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Early experiences with microsporidia of man and mammals

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Abstract. Comparative data are given of microsporidian infections in insects and mammals. Due to minute size and diffuse distribution in mammals microsporidia were recognized in vertebrates only during search for virus infections. Early infections with *Encephalitozoon* in rearings of laboratory animals contaminated many isolates of other protozoan infections. Old separate descriptions of microsporidia in mice, rabbits or dogs have to be reevaluated with modern methods of mollecular analysis. Old cases of infection of man known now as *Encephalitozoon chagasi* or *Encephalitozoon* sp. Matsubayashi are not belonging to the genus *Encephalitozoon* and should be jointly studied with *Nosema connori* as eventually identical. With actual improvements of methods of diagnostics the net of detection of microsporidia in man should be extended on routine testing of urine, faecal samples, eye and nose biopsies and other materials including vaginal smears. The way of circulation of the infections in the host organisms must be studied.

Microsporidia infest as inapparent pathogens all groups of animals, but are most adapted to insects as hosts. Their role in warmblooded animals and man was underestimated due to their general limited survival at temperatures over 30°C. In insects microsporidian infections are apparent: the infected tissues are white and often hypertrophic. In dissections the spores are easily recognized. In vertebrates the minute spores scattered in large compact tissues were not recognized or mistaken for bacterial or fungal spores in secondary contaminations. Actually with the new interest in microsporidia in man, the experiences with microsporidia in insects can be of help. Therefore with some information on early descriptions of microsporidia from man also some experiences from infections of insects:

The development of microsporidia proceeds (with rare exceptions) in the cytoplasm of host cells. *Enterocytozoon salmonis,* closely related in morphology to *E. bieneusi* in man, is notoriously located in nuclei of kidney cells and lymphoblastosis cells of salmonid fishes. Microsporidia do not have mitochondria and they are adapted to use the metabolism of the host cell. Their metabolites are not antigenic and the parasite is not recognized as alien body by the host. Their speed of development is given by the speed of activity of host tissues. During diapause or hibernation, the development of the parasite is slowed with the activity of host cells. Increased temperatures bring the vegetative cycle of microsporidia in insects to a stop in their final stage, the spore, and are used (heating of eggs or pupae) for sterilization of infections in insects (Weiser 1976a).

The spore is the only stage surviving in the open air and serving to transmission to the new host in contaminated food. In water spores usually live more than one year at 4°C, in infected tissues they survive even longer. Spores are refringent, oval, usually all of the same size and shape. Sporoblasts are usually larger and their measurements together with mature spores bring disputed variability in descriptions of spore size. In Giemsa stained smears, sporoblasts have a visible stained nucleus in their interior, whereas in the mature spores the germ is stained in form of an „icecream cone" with an empty vacuole in the apical end. In ultrathin sections mature spores usually are dark and not differentiated in their interior. Only young spores are differentiated and we find there cross sections of the polar filament. The number of coils in cross section and the location (parallel coils, tilt) is characteristic for the species. Spores activated by their environment produce internal pressure which expells the polar filament which under pressure is hard enough to enter into cells as a hard needle. At the moment of maximum pressure the end of the filament breaks open and the germ is propelled through this tube into the target cell. This is usually the cell in the gut wall (the enterocyte in human infections). In insects some infections remain in the primary midgut epithelium, others cross the basal membrane of the midgut and enter the body cavity where they are phagocytized by lymphocytes. Analogously in human infections macrophages engulf free spores or receive germs by injection from extruded filaments. They do not recognize the parasite and the microsporidian produces schizonts and spores inside the lymphocyte. The migrating lymphocyte spreads the infection. When it sticks in narrow passages in the brain, the host cell changes in a pseudocyst, most common in *Encephalitozoon* (Fig.ld) or *Thelohania apodemi* (Fig. la, b). Lymphocytes burst open during their migration and spores are phagocytized again. There are target tissues where the microsporidia grow to the extent of the tissue. In insects some remain only in the gut wall, others invade silk glands,

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muscles, Malpighian tubules, the fat body or ovaries. In mammals typical development is in the kidneys, the pancreas (including Langerhans islets), the liver, and connective tissues. Normal development in neural ganglia, salivary glands etc. differs from formation of pseudocysts. The polar filament is of different length, the spores of species in man and mammals have rather short filaments of 15 to 50 μm and this is the optimum distance reached from the gut. The signal for activation of the spore and release of polar filament is the change of outer conditions (osmotic pressure, pH, ion content etc.).

Several microsporidia have in sporogony a morphological diversity in producing single binucleate larger spores or minor uninucleate spores in groups of eight. Some microsporidia in mosquitoes (*Amblyospora*, *Parathelohania*) produce visible masses of spores in octosporous pansporoblasts in male larvae, whereas the infection in female larvae remain in a few germs hidden till pupation and hatching of adults. After blood meal the microsporidian starts development, forms morphologically different spores and enters the egg follicles in the ovaries. In most mosquito populations up to 2% females have hidden microsporidian infection transmitted to eggs (Hazard and Weiser 1968). Among other activities of some microsporidia we may mention the induction of different metabolism in insects infected by *Vairimorpha*, where the host remains in larval stages, without pupation and formation of the adult (Weiser 1976a). Microsporidia of the genus *Glugea* in stickleback transform the infected macrophages into xenomas, giant cysts, where the interior is the hypertrophic host cell with abnormally segmented nucleus and in its centre are vegetative stages and spores of the microsporidian (Weiser 1976b).

In the early period of recognition of microsporidia in mammals diagnostics was badly influenced by their minute size. They were recognized first during search for viruses of encephalitis by Levaditi et al. (1924) and during evaluation of the Negri particles in dogs with rabies (Manouelian and Viala 1924). First it was not recognized that repeating findings of *Encephalitozoon* is actually an inapparent infection in laboratory strains of experimental animals and in many cases this infection spoiled also results of interesting studies. So Matsubayashi et al. (1959) tried to maintain the microsporidian from a human pacient in mice but received only an infection confused with *Encephalitozoon*. This situation was also in the Rockefeller Institute till 1962 when Trager (1961) identified the organism in mice for Nelson (1962) as a microsporidian. In Europe almost at the same time another study of mouse microsporidia appeared in Prague (Weiser 1965) and a description of another species, *Thelohania apodemi*, in field rodents in France (Doby et al. 1963). At that time mice produced in different farms had *Encephalitozoon* infections localized in different organs (see Table1).

Table 1. Detailed comparison of distribution of *Ecephalitozoon cuniculi* in laboratory mice, *Nosema chagasi* in man, and *Thelohania apodemi* in mouse. Early data. (After Weiser 1965) (Compare with Fig. 1.).

	1	2	3	4	5	6	7
Brain	++	+	–	+	++	++	++
Kidney	++	–	–	+	–	–	.
Ascites	+	++	++	++	.	.	.
Fat body	.	++	+	–	.	++	.
Heart	–	++	–	–	.	++	.
Muscle	–	+	–	–	.	++	+
Omentum	.	++	++	+	.	.	.
Pancreas	–	–	–	++	.	–	.
Salivary gland	–	–	–	–	++	.	.
Adrenal gland	–	–	–	+	.	.	.
Liver	+	++	++	+	.	–	.
Spleen	–	++	++	+	.	–	.
Lung	–	–	–	+	.	–	.
Connective tissue	.	+	++	++	+	.	.
	Mouse				Dog	Man	Mouse

Explanations: 1 - *Encephalitozoon cuniculi* Levaditi, 2 - *E. cuniculi* Weiser, 3 - *E. cuniculi* Nelson, 4 - *E. cuniculi* Innes, 5 - *E. cuniculi rabiei*, 6 - *Nosema chagasi*, 7 - *Thelohania apodemi*.
. = not inspected, – = inspected, negative, + = positive, ++ = frequent.

From a human host the first reported microsporidian was *Encephalitozoon* (*Nosema*) *chagasi* (Torres 1927). Its spores were larger in size and were distributed in different tissues in the human host. They could be identical with the material of Matsubayashi et al. (1959) and with *Nosema connori* (Fig.1c) reported from a human patient by Margileth et al. (1973). Starting with the dissertation of Petri (1969), who found *E. cuniculi* in routine cell cultures of the Yoshida's sarcoma in Denmark, reports from laboratory and domestic animals were more common, including the studies of Vávra's group in Prague.

With the use of immunosuppressive therapies during tissue transplants and with numerous immunodeficient patients in the AIDS epidemy, the reports on microsporidia in man are numerous. The immunological and molecular biological characters brought another quality into identifications of strains and species affecting man and animals, and we should now try to regroup early data from the times when identification in the actual manner was not possible. First of all we may find all ways of the distribution of individual species of microsporidia in their hosts, especially the identification of tissues where reproduction takes place in the same way as described by

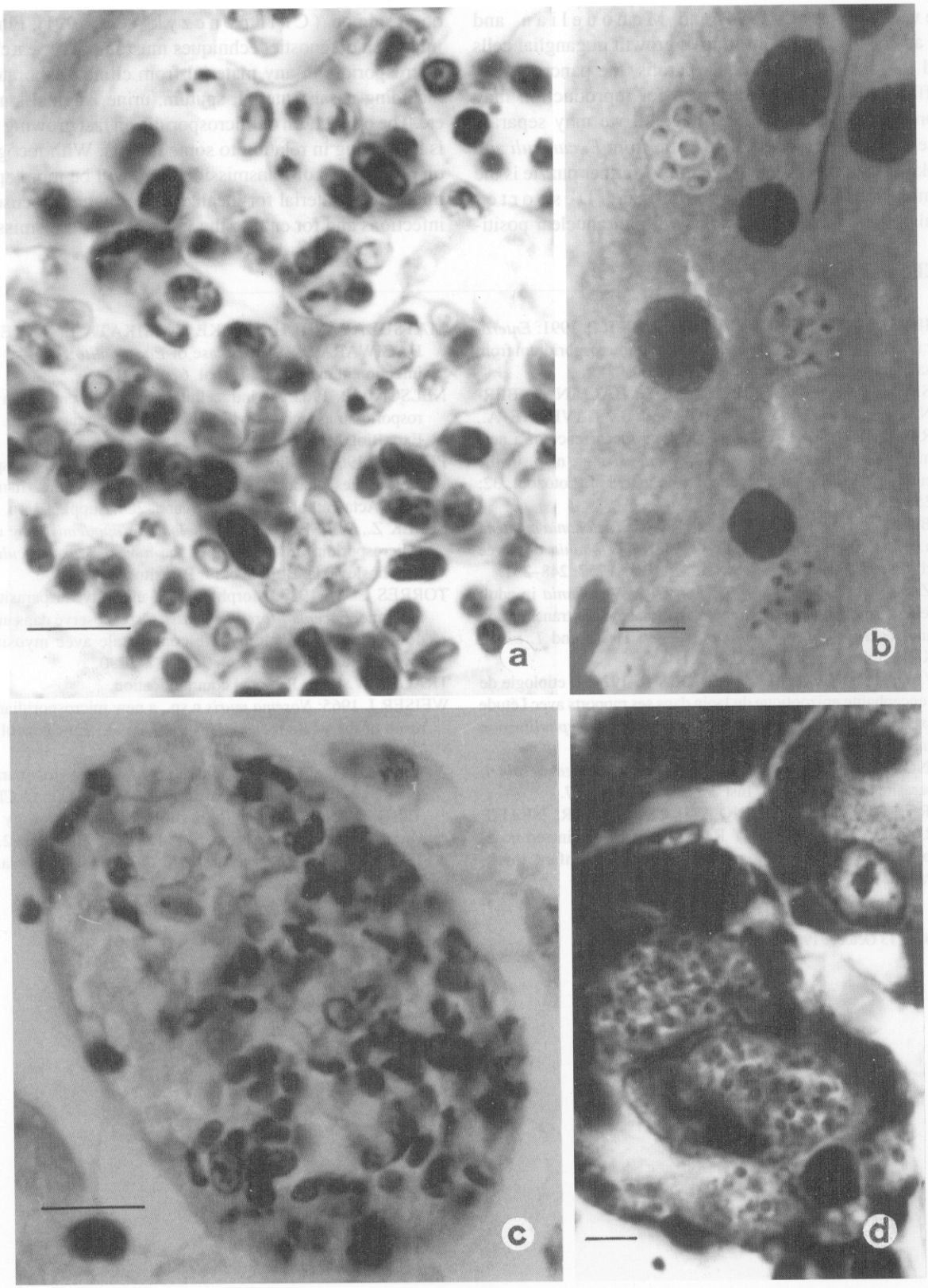

Fig. 1. a - *Thelohania apodemi*, multipansporoblastic pseudocyst. Groups of spores and sporoblasts in pansporoblastic membrane in the brain of *Apodemus sylvaticus*. Bourgbarré, France. **b** - *Thelohania apodemi* octospores and sporoblasts in a smear of the brain. *Mus musculus*, Olomouc, Czech Republic. **c** - *Nosema connori* in a hypertrophic macrophage. Sporoblasts and spores. **d** - *Encephalitozoon cuniculi*, Innes strain in the pancreas of *Mus musculus*. (a - d: bar = 10 µm). (Original micrographs dating from the sixties.)

Levaditi et al. (1924) and Manouelian and Viala (1924) with infiltrative growth in ganglial cells and the tissues like glandula parotis, the pancreas, etc. Differences in selective tissues for reproduction may represent different species. Second, we may separate cases with spores longer than 2 μm from *E. cuniculi* and look for their proper classification. A further puzzle is the comparison of *Enterocytozoon* in man (Desportes et al. 1985) and the *E. salmonis* with intranuclear position in salmon (Chilmonczyk et al. 1991). Finally, improved diagnostic techniques must improve search for microsporidia in any material from clinical laboratories including nasal material, sputum, urine, vaginal smears, etc. The orientation of microsporidia on fast growing cells is interesting in relation to some tumors. With recognition of the ways of transmission there will be more epidemiological material for localization of natural focuses of infections and for cutting the ways of their transmission.

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