Prevalence of *Setaria equina* microfilaraemia in horses in Hungary

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Peripheral blood samples were collected randomly from 195 horses in various parts of Hungary, and the presence of microfilariae was evaluated by the Knott technique. On the basis of morphological identification 18 of the horses (9·2 per cent) were infected with *Setaria equina*, and the infection was confirmed in 10 animals by PCR and sequencing. The level of microfilaraemia was between 1 and 1138 larvae in 2 ml of blood. There was no correlation between the time of sampling or the sex of the animals (stallions versus mares) and the prevalence of infection, but the prevalence decreased with age. There was a significant association between the prevalence of microfilaraemia and the presence of still waters; positive samples were collected either in the region of Lake Balaton, the largest lake in the country, or at places with nearby ponds.

Setaria equina is a filaroid nematode parasite of horses with a worldwide distribution (Coleman and others 1985). The adults are harmless inhabitants of the peritoneal cavity, and are generally found accidentally during pathological examinations (Hillyer and others 2001). First stage larvae (microfilariae) accumulate in the circulation. Discounting the exceptional establishment of *Dirofilaria immitis* (Klein and Stoddard 1977, Thurman and others 1984), only two filaroid species have microfilariae that appear regularly in the peripheral blood of horses (Kassai 1999): *S equina* and *Elaeophora boehmi*, the latter with morphologically different larvae and a geographically-restricted range (Supperer 1953). Mosquitoes belonging to the genera *Aedes* or *Culex* serve as vectors of *S equina* (Nelson 1959), the significance of which is due to microfilariae and/or inoculated third stage larvae that can occasionally reach the eye (Jirina 1959, Abu El-Magd and Ahmed 1994) or central nervous system (Orlov 1961, Frauenfelder and others 1980), where their presence or development can be associated with pathological changes. Skin lesions due to setariosis have also been reported (Yousif and others 1990). However, in such clinical cases, due to the aberrant migration of the developmental stages, the causative role of *Setaria digitata* – for which horses are abnormal hosts – should also be considered, justifying the need for molecular identification (Wijesundera and others 1999).

S equina infection has been reported in several European countries, including the UK (Hillyer and others 2001), Germany (Buchwalder and Schuster 1989), Poland (Bezubik and Furmaga 1964), the Czech Republic, Slovakia, Romania and Bulgaria (Jirina 1959), Italy (Ricci and Sabatini 1992), Greece (Sotiraki and others 1997) and Turkey (Oge and others 2003), but the prevalence is seldom reported and is mostly based on finding the adult worms. There is also little information in the literature on the density of larvae in the blood or on the age of the horses most commonly affected. Because there were no data on the extent or severity of the microfilaraemia caused by *S equina* in Hungary, a survey was conducted to screen samples of blood from horses in various regions of the country by the Knott method and PCR.

MATERIALS AND METHODS

The survey was conducted in June 2006, when blood was collected by jugular venepuncture from 195 horses. The randomly sampled animals were kept in 14 different locations, including six that were within 1 km of one or more ponds, two that were next to Lake Balaton, the largest lake in the country, two that were 25 km from Lake Balaton and four with only rivers or streams in their proximity. EDTA was used as an anticoagulant, and the blood was stored at 4°C until it was evaluated. The local veterinarians were consulted for relevant clinical signs of neurological or visual disorders.

The samples were first screened by the Knott method (Knott 1939). Three drops of 1 per cent saponin solution were added to 2 ml of blood, then (after shaking) 8 ml of distilled water were added, followed by thorough mixing and centrifugation at 500 *g* for five minutes. The sediment was removed with a 1 ml Pasteur pipette and examined with a light microscope at × 125 magnification for the presence of microfilariae. If positive, the material was washed into a 1·5 ml Eppendorf tube, and 10 smears were prepared from aliquots of the sediment, fixed with methanol, and stained with Giemsa for the identification of the larvae on the basis of their size and morphology. The number of microfilariae was counted in the Knott sediment.

DNA was extracted from 100 µl of the positive blood samples using a commercial kit (QIAamp DNA blood; QIAGEN). PCR reactions for the amplification of a portion of the 12S rDNA were performed using the general filarial primers 12SF and 12SR, according to the thermal profile described by Casiraghi and others (2004). These primers were designed on the basis of regions of 12S rDNA that are conserved among the nematode species *Onchocerca volvulus*, *Ascaris suum* and *Caenorhabditis elegans*, whose complete mitochondrial genome sequences are available in the databases (accession numbers: NC001861.1, NC001327.1 and U80438/CELT19B4, respectively). The PCR products were gel-purified and sequenced directly with an automated sequencer (ABI PRISM 310 Analyser; Applera Corporation). The sequences obtained were compared with those deposited in sequence databases by using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.nih.gov/BLAST).

Exact confidence intervals (CI) for the prevalence values were calculated by Sterne's method. The data were compared by using Fisher's exact test, and the mean microfilarial counts were compared by student's *t* test. Differences were regarded as significant when P was 0·05 or less.

RESULTS

Eighteen of the 195 horses (9·2 per cent; 95 per cent CI 5·6 to 14·2 per cent) had microfilariae in their blood. The length of the larvae ranged from 240 to 290 µm; the mean (sd) length of 100 individuals was 261·0 (11·7) µm. Although their sheath was not consistently visible (particularly in smears), they contained a more or less visible inner body. On the basis of these morphological features, which were characteristic of the larvae from all the infected horses, *S equina* was suspected. This diagnosis was confirmed by molecular tech-

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TABLE 1: Proximity to still water, age, sex, approximate time of sampling, numbers of microfilariae and PCR results for the 18 horses infected with *Setaria equina*

Proximity to still water	Approximate time of sampling	Number of microfilariae in 2 ml blood	PCR result from 100 µl blood	Age (years)	Sex
Within 1 km of a small pond	10.00	40	Negative	2	F
Within 1 km of a small pond	10.00	1138	Positive	5	F
Within 1 km of a small pond	10.00	6	Positive	19	F
Within 1 km of a small pond	10.00	41	Positive	9	F
Within 1 km of a small pond	16.00	121	Positive	1	M
Within 1 km of a small pond	17.00	158	Positive	4	M
Within 1 km of a small pond	13.00	1105	Positive	3	M
Within 1 km of a small pond	13.00	11	Negative	3	F
Within 1 km of a small pond	13.00	788	Positive	3	M
Within 1 km of a small pond	13.00	4	Negative	4	M
Within 1 km of a small pond	13.00	18	Negative	5	M
Within 1 km of a small pond	13.00	24	Negative	8	F
Within 1 km of Lake Balaton	10.00	1	Negative	7	M
Within 1 km of Lake Balaton	12.00	43	Negative	7	F
Up to 25 km from Lake Balaton	13.00	52	Positive	3	F
Up to 25 km from Lake Balaton	13.00	4	Negative	6	F
Up to 25 km from Lake Balaton	13.00	38	Positive	3	F
Up to 25 km from Lake Balaton	13.00	17	Positive	3	F

M Male, F Female

niques; *S equina* was identified in each of the 10 PCR-positive samples by sequencing.

The data for the infected animals are given in Table 1. The number of larvae in 2 ml blood ranged from one to 1138. The numbers of microfilariae were not correlated significantly with the age or sex of the horses, the time of sampling or the type of place where they were kept. The eight infected horses that were PCR-negative had small numbers of microfilariae in 2 ml of blood (mean 18·1 [16·4]) but the mean number was not significantly different from the mean number in the blood of the PCR-positive horses. Relatively more stallions (11·9 per cent: seven of 59) were infected than mares (8·1 per cent: 11 of 136) but this association was also not significant.

The percentage of infected horses decreased with age (Fig 1). None of the horses had visual disorders or neurological signs attributable to setariosis.

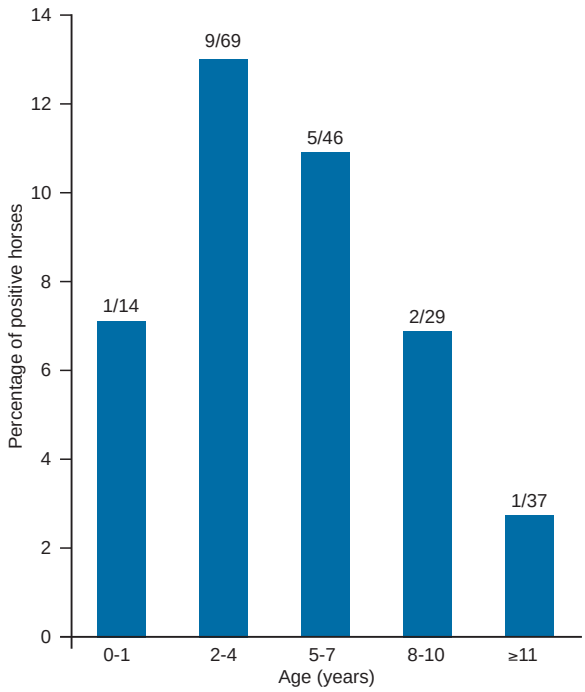

FIG 1: Numbers of horses of different ages that were infected with *Setaria equina* as a percentage of those in each age group that were sampled

Positive samples were collected from eight places in the country. The prevalence of setariosis was 17·6 per cent (95 per cent CI 9·5 to 28·8 per cent) in areas with nearby ponds, 11·1 per cent (95 per cent CI 1·4 to 34·7 per cent) in areas close to Lake Balaton and 9·5 per cent (95 per cent CI 2·7 to 22·6 per cent) in areas 25 km from Lake Balaton. No infected horses were identified in areas that were further away from still waters, with only rivers or streams in their proximity. Fourteen of 86 (16·3 per cent) of the horses that were kept closer than 1 km to ponds or Lake Balaton were infected, whereas only four of 109 (3·7 per cent) of the horses that were kept further from lakes or ponds were infected, indicating that there was a significant association ($P<0·005$) between setariosis and still waters.

DISCUSSION

This is the first study of the prevalence of *S equina* infection in horses in Hungary. In recent decades no data have been published in peer-reviewed journals on the prevalence of setariosis, in Europe, judged in terms of microfilaraemia, but the 9·2 per cent prevalence reported here appears to be high. Considering that the presence of adult worms in the peritoneal cavity is not always accompanied by microfilaraemia (Bezubik and Furmaga 1964, Coleman and others 1985, Oge and others 2003), the prevalence of the infection may be higher.

The results confirm that the Knott method is a sensitive technique for routine diagnostic investigations. On the other hand, when applying PCR, animals with a low level of microfilaraemia (less than 50 larvae in 2 ml of blood) may be overlooked, as occurred in the present study. To increase the sensitivity the amount of blood from which DNA is extracted should be increased to 200 µl or more. The problem is partly due to the uneven distribution or clumping of the larvae, even in thoroughly mixed specimens (S. Hornok, personal observations). At the same time the release of DNA from dead microfilariae could not be justified, as postulated by Wijesundera and others (1999), who obtained positive results for *S digitata* by PCR in samples of 50 µl of fresh blood that were negative by microscopical analysis.

Two of the horses had more than 1000 microfilariae in 2 ml of blood, a higher level of microfilaraemia than reported earlier (Coleman and others 1985, Oge and others 2003). Periodicity of the first stage larvae in the peripheral blood has been demonstrated in several filarial nematode species (Kassai 1999), but the phenomenon could not be demonstrated for *S equina* (Coleman and others 1985). In the present study the lack of correlation between the time of sampling and the detection of microfilaraemia is consistent with an absence of periodicity. The lack of a significant difference between the prevalence of the infection among the stallions and mares also supports previous findings (Oge and others 2003). The fact that the percentage of infected horses decreased with age is consistent with observations on the age-related resistance to setariosis (Coleman and others 1985).

Although the potential vectors, mosquitoes of the genera *Aedes* or *Culex*, are ubiquitous in Hungary (Mihályi 1955), their density and therefore the chances for transmission would be expected to be higher in the proximity of the most favoured habitats for larval development, that is, still waters, as observed with other *Setaria* species (Panaitescu and others 1999). In accordance with this likelihood, most of the horses infected with *S equina* were associated either with Hungary's largest lake or (more particularly) with smaller, nearby ponds.

In general, setariosis should be regarded as a benign, harmless parasitosis. Even high levels of microfilaraemia can

be well tolerated, as evidenced by the absence of relevant clinical signs in the infected horses. On the other hand, the condition may increase the risk of pathological changes due to the invasion of the central nervous system or the eye by microfilariae or inoculated third stage larvae (Jirina 1959, Orlov 1961, Frauenfelder and others 1980). Therefore, monitoring the factors that promote the transmission of the parasite by the vectors (especially the density of microfilariae in the peripheral blood and the availability of still water, which may increase the abundance of the vectors) may be important in all potentially affected areas.

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References

ABU EL-MAGD, M. M. & AHMED, Z. G. (1994) The occurrence of *Setaria equina* in donkeys' eyes and their treatment. *Assiut Veterinary Medical Journal* **31,** 86-90

BEZUBIK, B. & FURMAGA, S. (1964) Some observations on *Setaria equina* (Abildgaard, 1789). *Acta Parasitologica Polonica* **12,** 1-5

BUCHWALDER, R. & SCHUSTER, R. (1989) Finding *Setaria equina* in horses. *Angewandte Parasitologie* **30,** 127-130

CASIRAGHI, M., BAIN, O., GUERRIERO, R., MARTIN, C., POCACQUA, V., GARDNER, S. L., FRANCESCHI, A. & BANDI, C. (2004) Mapping the presence of *Wolbachia pipientis* on the phylogeny of filarial nematodes: evidence for symbiont loss during evolution. *International Journal for Parasitology* **34,** 191-203

COLEMAN, S. U., KLEI, T. R. & FRENCH, D. D. (1985) Prevalence of *Setaria equina* (Nematode: Onchocercidae) in Southeastern Louisiana horses. *Journal of Parasitology* **71,** 512-513

FRAUENFELDER, H. C., KAZACOS, K. R. & LICHTENFELS, J. R. (1980) Cerebrospinal nematodiasis caused by a filariid in a horse. *Journal of the American Veterinary Medical Association* **177,** 359-362

HILLYER, L., COLES, G. & RANDLE, T. (2001) *Setaria equina* in the UK. *Veterinary Record* **149,** 464

JIRINA, K. (1959) Über das Vorkommen der Setarien bei Pferden. *Deutsche Tierarztliche Wochenschrift* **66,** 439-441

KASSAI, T. (1999) Filarioidoses of animals. In Veterinary Helminthology. 1st edn. Oxford, Reed Educational and Professional Publishing. pp 119-126

KLEIN, J. B. & STODDARD, E. D. (1977) *Dirofilaria immitis* recovered from a horse. *Journal of the American Veterinary Medical Association* **171,** 354-355

KNOTT, J. (1939) A method for making microfilarial surveys on day blood. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **33,** 191-196

MIHÁLYI, F. (1955) Igazi szúnyogok – Culicidae. *Fauna Hungariae* **14,** 1-40

NELSON, G. S. (1959) The identification of infective filarial larvae in mosquitoes: with a note on the species found in wild mosquitoes on the Kenya Coast. *Journal of Helminthology* **33,** 233-256

OGE, S., OGE, H., YILDIRIM, A. & KIRCALI, F. (2003) *Setaria equina* infection of Turkish equines: estimates of prevalence based on necropsy and the detection of microfilaraemia. *Annals of Tropical Medicine and Parasitology* **97,** 403-409

ORLOV, A. I. (1961) Microfilariae of *Setaria* in the central nervous system of horses. *Helminthological Abstracts* **32,** 229

PANAITESCU, D., PREDA, A., BAIN, O. & VASILE-BUGARIN, A. C. (1999) Four cases of human filariosis due to *Setaria labiatopapillosa* found in Bucharest, Romania. *Romanian Archives of Microbiology and Immunology* **58,** 203-207

RICCI, M. & SABATINI, A. (1992) Parasitic helminths of the caecum and colon of equidae in Italy. *Parassitologia* **34,** 53-60

SOTIRAKI, S. T., BADOUVAS, A. G. & HIMONAS, C. A. (1997) A survey on the prevalence of internal parasites of equines in Macedonia and Thessalia. Greece. *Journal of Equine Veterinary Science* **17,** 550-552

SUPPERER, R. (1953) Filariosen der Pferde in Österreich. *Wiener Tierarztliche Monatsschrift* **40,** 193-220

THURMAN, J. D., JOHNSON, B. J. & LICHTENFELS, J. R. (1984) Dirofilariasis with arteriosclerosis in a horse. *Journal of the American Veterinary Medical Association* **185,** 532-533

WIJESUNDERA, W. S. S., CHANDRESEKHARAN, N. V. & KARUNANAYAKE, E. H. (1999) A sensitive polymerase chain reaction based assay for the detection of *Setaria digitata*: the causative organism of cerebrospinal nematodiasis in goats, sheep and horses. *Veterinary Parasitology* **81,** 225-233

YOUSIF, Y. A., HAYATEE, Z. G., SALEEM, A. N. & JOSHI, H. C. (1990) Haemato-biochemical changes in microfilariae affected horses under field conditions. *Journal of Veterinary Parasitology* **4,** 55-58