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First report of infection with metacestode stages of *Echinococcus multilocularis* in a kulan (*Equus hemionus kulan*) from Slovakia

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ABSTRACT

While the principle definitive host of the zoonotic cestode *Echinococcus multilocularis* in Europe is the red fox, several rodent species act as main intermediate hosts. Among others, e.g., humans, dogs, and pigs, also horses have been described to act as aberrant hosts in highly endemic regions. Here, a case of an *E. multilocularis* infection in a kulan (*Equus hemionus kulan*) is described. The five years old kulan from a zoo in Slovakia was transported to an animal park in Germany. The animal had to be euthanized within a few weeks after the import due to its poor general state of health. The pathological examination revealed a nodular mass in the liver as an incidental finding. By histological examination of the mass, a pyogranulomatous and necrotizing inflammation and intralesional fragments of amorphous eosinophil layers were detected. The suspected diagnosis of *E. multilocularis* infection was confirmed by PCR addressing parts of the genes 12S rRNA and the NADH dehydrogenase subunit 2, showing very high identities with isolates from France, Slovakia and the USA.

1. Introduction

As part of the sylvatic life cycle of *Echinococcus multilocularis*, red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) act as main final hosts, while rodent species as e.g., *Microtus* spp. serve as predominant intermediate hosts in Europe (Petersen et al., 2018). Nevertheless, other carnivores are able to function as final hosts also, e.g., dogs (*Canis lupus familiaris*), wolves (*Canis lupus*) and golden jackals (*Canis aureus*) (Citterio et al., 2021). Additionally, among others, humans, primates, or even dogs and pigs may act as aberrant hosts for the parasite's metacestode stage (Böttcher et al., 2013; Knapp et al., 2021). Alveolar echinococcosis is regarded as one of the most harmful parasitic zoonoses of the northern hemisphere, characterized by a mainly intrahepatic tumorlike growth of the larval stage of *E. multilocularis* in the intermediate host (Gottstein and Hemphill, 2008; Conraths et al., 2017). Regarding the occurrence of *Echinococcus* in equidae, infection is usually related to cystic echinococcosis caused by non-zoonotic *E. equinus* or even zoonotic *E. granulosus* sensu stricto, whereby both species seem to be able to produce fertile hydatid cysts (Boufana et al., 2014; Lahmar et al., 2014; Romig et al., 2015). Nevertheless, also *E. multilocularis* infection has already been described to cause nodular lesions in the liver of slaughtered horses from Japan and Poland (Goto et al., 2010; Ueno et al., 2012; Tomczuk et al., 2020). To date, echinococcosis in Slovakia was ascertained in humans and carnivores. The first case of an autochthonous alveolar echinococcosis in humans was diagnosed in a 69 years old woman in 2000 (Kinčeková et al., 2001). Initially reported in 1999, the presence of *E. multilocularis* infection in red foxes has been demonstrated in all districts of the country now (Dubinský et al., 1999; Miterpáková et al., 2006). Additionally, dogs from Slovakia have been identified as a potential source for human infection with *E. multilocularis* (Antolová et al., 2009). Herein, a case of infection with the larval stage of *E. multilocularis* in a kulan (*Equus hemionus kulan) from Slovakia* is reported for the first time.

2. Material and methods

2.1. Patient history, clinical presentation and diagnostics

A five years old male kulan was transported from Košice Zoo, Eastern Slovakia to an animal park in Chemnitz, Eastern Germany. The animal was reared in Košice Zoo since birth, and routinely vaccinated and treated with anthelminthics. Apart from that the animal never showed

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any signs of disease and therefore, the kulan did not receive any other treatment. During the transport, the kulan was restless, while in Košice no clinical signs or health issues had been observed. After arriving, the kulan showed clinical symptoms in terms of hypersalivation, inappetence, and dysphagia. In addition, a severe lameness of the left forelimb was noted. Since no improvement of the clinical presentation was achieved during the following two weeks, the donkey's skull and vertebrate column were examined by x-ray. Also, an endoscopic examination of the upper and lower respiratory passages as well as of the oesophagus, and stomach were undertaken. Thereby, hyperkeratosis and an ulcer were detected in the stomach. After the recovery phase of anaesthesia, a radial nerve paralysis was noticed, probably caused by the long period of lying during the examinations. Two days after, the kulan showed signs of multiple organ failure, and he was not able to get up; therefore, euthanasia was initiated soon.

2.2. Pathological examination

The animal was autopsied one day postmortem. Representative samples of altered tissue, e.g., cystic liver lesions as well as macroscopically unremarkable organs were collected and fixed in 4% neutral buffered formaldehyde for 3 days. Afterwards, several specimens were routinely processed for histopathological and histochemical examination.

2.3. Molecular diagnostics

For molecular analysis, DNA was extracted from cystic lesions of the native liver tissue using the NucleoSpin Tissue kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. To confirm the suspected diagnosis of echinococcosis, a nested PCR detecting a part of the mitochondrial 12S rRNA gene was performed using primers and PCR cycling conditions described previously (Dyachenko et al., 2008) with the following conditions: 0.3 μl of each primer, 0.1 U of DreamTaq DNA polymerase (ThermoFisher Scientific, Dreieich, Germany), 2.5 μl of DreamTaq™ Green Buffer (10x; ThermoFisher Scientific, Dreieich, Germany), and 0.5 μl of each dNTP. Furthermore, 3 μl of the sample DNA was used and a final volume of 25 μl was achieved by adding DNA-free, nuclease-free water. For sequencing and phylogenetic analysis, the NADH dehydrogenase subunit 2 (nad2) gene was addressed under conditions as described previously (Nakao et al., 2009; Delling et al., 2020). After running the gel-electrophoresis, ethidium bromide was used for the staining of the gel. Afterwards, bands were visualized by UV light. For sequence and phylogenetic analysis, the PCR product was then purified using the PCR Purification Kit (Jena Bioscience GmbH, Jena, Germany). Sequencing was performed in both directions by a commercial company (Microsynth Seqlab, Leipzig, Germany). The obtained sequences were aligned, and afterwards, compared with those available in the GenBank database by BLASTn analysis optimized for highly similar sequences (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Alignment with sequences of high identity was performed using the Muscle tool of MEGA version X (Kumar et al., 2018). Alignment was refined on https://ngphylogeny.fr (Criscuolo and Gribaldo, 2010; Katoh and Standley 2013; Lemoine et al., 2019). For phylogenetic analysis, Neighbor-Joining method was conducted by using MEGA version X (Felsenstein 1985; Saitou and Nei 1987; Kumar et al., 2018). The bootstrap consensus tree inferred from 1000 replicates and Kimura 2-parameter method was used to compute the evolutionary distances (Kimura 1980).

3. Results and discussion

The postmortem examination of the kulan mainly revealed multifactorial inflammatory lesions of the skin and subcutis, as well as of the skeletal muscles of the hind limbs and the gastrointestinal tract. Moreover, there was mineralization of several muscle fibres of the skin, the skeletal muscles and the muscular tissue of internal organs. In the liver a smooth and firm, grey-white to ivory-coloured nodular mass of about 6 cm in diameter was detected (Fig. 1). Besides fewer cystic areas of up to 3 mm in diameter, the cut surface was mainly solid and the lesion was bounded by a 1–2 mm thick capsule. Pyogranulomatous to necrotizing hepatitis with intralesional fragments of amorphous eosinophil and periodic acid-Schiff (PAS)-positive layers up to 15 μm thick was found in the mass by microscopical examination (Fig. 2). These findings were consistent with alveolar echinococcosis, nevertheless, protoscoleces were not observed within the microscopically examined sections. The sequence analysis addressing the nad 2 gene confirmed the pathological findings and revealed very high identities (99.89–100%; query cover: 92–99%) of the 952 bp long sequence obtained in this study (accession number: OR098882) with sequences from France (e.g., accession number: OQ599967.1), the USA (e.g., accession number: OK268248.1) as well as from Slovakia (e.g., accession number: OP277525.1), which were revealed from the GenBank. Phylogenetic analyses of the relations between the herein found isolate from the kulan and sequences with high identities hint at a close relationship to isolates from France. This result fits with former analyses where *E. multilocularis* isolates from foxes captured in 19 districts of Slovakia shared all sequences with two French isolates from wild boar (Šnábel et al., 2006) (Fig. 3). Furthermore, phylogenetic analysis assorts the suggestion of European strains in northern America (Jenkins et al., 2012).

This is the first report of the infection with metacestode stages of *E. multilocularis* in a kulan from Slovakia. Among zebras (*E. grevyi*, *E. burchelli*, *E. zebra*), horses (*Equus caballus*), and African asses (*E. asinus*), the Asiatic asses (*E. hemionus kulan*, *E. kiang*) belong to the family Equidae, and serve as intermediate hosts of *E. equinus* or may act also as a reservoir for *E. granulosus* s. s. (G1) (Musilova et al., 2009; Lahmar et al., 2014). Cystic echinococcosis in Equidae is reported to occur worldwide and hydatid cysts usually develop in liver, lungs, or both (Blutke et al., 2010; Romig et al., 2015). Nevertheless, additionally to other helminth infections inducing alterations within the liver parenchyma, also *E. multilocularis* is reported to cause hepatic nodular lesions in horses (Goto et al., 2010; Tomczuk et al., 2020; Hifumi et al., 2021). Infected by the oral ingestion of *E. multilocularis* eggs, horses serve as aberrant hosts for the larval stage among others, e.g., humans and pigs (Hifumi et al., 2021), and previous reports of infection in horses originated from highly endemic regions (Goto et al., 2010; Tomczuk et al., 2020). In Japan, the larval infection with *E. multilocularis* has been described in slaughtered race horses which originated from or were reared in Hokkaido, where echinococcosis is endemic. Therefore, the environment of the horses has probably become contaminated due to the high infestation in foxes (Goto et al., 2010), which is manifested in

Fig. 1. Liver showing a single nodular encapsulated mass of about 6 cm in diameter. The mainly solid cut surface reveals several small cystic areas (arrows).

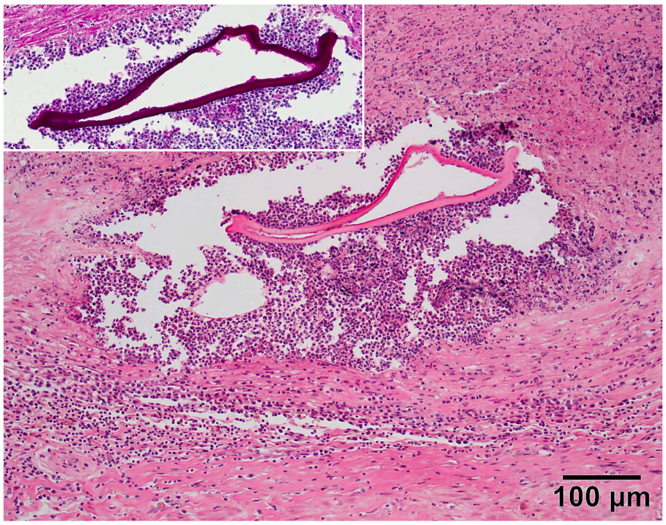

Fig. 2. Photomicrograph of the hepatic nodular lesion showing a pyogranulomatous to necrotizing inflammation with intralesional fragments of amorphous band-like eosinophil and strongly PAS-positive (inset) structures, consistent with the laminated layer of the larval stage of *Echinococcus multilocularis*. Hematoxylin-eosin stain and Periodic acid-Schiff stain (inset), respectively.

prevalence rates between 30 and 40% (Irie et al., 2019). Additionally, in another study from Japan parasitic stages in the liver tissue of imported horses have been examined, and identities of 99–100% to European haplotypes have been described, indicating that infection took place in the also endemic region of Alberta in Canada, where the horses had been raised (Hifumi et al., 2021). In Poland, 365 slaughtered horses were examined, which originated from central, eastern and southern Poland, and the overall prevalence rate of alveolar echinococcosis was estimated to be between 4.7% and 14.8% (Tomczuk et al., 2020). Poland has also been described to be an endemic area and infection rates in red foxes vary regionally with the highest prevalence in the eastern parts (up to 42.7%) (Karamon et al., 2018; Tylkowska et al., 2019; Tomczuk et al., 2020).

To date, no reports of *E. multilocularis* infection in Equidae from Slovakia or other European countries except Poland have been published (Tomczuk et al., 2020). However, the occurrence of echinococcosis in Slovakia has been examined in foxes, dogs, wolves, and humans. First findings of *E. multilocularis* were described in Košice-surroundings among other regions in 1999 (Dubinský et al., 1999). From 2000 to 2010, 4761 red foxes were examined for the presence of infection, and great differences of the parasite's prevalence and infection intensity was described between regions and years for this time period (Miterpáková et al., 2003, 2006; Miterpáková and Dubinský, 2011). Suggesting climate and environmental factors as important influencing variables regarding eggs' survival and spread of infection, tight relationships between yearly rainfall amounts, potential intermediate hosts and parasites' distribution in red foxes were shown (Miterpáková et al., 2006). The mean prevalence rate of *E. multilocularis* infection in red foxes from Košice was estimated to be 18.4%, with the highest prevalence recorded in 2010 (39.5%) (Miterpáková and Dubinský, 2011), while in the northern part of the country prevalence rates up to >60% were reported occasionally (Miterpáková et al., 2006; Miterpáková and Dubinský, 2011; Antolova et al., 2014). Examining 112 wolf samples from Slovakia, 35.7% of them were found to contain *E. multilocularis* DNA, mirroring the extensive dissemination of this parasite in wildlife (Jarošová et al., 2020). However, 289 dogs originating mainly from a highly endemic region in northeastern Slovakia as well as from the Košice region were examined for an infection with *E. multilocularis*. Eight of them were detected to be infected (2.8%), whereby no association between origin and infection status could be shown (Antolová et al., 2009). The authors of this study concluded that dogs contributed substantially to the transmission of *E. multilocularis* in the examined territory in Slovakia. In humans, the first case of alveolar echinococcosis was diagnosed in the year 2000, since then, the number of cases has increased continuously (Šimeková et al., 2021).

By now, no other cases of alveolar echinococcosis at the *Košice Zoo have been reported.* The kulan presented in this case report was born and raised in the *zoo*. Since the animal did not leave this place before, infection must have occurred at the zoo. The studies mentioned above underline the high prevalence of *E. multilocularis* in wild carnivores in Slovakia, so the occurrence of this parasite in aberrant hosts like Equidae seems to be reasonable since this has also been reported from other endemic regions before (Goto et al., 2010; Tomczuk et al., 2020). Cleaning and disinfection of open-air enclosures as well as keeping away wild carnivores from the zoo area are impractical, therefore, environmental contamination is difficult to prevent. However, as described in former studies of alveolar echinococcosis in Equidae (Tomczuk et al., 2020), no protoscoleces were detected in the altered liver tissue in this case, emphasizing the kulan's role as a dead-end host. Nevertheless, the report of *E. multilocularis* in those aberrant hosts may be a good epidemiological indicator mirroring the environmental contamination pressure with the parasite's infectious stages (Knapp et al., 2021).

4. Conclusion

This is the first report of *E. multilocularis* infection in a kulan, and furthermore, in Equidae from Slovakia. Sequence analysis revealed very high identities to isolates from France, Slovakia, and the USA. Although acting as dead-end hosts, findings of *E. multilocularis* in Equidae from the zoo, give insights in the epidemiological situation of the parasite's occurrence. Considering the presence of some highly endemic regions in Slovakia, the number of unknown cases of alveolar echinococcosis in aberrant hosts including humans may be relatively high in those areas.

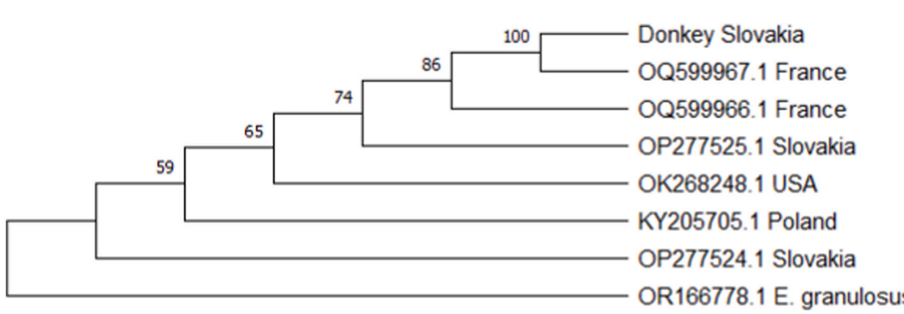

Fig. 3. Phylogenetic analysis of the herein obtained sequence (Donkey_Slovakia) and exemplary sequences from GenBank (e.g., OK268248.1; OP277525.1) using the Neighbor-Joining method, bootstraps consensus tree inferred from 1000 replicates. A sequence of *E. granulosus* was used as an outgroup (OR166778.1).

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Declaration of competing interest

All authors concur with the submission and the material submitted is not under consideration for publication elsewhere. The authors declare no conflict of interest.

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References

Antolová, D., Reiterová, K., Miterpáková, M., Dinkel, A., Dubinský, P., 2009. The first finding of *Echinococcus multilocularis* in dogs in Slovakia: an emerging risk for spreading of infection. Zoonoses Public Health 56, 53–58. https://doi.org/10.1111/j.1863-2378.2008.01154.x.

Antolova, D., Miterpakova, M., Radoňak, J., Hudačkova, D., Szilagyiova, M., Začek, M., 2014. Alveolar echinococcosis in a highly endemic area of Northern Slovakia between 2000 and 2013. Euro Surveill. 19, 20882. PMID: 25188612.

Blutke, A., Hamel, D., Hüttner, M., Gehlen, H., Romig, T., Pfister, K., Hermanns, W., 2010. Cystic echinococcosis due to *Echinococcus equinus* in a horse from southern Germany. J. Vet. Diagn. Invest. 22, 458–462. https://doi.org/10.1177/104063871002200323. PMID: 20453228.

Böttcher, D., Bangoura, B., Schmäschke, R., Müller, K., Fischer, S., Vobis, V., Meiler, H., Wolf, G., Koller, A., Kramer, S., Overhoff, M., Gawlowska, S., Schoon, H.A., 2013. Diagnostics and epidemiology of alveolar echinococcosis in slaughtered pigs from large-scale husbandries in Germany. Parasitol. Res. 112, 629–636. https://doi.org/10.1007/s00436-012-3177-2.

Boufana, B., Lahmar, S., Rebaï, W., Ben Safta, Z., Jebabli, L., Ammar, A., Kachti, M., Aouadi, S., Craig, P.S., 2014. Genetic variability and haplotypes of *Echinococcus* isolates from Tunisia. Trans. R. Soc. Trop. Med. Hyg. 108, 706–714. https://doi.org/10.1093/trstmh/tru138.

Citterio, C.V., Obber, F., Trevisiol, K., Dellamaria, D., Celva, R., Bregoli, M., Ormelli, S., Sgubin, S., Bonato, P., Da Rold, G., Danesi, P., Ravagnan, S., Vendrami, S., Righetti, D., Agreiter, A., Asson, D., Cadamuro, A., Ianniello, M., Capelli, G., 2021. *Echinococcus multilocularis* and other cestodes in red foxes (Vulpes vulpes) of northeast Italy, 2012–2018. Parasites Vectors 14, 29. https://doi.org/10.1186/s13071-020-04520-5.

Conraths, F.J., Probst, C., Possenti, A., Boufana, B., Saulle, R., La Torre, G., Busani, L., Casulli, A., 2017. Potential risk factors associated with human alveolar echinococcosis: systematic review and meta-analysis. PLoS Neglected Trop. Dis. 11, e0005801 https://doi.org/10.1371/journal.pntd.0005801.

Criscuolo, A., Gribaldo, S., 2010. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. BMC Evol. Biol. 10, 210. https://doi.org/10.1186/1471-2148-10-210.

Delling, C., Böttcher, D., Cabrera-García, I.A., Kiefer, I., Helm, C., Daugschies, A., Heilmann, R.M., 2020. Clinical, pathological and parasitological examinations of a German spaniel with alveolar echinococcosis, Germany, 2018. Vet Parasitol Reg Stud Reports 20, 100403. https://doi.org/10.1016/j.vprsr.2020.100403.

Dubinský, P., Svobodová, V., Turčeková, L., Literák, I., Martínek, K., Reiterová, K., Kolářová, L., Klimeš, J., Mrlík, V., 1999. *Echinococcus multilocularis* in Slovak Republic: the first record in red foxes (*Vulpes vulpes*). Helminthologia 36, 105–110.

Dyachenko, V., Pantchev, N., Gawlowska, S., Vrhovec, M.G., Bauer, C., 2008. *Echinococcus multilocularis* infections in domestic dogs and cats from Germany and other European countries. Vet. Parasitol. 157, 244–253. https://doi.org/10.1016/j.vetpar.2008.07.030.

Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783–791.

Goto, Y., Sato, K., Yahagi, K., Komatsu, O., Hoshina, H., Abiko, C., Yamasaki, H., Kawanaka, M., 2010. Frequent isolation of *Echinococcus multilocularis* from the livers of racehorses slaughtered in Yamagata, Japan. Jpn. J. Infect. Dis. 63, 449–451.

Gottstein, B., Hemphill, A., 2008. *Echinococcus multilocularis*: the parasite-host interplay. Exp. Parasitol. 119, 447–452. https://doi.org/10.1016/j.exppara.2008.03.002.

Hifumi, T., Tanaka, T., Hernandez, E.P., Akioka, K., Yamada, K., Imamura, Y., Hatai, H., Miyoshi, N., 2021. Relationship between hepatic grayish-white solid nodules in horses imported from Canada and larval *Echinococcus multilocularis* infection. Can. Vet. J. 62, 285–288.

Irie, T., Yamada, K., Morishima, Y., Yagi, K., 2019. High probability of pet dogs encountering the sylvatic cycle of *Echinococcus multilocularis* in a rural area in Hokkaido, Japan. J. Vet. Med. Sci. 81, 1606–1608. https://doi.org/10.1292/jvms.19-0307.

Jarošová, J., Antolová, D., Šnábel, V., Guimarães, N., Štofík, J., Urban, P., Cavallero, S., Miterpáková, M., 2020. The fox tapeworm, *Echinococcus multilocularis*, in grey wolves and dogs in Slovakia: epidemiology and genetic analysis. J. Helminthol. 94, e168. https://doi.org/10.1017/S0022149X20000528.

Jenkins, E.J., Peregrine, A.S., Hill, J.E., Somers, C., Gesy, K., Barnes, B., Gottstein, B., Polley, L., 2012. Detection of European strain of *Echinococcus multilocularis* in north America. Emerg. Infect. Dis. 18, 1010–1012. https://doi.org/10.3201/eid1806.111420.

Karamon, J., Dąbrowska, J., Kochanowski, M., Samorek-Pieróg, M., Sroka, J., Różycki, M., Bilska-Zając, E., Zdybel, J., Cencek, T., 2018. Prevalence of intestinal helminths of red foxes (*Vulpes vulpes*) in central Europe (Poland): a significant zoonotic threat. Parasites Vectors 11, 436. https://doi.org/10.1186/s13071-018-3021-3.

Katoh, K., Standley, D.M., 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol. Biol. Evol. 30, 772–780. https://doi.org/10.1093/molbev/mst010.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.

Kinčeková, J., Auer, H., Reiterová, K., Dubinský, P., Szilágyiová, M., Lauko, L., Aspöck, H., 2001. The first case of autochtonous human alveolar echinococcosis in the Slovak Republic (Case report). Mittl. Osterreichischen Ges. Tropenmed. Parasitol. 23, 33–38.

Knapp, J., Meyer, A., Courquet, S., Millon, L., Raoul, F., Gottstein, B., Frey, C.F., 2021. *Echinococcus multilocularis* genetic diversity in Swiss domestic pigs assessed by EmsB microsatellite analyzes. Vet. Parasitol. 293, 109429 https://doi.org/10.1016/j.vetpar.2021.109429.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35, 1547–1549. https://doi.org/10.1093/molbev/msy096.

Lahmar, S., Boufana, B., Jebabli, L., Craig, P.S., Ayari, H., Basti, T., Dhibi, M., Torgerson, P.R., 2014. Modelling the transmission dynamics of cystic echinococcosis in donkeys of different ages from Tunisia. Vet. Parasitol. 205, 119–124. https://doi.org/10.1016/j.vetpar.2014.06.007.

Lemoine, F., Correia, D., Lefort, V., Doppelt-Azeroual, O., Mareuil, F., Cohen-Boulakia, S., Gascuel, O., 2019. NGPhylogeny.fr: new generation phylogenetic services for non-specialists. Nucleic Acids Res. 47 (Issue W1), W260–W265. https://doi.org/10.1093/nar/gkz303.

Miterpáková, M., Dubinský, P., Reiterová, K., Machková, N., Várady, M., Šnábel, V., 2003. Spatial and temporal analysis of the *Echinococcus multilocularis* occurrence in the Slovak Republic. Helminthologia 40, 217–226.

Miterpáková, M., Dubinský, P., Reiterová, K., Stanko, M., 2006. Climate and environmental factors influencing *Echinococcus multilocularis* occurrence in the Slovak Republic. Ann. Agric. Environ. Med. 13, 235–242.

Miterpáková, M., Dubinský, P., 2011. Fox tapeworm (*Echinococcus multilocularis*) in Slovakia- summarizing the long-term monitoring. Helminthologia 48, 155–161.

Musilova, P., Kubickova, S., Horin, P., Vodicka, R., Rubes, J., 2009. Karyotypic relationships in Asiatic asses (kulan and kiang) as defined using horse chromosome arm-specific and region-specific probes. Chromosome Res. 17, 783–790. https://doi.org/10.1007/s10577-009-9069-3.

Nakao, M., Xiao, N., Okamoto, M., Yanagida, T., Sako, Y., Ito, A., 2009. Geographic pattern of genetic variation in the fox tapeworm *Echinococcus multilocularis*. Parasitol. Int. 58, 384–389. https://doi.org/10.1016/j.parint.2009.07.010.

Petersen, H.H., Al-Sabi, M.N.S., Enemark, H.L., Kapel, C.M.O., Jørgensen, J.A., Chriél, M., 2018. *Echinococcus multilocularis* in Denmark 2012-2015: high local prevalence in red foxes. Parasitol. Res. 117, 2577–2584. https://doi.org/10.1007/s00436-018-5947-y.

Romig, T., Ebi, D., Wassermann, M., 2015. Taxonomy and molecular epidemiology of *Echinococcus granulosus sensu lato*. Vet. Parasitol. 213, 76–84. https://doi.org/10.1016/j.vetpar.2015.07.035.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Šimeková, K., Rosoľanka, R., Szilágyová, M., Antolová, D., Nováková, E., Novák, M., Laca, Ľ., Sadloňová, J., Šoltys, J., 2021. Alveolar echinococcosis of the liver with a rare infiltration of the adrenal gland. Helminthologia 58, 100–105. https://doi.org/10.2478/helm-2021-0002.

Šnábel, V., Miterpakova, M., D'Amelio, S., Busi, M., Ševcová, D., Turčeková, L.'., Maddox-Hyttel, C., Skuce, P., Dubinsky, P., 2006. Genetic structuring and differentiation of *Echinococcus multilocularis* in Slovakia assessed by sequencing and isoenzyme studies. Helminthologia 43, 196–202. https://doi.org/10.2478/s11687-006-0037-6.

Tomczuk, K., Hirzmann, J., Köhler, K., Szczepaniak, K., Studzinska, M., Demkowska-Kutrzepa, M., Roczeń-Karczmarz, M., Bauer, C., 2020. *Echinococcus multilocularis* infection in horses in Poland. Vet Parasitol Reg Stud Reports 22, 100486. https://doi.org/10.1016/j.vprsr.2020.100486.

Tylkowska, A., Pilarczyk, B., Pilarczyk, R., Zyśko, M., Tomza-Marciniak, A., 2019. Presence of tapeworms (cestoda) in red fox (*Vulpes Vulpes*) in north-western Poland, with particular emphasis on *Echinococcus multilocularis*. J Vet Res 63, 71–78. https://doi.org/10.2478/jvetres-2019-0005.

Ueno, M., Kuroda, N., Yahagi, K., Ohtaki, T., Kawanaka, M., Analysis of antibody responses by commercial western blot assay in horses with alveolar echinococcosis. J. Vet. Med. Sci.. 74:813-815. doi: 10.1292/jvms.11-0552.