15**
nymphs	97	30	**127**
engorged ticks	9	0	**9**
total	**128**	**49**	**177***

* No TBEV RNA was detected.

The goat with TBE-specific antibodies remained seropositive by ELISA (Tab. 1A) over the whole period of investigation. TBEV positive antibodies by ELISA, but at a very low level, were also detected in two milk samples of this goat collected in May and June 2009 and were confirmed to be specific by SNT. The persistence of TBEV antibodies over more than one year indicated that goat serum might be a simpler and far more inexpensive tool for detecting TBEV contact than the collection and analysis of ticks. It would also be suitable for areas with no or an only low TBE risk such as the region of Suhl. In addition, other free ranging ruminants like sheep could be used as sentinel animals.

The results obtained from the two immunized goats were very different (Fig. 1B). It is known that goats develop TBEV antibody titres after contact with TBEV which was indeed observed in the naturally infected goat from Suhl, and in goat No. 12. The reason for the delayed and reduced development of TBEV-specific antibodies in goat No. 11, even after repeated contact with the antigen, is unclear. It might be that this case is an example for the so called "low responder" phenomenon, which is also observed in isolated human cases after immunization against TBEV (Lindquist and Vapalahti, 2008).

Finally, no TBEV RNA was detected by RT-PCR in the samples collected from the goats. Most of the collected ticks were nymphs (127), followed by adults (41), and no larvae were found. No pool was found to be positive for TBEV-RNA (Tab. 1B).

Large domestic animals such as goats, sheep and cattle are potential hosts for *I. ricinus*. These animals are viraemic over a very short period only (Van Tongeren, 1955). Especially goats and sheep, more rarely cattle, are of importance for the so-called alimentary TBE. During the viraemic stage, the virus is excreted in milk and can be ingested orally by consumption of non-pasteurized milk or cheese produced from raw milk. The occurrence of individual cases or small group outbreaks in humans (e. g. in connection with holidays on a farm) may be the consequence. Former experimental investigations showed that domestic animals can excrete TBEV into milk for 3–7 days (Van Tongeren, 1955; Gresikova, 1958a; Gresikova 1958b; Gresikova and Rehacek, 1959). After consumption of virus contaminated raw milk, the course of the disease is mostly biphasic, whereas in TBE induced by a tick bite, a biphasic course is observed in only 20 to 30% of cases. Meningoencephalitis, with mild meningeal and parenchymal symptoms, is also characteristically. Differences in clinical manifestations between TBE contracted by tick bite or through the alimentary tract are explained by differences in the host immune response that depend on the route of virus penetration and the initial concentration of virus. (Nuttall and Labuda, 2005). TBE in humans caused by virus-infected milk occurred over the past few years in Slovakia, Lithuania, Latvia, Poland, Russia, Albania, Hungary and Austria (Blaškovič, 1954; Gresikova, 1958a, b; Levkovich and Pogodina, 1958; Nosek et al, 1967; Gresikova et al.,1975; Kohl et al., 1996; Matuszczyk et al, 1997; Labuda et al., 2002, Kerbo et al., 2005; Balogh et al, 2010, Holzmann et al, 2009) but has not been relevant in Germany during the last few decades.

In Suhl, the investigations indicate that a contact with TBEV occurred in the goat flock before October 2008 and that TBEV evidently was present during this time in a so defined TBE non-risk area. Although it was not possible to find TBEV-RNA in sera or milk, the specific antibody titre persisting for more than one year demonstrated the presence of TBEV in this area. Consequently, this case demonstrates that it is recommended to avoid the consumption of raw milk and raw milk products from goats not only in distinct risk areas but also in areas with a low TBE risk. As the viraemic period in goats is very short, testing of specific antibodies could be a better tool than TBEV-RNA detection by PCR in milk or blood for determining the specific epidemiological situation of a flock, especially when it is used for milking. TBEV seroprevalence in animals might be useful as an additional tool for characterizing a TBE risk area and goats and other free ranging ruminants can serve as sentinels for TBEV occurrence. Especially in regions with a very low or unknown risk goats as sentinels may be a more suitable tool than TBEV prevalence in ticks because the prevalence in ticks is very low. Thus it is necessary to collect a large number of ticks to avoid false negative results which is time-consuming and requires suitable weather (not too cold or too wet). Therefore, further investigations should be carried out on the TBE seroprevalence in animals grazing on the meadows over a longer period of the year to verify if goats and other free ranging ruminants can be used as sentinels for tick-borne encephalitis, also in non-endemic areas with a very low prevalence of TBEV in ticks.

Acknowledgement

The authors are very thankful to Prof. Dr. Franz X. Heinz, Department of Virology, Medical University of Vienna, Austria, for modifying the serum neutralization test and Dr. Werner Müller for adapting and evaluating the ELISA kit Immunozym FSME IgM kit (Progen GmbH, Heidelberg, DE) in dogs. We would also like to thank to Katja Bauer, Elisabeth Hasse, Doreen Reichelt, Sigrun Kühner and Wolfram Maginot for their excellent technical assistance. We are grateful to the anonymous reviewers who made many helpful suggestions.

The authors confirm that no competing financial interests exist.

References

Balogh Z, Ferenczi E, Szeles K, Stefanoff P, Gut W, Szomor K, Takacs M, Berencsi G (2010): Tick-borne encephalitis outbreak in Hungary due to consumption of raw goat milk. J Virol Methods 163: 481–485.

Blaškovič D (1954): The epidemic of encephalitis in the Roznava natural focus of infection. Slov Acad Sci 9: 314 (in Slovak, with English summary).

Grabner A (1993): Klinische Differentialdiagnose infektiös bedingter Krankheiten des ZNS beim Pferd. Collegium veterinarium 24: 27–31.

Gresikova M (1958a): Recovery of the tick-borne encephalitis virus from the blood and milk of subcutaneously infected sheep. Acta Virol 2: 113–119.

Gresikova M (1958b): Excretion of the tick-borne encephalitis virus in the milk of subcutaneously infected cows. Acta Virol 2: 188–192.

Gresikova M, Rehacek J (1959): Isolation of the tick-borne encephalitis virus from the blood and milk of domestic animals (sheep and cow) after infection by ticks of the family *Ixodes ricinus*. Arch Gesamte Virusforsch 9: 360–364.

Gresikova M, Sekeyova M, Stupalova S, Necas S (1975): Sheep milk-borne epidemic of tick-borne encephalitis in Slovakia. Intervirology 5: 57–61.

Holzmann H, Kundi M, Stiasny K, Clement J, McKenna P, Kunz C, Heinz FX (1996): Correlation between ELISA, hemagglutination inhibition, and neutralization tests after vaccination against tick-borne encephalitis. J Med Virol 48: 102–107.

Holzmann H, Aberle SW, Stiasny K, Werner P Mischak A, Zainer B, Netzer M, Koppi S, Bechter E, Heinz FX (2009): Tick-borne encephalitis from eating goat cheese in a mountain region of Austria. Emerg Infect Dis 15: 1671–1673.

Klaus C, Hoffmann B, Hering U, Mielke B, Sachse K, Beer M, Süss J (2010a): Tick-borne encephalitis (TBE) virus prevalence and virus genome characterization in field-collected ticks (*Ixodes ricinus*) in risk, non-risk, and former risk areas of TBE, and in ticks removed from humans in Germany. Clin Microbiol Infec 16: 238–244.

Klaus C, Hoffmann B, Beer M, Müller W, Stark B, Bader W, Stiasny K, Heinz FX, Süss J (2010b): Seroprevalence of tick-borne encephalitis (TBE) in naturally exposed monkeys (*Macaca sylvanus*) and sheep and prevalence of TBE virus in ticks in a TBE endemic area in Germany. Ticks Tickborne Dis 1: 141–144, doi:10.1016/j.ttbdis.2010.06.001.

Kohl I, Kozuch O, Elecková E, Labuda M, Zaludko J (1996): Family outbreak of alimentary tick-borne encephalitis in Slovakia associated with a natural focus of infection. Eur J Epidemiol 12: 373–375.

Korenberg E (2009): Recent epidemiology of tick-borne encephalitis: an effect of climate change? Adv Virus Res 74: 123–144, DOI= 10.1016/S0065-3527(09)74004–7.

Kerbo N, Donchenko I, Kutsar K, Vasilenko V (2005): Tick-borne encephalitis outbreak in Estonia linked to raw goat milk. Euro Surveill 10: 2–4.

Kupča AM, Essbauer S, Zoeller G, de Mendonca PG, Brey R, Rinder M, Pfister K, Spiegel M, Doerrbecker B, Pfeffer M, Dobler G (2010): Isolation and molecular characterization of a tick-borne encephalitis virus strain from a new tick-borne encephalitis focus with severe cases in Bavaria, Germany. Ticks Tickborne Dis 1: 44–51.

Labuda M, Elecková E, Licková M, Sabó A (2002): Tick-borne encephalitis virus foci in Slovakia. Int J Med Microbiol 291, S33: 43–47.

Leschnik MW, Kirtz GC, Thalhammer JG (2002): Tick-borne encephalitis (TBE) in dogs. Int J Med Microbiol 291 S33: 66–69.

Leutloff R, Nübling M, Neumann-Haefelin D, Rieger MA (2006): Cows as indicators for TBE endemic regions: suitability of testing for antibodies in serum and milk. Int J Med Microbiol 296 S40: 87–88.

Levkovich EN, Pogodina VV (1958): Infection through the alimentary tract with tick-borne encephalitis. Vopr Virusol 3: 145–150 (in Russian, with English abstract).

Lindquist L, Vapalahti O (2008): Tick-borne encephalitis. www.thelancet.com 371: 1861–1871.

Matuszczyk I, Tarnowska H, Zabicka J, Gut W (1997): The outbreak of an epidemic of tick-borne encephalitis in Kielce province induced by milk ingestion. Przegl Epidemiol 51: 381–388.

Müller, W (1997): Tick-borne encephalitis in dogs – serological-epidemiological investigations, Abstractbook, 4th International Potsdam Symposium on Tick-borne diseases, Berlin: 204–218.

Nosek J, Kozuch O, Ernek E, Lichard M (1967): The importance of goats in the maintenance. Tick-borne encephalitis virus in nature. Acta Virol 11: 470–472.

Nuttall PA, Labuda M (2005): Tick-borne encephalitis. In: JL Goodman, DT Dennis, DE Sonenshine (eds.), Tick-borne diseases of humans. ASM Press Washington, DC, USA, 150–163.

Robert-Koch-Institut (2007): FSME: Risikogebiete in Deutschland. Epid Bull 15: 129–135.

Robert-Koch-Institut (2010): FSME: Risikogebiete in Deutschland. Epid Bull 17: 147–154.

Schwaiger M, Cassinotti P (2003): Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA, 2003. J Clin Virol 27: 136–145.

Süss J, Schrader C, Falk U, Wohanka N (2004): Tick-borne encephalitis (TBE) in Germany – epidemiological data, development of risk areas and virus prevalence in field-collected ticks and in ticks removed from humans. Int J Med Microbiol 293 S37: 69–79.

Süss J, Gelpi E, Klaus C, Bagon A, Liebler-Tenorio EM, Budka H, Stark B, Müller W, Hotzel H (2007): Tickborne encephalitis in naturally exposed monkey (*Macaca sylvanus*). Emerg Infect Dis 13: 905–907.

Süss J, Dobler G, Zöller G, Essbauer S, Pfeffer M, Klaus C, Liebler-Tenorio EM, Gelpi E, Stark B, Hotzel H (2008): Genetic characterisation of a tick-borne encephalitis virus isolated from the brain of a naturally exposed monkey (*Macaca sylvanus*). Int J Med Microbiol 298 S1/S44: 295–300.

Süss J, Kahl O, Aspöck H, Hartelt H, Vaheri A, Oehme R, Hasle G, Dautel H, Kunz C, Kupreviciene N, Randolph S, Zimmermann H-P, Atkinson B, Dobler G, Kutsar K, Heinz FX, Steffen R (2010):. Tick-borne encephalitis in the age of general mobility. Wien Med Wochenschr 160: 1–7.

Van Tongeren HA (1955): Encephalitis in Austria. IV. Excretion of virus by milk of the experimentally infected goat. Arch gesamte Virusforsch 6: 158–162.

Waldvogel K, Matile H, Wegmann C, Wyler R, Kunz C (1981): Zeckenenzephalitis beim Pferd. Schweiz Arch Tierh 123: 227–233.

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