

Three important endoparasites of laboratory woodchucks (*Marmota monax*) caught in the wild: *Capillaria hepatica*, *Ackertia marmotae*, and *Taenia crassiceps*

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Summary

Wild animals kept in laboratories are potential carriers of viruses, bacteria and parasites. These might be a risk to people who have contact with those animals. We demonstrate this by the example of the American laboratory woodchuck (*Marmota monax*) which has been kept in our laboratory for 6 years (n=155). Beside *Capillaria hepatica*, the filaria *Ackertia marmotae* and the cestode *Taenia crassiceps* have been found. These three species were recognised outside the routine monitoring for parasites. As *C. hepatica* and *T. crassiceps* are human pathogens, the potential for transmission to humans and other woodchucks is estimated. Precautionary measures such as treatment to eradicate and hygiene instructions are discussed.

Introduction

The daily routine in an animal laboratory is orientated towards protecting animals from infections, which are free from pathogenic germs. A different strategy is required when animals caught in the wild are kept in a laboratory. In principle, they may all be carriers of parasites, which might be dangerous to other animals or humans. But information is normally not available concerning the potential spectrum of parasites of the population, from which the animals have been taken. Therefore, it is difficult to assess the risk to people, when they working with such animals.

Woodchucks (Rodentia, Sciuridae) are often carriers of a particular hepadnavirus (Woodchuck Hepatitis Virus). Because of the similarity to human viral hepatitis, this species is regularly used as an animal model to examine different aspects of hepatitis (Young & Sims 1979, Snyder 1985, Marion 1988, Korba et al. 1989, Lu et al. 1999). The breeding of woodchucks in captivity is very difficult. Purpose-bred animals are unobtainable yet. Therefore nearly all experiments were carried out with

animals caught in the wild. The present study reports three cases of different infections that regularly occurred in our laboratory. Together with a short review of the corresponding literature advices for laboratory animal facilities are given.

Animal keeping

In our laboratory 155 woodchucks (*Marmota monax* Linné, 1758), caught in the wild in the USA (Northeastern Wildlife, South Plymouth) were kept since 1991 (Figure 1). Animals were housed under



Fig. 1 The laboratory Woodchuck (*Marmota monax*).

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standardised laboratory conditions: constant 12L:12D photoperiod, room temperature $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$, humidity $50\% \pm 5\%$. Guinea pig diet were provided ad libitum, together with salad and fruits. Cages (100cm x 65cm x 89cm) were subdivided into two parts (length 50cm each): one filled with straw for shelter, in the other one water and food was available.

Case reports

The infections of woodchucks with *Taenia crassiceps* (Cestoda, Taeniidae), *Capillaria hepatica* (Nematoda, Capillariidae) and *Ackertia marmotae* (Nematoda, Onchocercidae) which will be reported here, were discovered during health control.

Taenia crassiceps (Zeder, 1800). Two animals showed conspicuous swellings: one at the left hind leg, the other at the jaw-bone. Because it was presumed at the beginning that there were tight abscesses, the animals were anaesthetised and investigated. The left hind extremity of the first affected animal showed a clear swelling and was nearly unmoveable. After the skin was cut, an opaque mass was revealed in the subcutis, in which single vesic-

les could be distinguished on closer inspection (Figure 2). It was suspected that the vesicles could be cestodal stages but the large number was confusing. More than thousand of these cysts were found inside the subcutis and also deeper in the muscular system of the thigh. The cysts varied in shape, from round to egg shape (Figure 3) and moved quite quickly when they were alive. Under the microscope, most of the scolices were just recognizable as unclear whitish areas. Some cysts were ready to turn their scolices (S) out and in others budding processes (C) could be observed (Figure 3).

The second animal showed the same symptoms. The location of the infection were the muscles of the left lower jaw-bone and the swelling reached the neck region. Therefore the animal was not able to close the mouth completely. Due to the poor condition of the two animals and the initially unclear diagnosis - it could not be ruled out that the animal keepers might be put in danger at this point - the animals were killed under anaesthesia by an overdose of Ketamin and Xylazin. Material was taken for a later determination, that proved that the cysts were cysticerci of the cestode *Taenia crassiceps*.

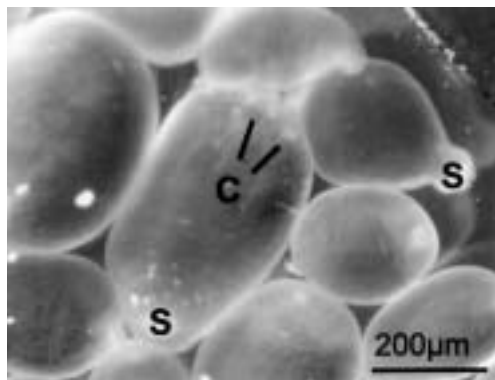


Fig. 2-3 Subcutaneous infection with cysticerci of *Taenia crassiceps*. –

- 2) Opened hind extremity: some hundred cysticerci become evident (overview);
- 3) Cysticerci, detail (S: scolex, C: budding cysts).

Ackertia marmotae (Webster, 1967). - When skin cells of 3 woodchucks were cultivated, many sheathed microfilaria of *Ackertia marmotae* were discovered in cell cultures. Due to their pointed tail ends, they were easy to identify (Figure 4). Together with larval stages, the adults were also observed. The adult nematodes were very mobile and stayed in the culture for several days. Blood smears (Giemsa staining) from the 3 affected animals, revealed *A. marmotae* in only one animal.



Fig. 4 Sheathed microfilaria of *Ackertia marmotae*, the pointed tail end is clearly recognisable.

Capillaria hepatica (Bancroft, 1893). - During the histological examination of a liver biopsy, an infection of the liver parenchyma with *Capillaria hepatica* was diagnosed in three animals (Figure 5). The liver showed a whitish-yellow focus, comprising clusters of eggs. The infection was identified by the characteristic eggs (length 48-62 µm, width 29-

37 µm), which have two pole plugs (Figure 6, P). The affected areas of the liver had become granulo-ma-like, with leuco-lymphocytic infiltration (Figure 5).

Discussion

Parasite biology and human pathogenicity

Taenia crassiceps. - The infection with *T. crassiceps* is caused by the ingestion of the intermediate host (rodents), which carries cysticerci. The prepatent period lasts 31-42 days and the patent period is 2 to 5 years (Mehlhorn *et al.* 1993). Cysticerci can reproduce asexually by shooting at the distant scolex pole (Figure 3), even single cells can turn into complete cysts (Toledo *et al.* 1997). Reproduction in the abdominal cavity has been reported (Chernin & McLaren 1983, Toledo *et al.* 1997). Carnivores like dogs, foxes, cats, and pigs can be infected with *T. crassiceps* as definitive hosts (Blair & Campbell 1976, Rietschel 1981, Kunstjör 1992, Chermette *et al.* 1993, Schuster *et al.* 1993, Alvarez *et al.* 1995). The cysticercosis has been associated with immune suppression (Villa & Kuhn 1996). Human infection has been known and Aids patients appear to be particularly at risk (Klinker *et al.* 1992). Two cases have been reported of cysticerci embedding themselves in the front eye chamber of children (Shea *et al.* 1973, Arocker-Mettinger *et al.* 1992). One of these infections was transmitted by a pet dog (Arocker-Mettinger *et al.* 1992).

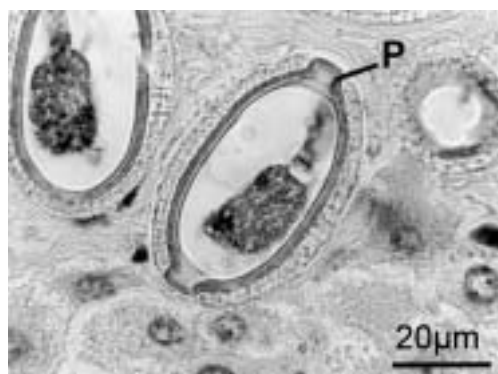
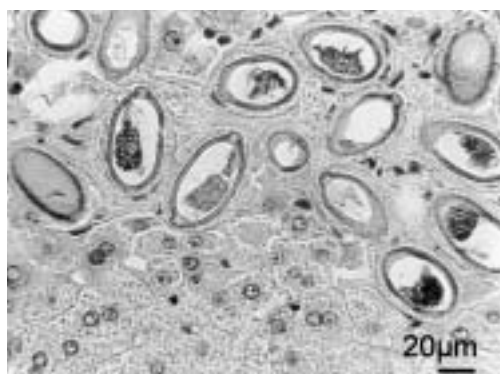


Fig. 5-6 *Capillaria hepatica*. - 5) Affected liver parenchyma, overview; 6) Length section of eggs (P: pole plug)

Capillaria hepatica. - The life cycle of *C. hepatica* has been well investigated (Müller *et al.* 1990). The adult stages live in the liver parenchyma directly under the surface of the organ and the eggs remain there too for the entire life of the host (Mehlhorn *et al.* 1993). As a rule, rodents, especially rats and mice are the most important hosts of *C. hepatica* (Farhang-Azad 1977a, Kunstjör 1992, Ruempler 1995). In the case of rats, cannibalism has been described as the most important method of transmission (Farhang-Azad 1977b). However, a far larger spectrum of hosts exists as monotremata (2 species), marsupials (67 species), rabbits, cats, dogs, foxes and primates are also affected (Kunstjör 1992, Singleton *et al.* 1991, Mehlhorn *et al.*, 1993). Infections with deadly courses have also been reported in primates and humans (Kunstjör 1992, Gonzales Baranco *et al.* 1996, El Nassery *et al.* 1996).

Ackertia marmotae. - The incubation period of *A. marmotae* lasts for several months, the prepatent period 18 months and the patent period lasts some years. According to Cohn *et al.* (1986), 92% of woodchucks captured in the wild are infected by *A. marmotae*. When kept under laboratory conditions, the number of infected animals was reduced continuously even without treatment. However, after 39 months, 53 % of the animals were still affected. The development of microfilariae to the third larval stage stops in the laboratory, because their intermediate host (*Ixodes cooki*), is not present (Ko 1972, Cohn *et al.* 1986). For this reason, animals born in the laboratory show no infection. There have been reports (Mehlhorn *et al.*, 1993) that filaria cause hyalinized hardening areas in affected zones, oedema, fistula, general condition disorders, shaggy fur, emaciation, visual impairment (due to microfilaria entering the eyes). Slight infection is often symptomless. The effects of *A. marmotae* on the host are unknown.

Significance for Animal Laboratories

Until now just a few authors (*e. g.* Albert *et al.* 1972, Snyder 1985, Cohn *et al.* 1986) have investigated woodchuck parasites and human pathogenic parasites

have only been reported in a few cases. Therefore personnel who are in contact with these animals and their organs are often unaware of the health risks. However, our case report shows that when animals captured in the wild are used, great care should be taken. The probability of human infection may be small, but the consequences are considerable if infected. The findings can also be applied to other animals captured in the wild. Two of the parasites which have been proven here to infect woodchucks (*C. hepatica*, *T. crassiceps*) can also be brought into the laboratory by other rodents. The woodchuck which was infected by *T. crassiceps* had already been kept in the Central Animal Laboratory for 11 months. Ruempler (1995) stated that treatment against the cysticerci of *Taenia* is not possible. Campbell & Blair (1974) report, on the other hand that cyst stages are sensitive to thiabendazole. However, for safety reasons it seems appropriate to kill the affected woodchuck, as recidive could appear (Chermette *et al.* 1993). Because in the case of *T. crassiceps*, transmission can also occur without an intermediate host, this cestode represents a potential risk to other woodchucks (cannibalism) as well as to humans. Since even isolated cells can lead to infection (Toledo *et al.* 1997) great caution should be exercised, particularly when handling organ material. Technical or scientific employees, who work with liver cells of woodchucks can also be infected by *C. hepatica*, if they contravene rules of hygiene. On the other hand, the danger that woodchucks could infect each other is considered remote (cannibalism). However, as the eggs remain in the host life long, if not treated, the danger of infection is potentially present for years.

Consequences

Obviously, not all the infected animals are detected by routine examination. This is why staff are ordered to observe strict hygiene rules. Newly delivered woodchucks are kept in quarantine in the Central Animal Laboratory Essen for three months before being used in experiments. Because many woodchucks are frequently infected with *A. marmotae*

(compare Cohn *et al.*, 1986), treatment is imperative. According to several authors, ivermectin is suitable for the treatment of *A. marmotae* as well as *C. hepatica* (Mehlhorn *et al.* 1993, El Nassery *et al.* 1996, Kirkpatrick & Nelson 1987). Therefore all newly delivered animals are preventively treated with ivermectin (ivomec®, MSD AGVET, 0.2 mg/kg body mass). In addition, the introduction of *A. marmotae* into the laboratory is stopped by using an ectoparasiticum (for example Fipronil [Frontline®, Merial]) to fight the intermediate host. Additionally, woodchucks are given praziquantel (Droncit®, 1 x 5 mg/kg body mass, s.c.) to prevent cestodal infection. However, it must be assumed that not all parasital stages can be successfully defeated by these treatments. This is why the measures of hygiene include wearing coats, gloves and face masks. In order to recognise illness as early as possible, our animals are regularly weighed and examined.

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