SPONTANEOUS OCCURRENCE OF ANTIBODIES AGAINST PATHOGENIC AMOEBAE OF THE LIMAX GROUP IN DOMESTIC ANIMALS

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Abstract. Sera of 1 218 animals were examined using indirect haemagglutination reaction with antigens of Naegleria fowleri and Acanthamoeba culbersoni. Rabbit sera (214 samples) gave negative reaction with both antigens. Bovine sera (593 samples) reacted positively in 1.5%, particularly with the antigen of A. culbersoni. Out of the 411 pigs examined 12% were positive with one or both of the antigens. In one of the pig farms of a modern type the positivity was as much as 32.2%. The titres of positive animal sera have never been higher than 1 : 320. The amoebae of the limax group do not seem to be involved in the pathogenesis of diseases of cattle and rabbits. A sporadic occurrence of peracute cases is possible. In case of a sudden death, animals with symptoms of encephalitis or pneumonia should be examined for the presence of amoebae. Hypersensitivity should be considered as a possible cause of pig diseases.

The group of small free-living limax amoebae has lately been found to include species with biological abilities enabling them to pass from the usual saprophytic way of living in water and soil to a parasitic life in organs of warmblooded animals and man. For the time being, this ability has been proved in Naegleria fowleri, Acanthamoeba culbersoni and A. polyphaga. N. fowleri is known as the agent of a peracute fatal disease of man — primary amoebic meningoencephalitis. A similar disease with a protracted course may be induced also by amoebae of the genus Acanthamoeba, which have repeatedly been reported also as etiological agent of keratoconjunctivitis.

One of the characteristic peculiarities in the pathogenesis of diseases caused by pathogenic limax amoebae is the port of entry through which the agent enters the host organism. The amoebae are capable of penetrating actively through the nasal mucous membrane into the submucosa and then, owing to its affinity to the nerve tissue, reach the brain basis passing through the processes of olfactory nerves. The primary amoebic meningoencephalitis infection in man therefore occurs after bathing or washing, when the infectious water had been aspirated into nasal cavities.

The pathogenic limax amoebae do not exhibit any host specificity and are capable of inducing fatal infections in all species of laboratory animals under experimental conditions. In some species of economically important animals, the conditions for the penetration of amoebae into nasal mucosa are theoretically very favourable. The grazing animals move with the nostrils close above the earth and the air flow at breathing enables the aspiration of small soil particles. Also in pigs the nostrils are exposed to the infectious agent penetrating from the outer environment. The fodder of stall-fed cattle may also be contaminated by cysts or vegetative stages of amoebae from soil.

Nevertheless, spontaneous infections with amoebae of the limax group have been identified only rarely in free-living and domestic animals. The genus Acanthamoeba was found to cause pneumonia in cattle in the U.S.A. and India (McConnell et al.
1968, Dwivendi 1965), spontaneous acanthamoebiasis in dog was reported by Ayers et al. (1972). However, the cases reported were only occasional and there are no results available of systematic and extensive studies on limax amoebae in animals.

The occurrence of antibodies in animals could indicate a participation of the amoebae of the limax group in the pathogenesis of some of their diseases. Spontaneous CF antibodies against A. culbertsoni were found in monkeys, dogs and cattle by Culbertson (1961) and Culbertson et al. (1959). A specific character of antibody response in guinea pigs and rabbits inoculated with cultures of A. culbertsoni and N. fowleri was demonstrated in previous experiments (Čerňa 1967, 1971).

The extensive studies on the occurrence of spontaneous antibodies against two species of pathogenic amoebae of the limax group were performed in order to ascertain whether the pathogenic limax amoebae may participate significantly in the origin of diseases in economically important animals and whether these animals can serve as a reservoir of infection produced by amoebae of the limax group in man.

MATERIAL AND METHODS

Samples of fresh animal sera were obtained from samples intended for other biochemical or serological examinations and collected by veterinary workers in various large-scale breeding farms. Till the time of examination the sera were deeply frozen.

The basic method used for the examination was the indirect haemagglutination reaction (IHA) with antigens prepared from axenic cultures of Acanthamoeba culbertsoni, strain A1 and Naegleria fowleri, strain Vitek. The preparation of antigens and the reaction with formalinized sheep erythrocytes have been described elsewhere (Čerňa 1977). All sera were examined in the dilutions of 1:40, 1:80, 1:160, 1:320 and 1:640. The IHA reaction was performed in polystyrene Microtiter-System panels with rounded bottom and final total volume of 0.05 ml. Control positive and negative sera were examined parallelly. Only titres of 1:80 and higher were considered a positive result of the reaction.

RESULTS

The serological examinations were carried out in 1218 animals. A survey of the examined material and results are given in Table 1. The number of positive animals was very low in cattle, 1.5% on the average. A majority of animals reacted in the basic titre of 1:80, only with A. culbertsoni antigen. A medium titre of 1:320 was repeatedly detected in a single cow. According to the veterinarian it was a quite healthy animal.

The highest frequency of positive animals occurred with pigs. Of the 411 specimens examined 50 (12.1%) reacted with at least one of the tested antigens in the titres up to 1:160. In contrast to the cattle, most of the positive pigs reacted with N. fowleri antigen and almost half of all positive animals reacted with both antigens. A great majority of pigs originated from a large farm. The results shown in Table 2 are divided into two groups according to the type of the pig farm in which the animals were bred. The first group includes animals from a new, modern hall with air condition, automatic feeding and watering and washing of wastes. Animals of the other group were bred in a pig farm of the old type with a central corridor for the distribution of food and transport of wastes. The differences in the occurrence of positive animals are very significant. In the pig farm of the new type, the number of positive animals was not only four times higher, but more than half of them
Table 1. Results of the IHA test with animal sera

<table>
<thead>
<tr>
<th>Animal</th>
<th>No. of examined animals</th>
<th>Positive with antigen of</th>
<th>Total positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A. culbertsoni</td>
<td>N. fowleri</td>
</tr>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Cattle</td>
<td>593</td>
<td>8 1.3</td>
<td>2</td>
</tr>
<tr>
<td>Pig</td>
<td>411</td>
<td>28 6.8</td>
<td>43</td>
</tr>
<tr>
<td>Rabbit</td>
<td>214</td>
<td>0   0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Results of the IHA test with sera of pigs from different types of pig farms

<table>
<thead>
<tr>
<th>Pig farm — No. of animals examined</th>
<th>Positive with antigen of</th>
<th>Positive with both antigens</th>
<th>Total positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. culbertsoni</td>
<td>N. fowleri</td>
<td></td>
</tr>
<tr>
<td>New type (96 animals)</td>
<td>22 22.9</td>
<td>27 28.1</td>
<td>18 58</td>
</tr>
<tr>
<td>Old type (170 animals)</td>
<td>5  2.9</td>
<td>10  5.8</td>
<td>2  15</td>
</tr>
</tbody>
</table>

reacted with both antigens. The frequency of positive animals in the farm of the old type corresponds to average values obtained in other localities examined.

The sera of rabbits originated from two farms from the vicinity of Prague. None of them reacted with the used antigens even in the lowest titres.

**DISCUSSION**

Our results of serological examinations of domestic animals supply first information on the occurrence of spontaneous antibodies against known pathogenic species of amoebae of the limax group in these animals. The conclusions which can be deduced show that the significance of amoebae in the disease occurrence and productivity of cattle and rabbits is not very high. Of interest is the very low occurrence of specific antibodies in cattle, which must very often come in contact with the vegetative stages and cysts of amoebae from the soil and water. Chronical disease caused by amoebae do not seem to occur in cattle, at least they are not common. However, there is a possibility of peracute infections which might lead to a sudden death of the animals with symptoms of pneumonia or encephalitis. In these cases the diagnosis could be made only after a detailed microscopic examination of the post mortem material. Otherwise it is probable — like in cases of primary amoebic meningoencephalitis in man — that the amoebae will not be found in the histological preparations.

The frequency of positive reactions in pigs corresponds rather to theoretical presumptions. The extremely high percentage of positive animals in the pig farm of the new type may be due to the fact that a large quantity of pure water is used in the modern technology, for example under the floor grate of breeding boxes and in air condition equipment. All surfaces moistened with water provide suitable conditions for the multiplication of amoebae. Both active and passive immunization or even sensibilization of animals can arise from these sources also through aerosols. A similar
phenomenon was observed with persons using air moisteners in households. It was found that pneumoniae occurring in some of these persons had been caused by a hypersensitivity to the antigen from Naegleria amoebae reproducing in the evaporating pans (Edwards et al. 1976). It remains to be solved whether such allergic diseases may occur also in pigs.

The negative reactions in rabbit sera may be explained by the breeding regime of these animals which are fed only by pellets. This considerably reduces the possibility of contact with amoebae on forage plants.

On the basis of the results obtained it may be supposed that the breeding farms of domestic animals cannot play a role in the epidemiology of primary amoebic meningoencephalitis of man.

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