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BABESIA DIVERGENS BECOMING EXTINCT IN CATTLE OF NORTHEAST HUNGARY: NEW DATA ON THE PAST AND PRESENT SITUATION

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Previously unpublished data from 1958 to 1967 attest the occurrence of *Babesia divergens* in cattle in several endemic foci of Northeast Hungary. During that period the number of clinical cases showed fluctuation with intervals of 4–5 years and monophasic seasonality (peaking in June). In order to assess the current status of bovine babesiosis in that region, blood samples were collected from 654 cattle on 44 farms of 36 settlements in or near the endemic area during 2005, and serum levels of IgG antibodies to *B. divergens* were measured by indirect fluorescent antibody test (IFAT). Only 2 samples (0.3%) showed positivity. In one village clinical babesiosis was observed over the past few years. Animals brought into the endemic area during the spring developed haemoglobinuria in the summer of the same year, but those introduced during the summer or autumn showed clinical signs only after two years. Sampled animals born and raised locally had neither haemoglobinuria nor seroconversion. Reduction in the number of cases during the past decades may have been influenced by the availability of hosts (i.e. decrease of cattle breeding) and the activity of vectors associated with climate-related changes (e.g. increase of annual sunlight hours in the endemic area). This is the first report on the prevalence of antibodies to *B. divergens* in cattle in Hungary.

Key words: *Babesia divergens,* prevalence, seasonality, haemoglobinuria, climate changes

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Babesia divergens is a small piroplasm, infecting red blood cells and transmitted by the common tick, *Ixodes ricinus*. Its primary hosts are cattle. Due to its pathogenicity and widespread occurrence, *B. divergens* is the most important agent in the aetiology of bovine babesiosis in Europe. The significance of this species is further substantiated by its zoonotic potential, causing infections in humans, particularly in splenectomised individuals (for review see Zintl et al., 2003).

Regarding its geographical distribution on the continent (Mainland Europe), infection of animals with *B. divergens* was reported from France, Belgium, the Netherlands, Scandinavia, Germany, Switzerland and Austria. Several later accounts from these countries describe only sporadic infections or (seasonal) outbreaks, suggesting that the epidemiology might have changed over the past years (Zintl et al., 2003).

Bovine piroplasmosis used to be endemic in Northeast Hungary (Bernard, 1963). Although some cases were attributed to *B. bigemina* (Kotlán et al., 1959), *B. divergens* remained the most important causative agent. However, during the past 40 years cattle breeding in Hungary suffered enormous losses as the number of animals decreased to one third of the original (FAOSTAT database, 2004). It is also likely that tick species have undergone shifts in their proportion and habitat density (Sréter et al., 2005).

Since previously unpublished data have become available from the period of 1958–1967 on the occurrence of *B. divergens* in Northeast Hungary, the aim of the present study was twofold. First, to assess the prevalence of antibodies to this piroplasm in relevant regions. Second, to compare these results with the past situation in order to evaluate the impact of the above changes on the endemicity of bovine babesiosis during the past decades.

Materials and methods

B. divergens infections in the period of 1958–1967, reported here for the first time, were recognised on the basis of clinical signs and verified in blood smears during parasitaemia.

In the summer of 2005 blood samples were collected on 44 farms of 36 settlements from 654 beef and dairy cattle (8 bulls and 646 cows) kept under extensive conditions and belonging to 12 breeds. Data of animals regarding their age, sex, breed and keeping place, as well as relevant clinical signs (mainly haemoglobinuria) in the past years were recorded. Serum was obtained by centrifugation not more than two days after bleeding, then the samples were stored at –20 °C until evaluation.

Sera were tested for the presence and level of IgG antibodies against *B. divergens* by indirect fluorescent antibody test (IFAT) as described elsewhere

(Edelhofer, 1995). All sera were screened at dilutions of 1:8 and 1:16 in PBS. Fluorescein-isothiocyanate- (FITC-) labelled goat anti-bovine IgG (heavy and light chains) immunoglobulins (Jackson Immunoresearch Laboratories Inc., USA) were used as conjugate, at a dilution of 1:100 in PBS. Serum samples showing fluorescence at the dilution of 1:16 (cut-off value) were further titrated using 2-fold serial dilutions. Sera from cattle naturally infected with *B. divergens* were used as positive controls.

Exact confidence intervals for the prevalence rate were calculated according to Sterne's method.

Results

Past situation

B. divergens infections used to be endemic in the northern part of Borsod county, where 283 cases occurred in 21 villages between 1958 and 1967 (Fig. 1). The number of affected cattle showed yearly fluctuation, with peaks in 1958, 1962 and 1967 (Fig. 2). A clear seasonality was observed, as most cases occurred between May and September, culminating in June. Bovine piroplasmosis was also diagnosed in at least 3 regions of Nógrád county from 1963 onwards (Fig. 1).

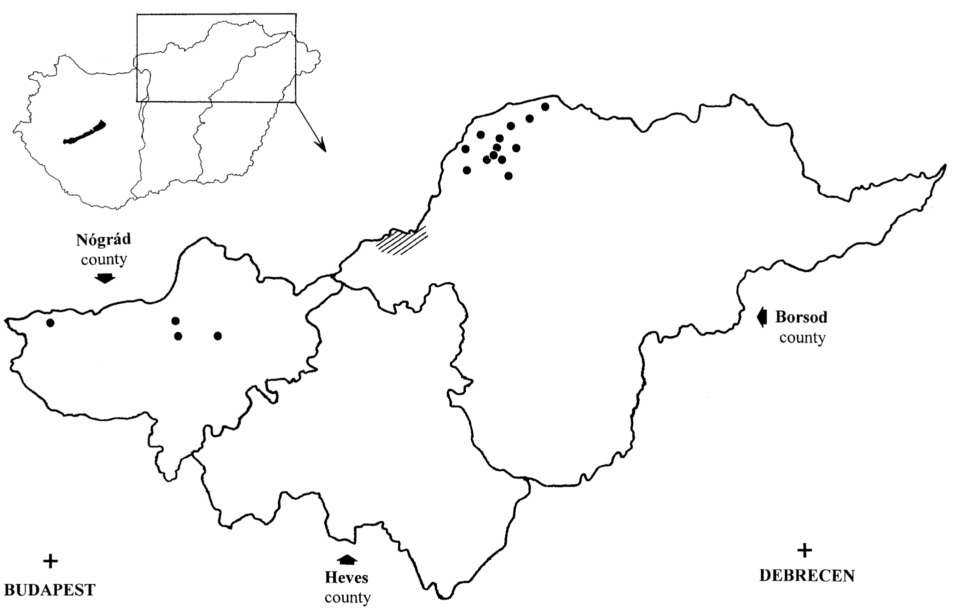

Fig. 1. Places where babesiosis was diagnosed in Nógrád county from 1963 onwards, and in Borsod county from 1958 to 1967. In the northern region of the latter only those 13 villages are shown, where the number of cases exceeded 10 during that decade (an endemic area with sporadic cases is shaded)

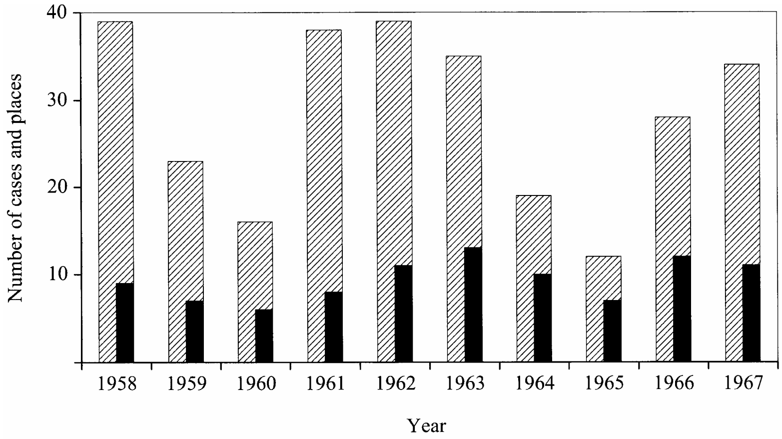

Fig. 2. Yearly changes in the number of cases with *B. divergens* between 1958 and 1967 in northern Borsod county (shaded columns) and the number of affected villages (black columns)

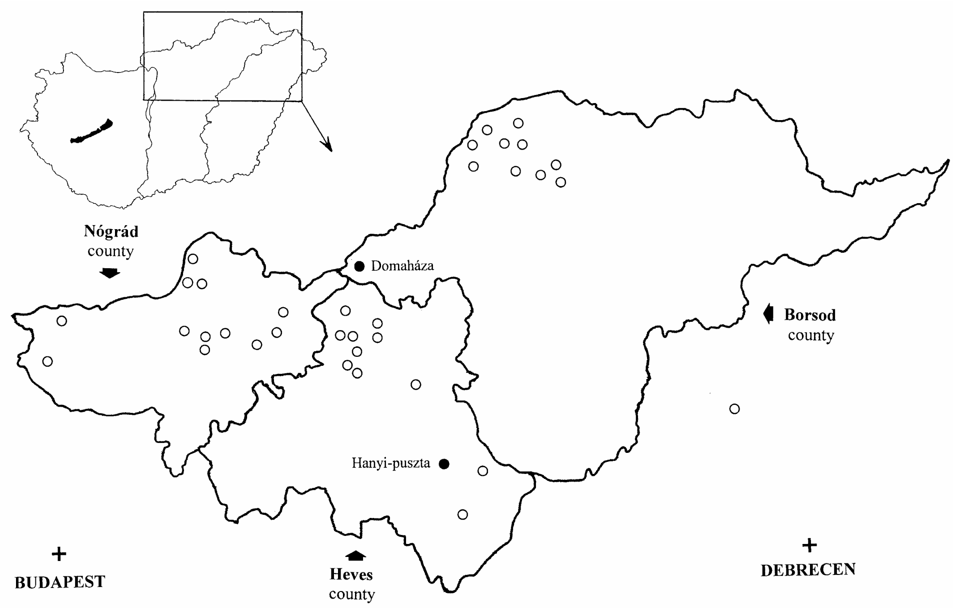

Fig. 3. Places of sampling (open dots) and location of seropositive cattle (filled dots) in 2005

Present situation

Of the 654 cattle tested in this survey only 2 had antibodies to *B. divergens* (0.3%; 95% confidence interval: 0.04–1.1%). The age of cattle with seroconversion (7.6 and 8.6 years) was higher than the mean age of seronegative animals (6 years). Both animals were females and belonged to the same breed (Hungarian Pied). One cow had a titre of 1:512 without clinical signs. This animal was kept in the past 1.5 years on a large farm (Hanyi-puszta, Heves county) south of the endemic area (Fig. 3), and prior to that she had lived 50 km to the west in the same county. In her current herd no haemoglobinuria was observed in the previous years.

However, in Domaháza (Fig. 3), a village in the neighbouring Borsod county, the history of haemoglobinuria was reported over the past few years on 4 of the 9 small farms, where household cattle were sampled. Depending on whether these animals were brought into the endemic area during the spring or later, they developed clinical signs either in the summer of the same year or only after two years (Table 1). Only one of them had haemoglobinuria this year, and was found seropositive with a titre of 1:64 in the IFAT 18 days after the onset of clinical signs, and 1:1024 two weeks later. Sampled animals born in that place had neither haemoglobinuria nor seroconversion (Table 1).

In the third, formerly endemic county (Nógrád) no cattle with babesiosis could be identified.

Table 1

Data of animals on 4 household farms in the endemic area (Domaháza, western Borsod county)

Registration number of place/cattle	Age of cattle (years)	Born, raised locally	Brought here: time of arrival	History of summer haemoglobinuria	Serological status for *B. divergens* in 2005
1.1	8.3	–	April 2003	in 2003	–
2.1	9	yes	–	–	–
2.2	7.4	yes	–	–	–
2.3	8	–	March 2003	in 2003	–
3.1	0.9	yes	–	–	–
3.2	0.9	yes	–	–	–
3.3	8.6	–	June 2003	in 2005	+
3.4	10.2	–	August 2002	in 2004	–
3.5	11.9	–	September 2002	in 2004	–
4.1–4.4	Unknown	–	March 2003	in 2003	Not tested

Discussion

This is the first report on the prevalence of *B. divergens* infections in Northeast Hungary. The occurrence of this piroplasm in any region depends on two factors: the habitat availability for the vector, *Ixodes ricinus*, and its capability to become infected and to transmit the babesiae in the next generation. Since *I. ricinus* had been widespread in Hungary in the 1950s (Janisch, 1959), the second factor seems to be more important in the maintenance of endemicity. Regarding its past geographical distribution, bovine babesiosis was diagnosed along the Slovakian border, possibly as the southernmost part of a larger endemic area to the north. A few of these cases were reportedly caused by *B. bigemina* (Kotlán et al., 1959). However, the possibility of natural infections of cattle with this large piroplasm (and the history of its indigenous status) in Hungary is questionable: the assumed vector role of *Haemaphysalis punctata* occurring in the country could not be proved experimentally as transmission trials were not successful (Yin et al., 1996). On the other hand, *Boophilus* spp. and *Rhipicephalus bursa*, the true vectors of *B. bigemina*, have not been identified in Hungary. Therefore, these infections were most likely caused by *B. major*, transmitted by *H. punctata*.

Yearly changes in the number of cases of bovine babesiosis between 1958 and 1967 may have been influenced by the amount of rainfall, as the life cycle of *Ixodes* spp. is largely dependent on moisture (Loye and Lane, 1988). For instance, a peak rate of *B. divergens* infections was diagnosed in 1958 which was one of the rainiest years in Hungary (data from the Hungarian Meteorological Institute). On the other hand, seasonality was previously described by Bernard (1963), corresponding to vector tick activity (high spring, low autumn peak). Annual changes in the number of seropositive animals and their level of antibodies to *B. divergens* has also been reported by others (Gern et al., 1988; L'Hostis et al., 1997).

Only those beef and dairy cattle were included in the present survey which were kept extensively, grazing in potential tick habitats. Nevertheless, stabled animals may also be at risk of babesiosis on account of tick-contaminated forest plants in their feed (Szotáczky, 1962, unpublished observation). The current 0.3% prevalence of antibodies to *B. divergens* in Northeast Hungary is low compared to other countries. For instance, in Germany the rate of infection was 1.3–5% on farms with a previous history of babesiosis (Niepold, 1990).

The two cows with seroconversion in this study were advanced in their age, in accordance with the well-documented inverse age resistance to babesiosis (Christensson, 1989). Seropositivity detected in the first cow staying permanently outside the endemic region (Heves county) may have resulted from an incidental import of infected tick stage(s) into the place of its keeping. Regarding those cattle which were sampled during the present survey and showed typical clinical signs of babesiosis in previous years (Borsod county), the causative role of *B. divergens* is strongly suspected, since these animals lived in the endemic area, their haemoglobinuria occurred seasonally and responded well to treatment with imidocarb.

Calves born and raised locally may have encountered *B. divergens* during the first 9 months of their life, when – presumably due to the transfer of maternal immunity and stimulation by babesial antigens – they are naturally resistant to the disease (Gern et al., 1988). In this way they could also develop immunity to subsequent homologous challenge infections, as indicated by the lack of clinical signs in local cattle which grazed the same area as their haemoglobinuric companions. On the other hand, calves raised in a non-endemic area and later exposed to *B. divergens* usually exhibit symptoms and/or increase of antibody levels, as was observed in the case of newly purchased cows within 3 months after their introduction into a new herd (L'Hostis et al., 1997). Similarly, animals of this study brought into the endemic area in the spring developed acute babesiosis, whereas those arriving later consistently had clinical signs after two years, either due to a transient protection elicited by a low rate of infection or they just did not encounter infected ticks during that time. Regarding the latter, the manifestation of the disease depends on the availability and vector capacity of ticks in yet another way, because only adults of *I. ricinus* can take up *B. divergens* developmental stages (Donnelly and Peirce, 1975), and although larvae and nymphs of the next generation can inoculate them, these younger stages do not normally suck blood on cattle (Milne, 1947). Taking into account that the life cycle of the ticks can extend to several years, this could also contribute to the absence of symptoms of some animals in certain years.

Since previous haemoglobinuria of recently seronegative cattle was most likely caused by *B. divergens*, detectable levels of antibodies must have been eliminated from their circulation after one year. As reported, seasonal decrease in the percentage of positive animals and loss of positivity can occur in the case of most *B. divergens* infected cattle during a 2-year period (L'Hostis et al., 1997). IFAT titres can even decline within a few months postinfection (Ganse-Dumrath, 1986).

In the light of previous data the current status of bovine babesiosis suggests that it is becoming extinct in Northeast Hungary, since yearly fluctuation in prevalence alone cannot explain such reduction in the number of cases (or complete disappearance) over the past decades in foci of the (formerly) endemic area. This dramatic change must have been triggered by at least two major factors. Cattle population decreased to one third of its original size (FAOSTAT database, 2004), and it is also possible that fewer susceptible animals were imported into the region, thus reducing the chance for infected ticks to find a suitable host for the transmission of this piroplasm within the appropriate time. Simultaneously, shifts in the population density (Sréter et al., 2005) or activity of relevant vectors can be supposed. The effect of weather mediated by the activity of ticks was formerly demonstrated to be the only significant determinant of the epidemiology of *B. divergens* infection in cattle (Donnelly, 1973). According to the same study, disease prevalence correlates well with mean temperatures, and the annual mean temperature in Hungary rose by more than 1 °C over the past 30 years (data from the Hungarian Meteorological Institute). Since all life cycle stages of *I.*

ricinus also depend on the photoperiod and/or sunny hours (Perret et al., 2003), it is noteworthy that the endemic area was formerly (1958–1988) situated in a zone of less than 1900 annual sunlight hours. However, this zone disappeared from Northeast Hungary by 2004 and the region is now experiencing 1900–2000 sunny hours per year (data from the Hungarian Meteorological Institute). The effect of similar changes is further supported by variations in the distribution pattern of other important pathogens transmitted by *I. ricinus* depending on climate-related changes, reported from several countries in the same period. Differences in the endemicity of relevant diseases were seen due to the occurrence of these ticks at higher altitudes (Danielova et al., 2004) and to a northward expansion of their geographical range (Lindgren et al., 2000). Although such observations confirm the tendency of vector-borne diseases to emerge outside the former endemic areas in Europe (Kampen, 2001), the present results indicate that this may also imply a collateral disappearance of the same infections from certain regions.

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