INFECTIVITY OF *TRICHINELLA* SPP. RECOVERED FROM DECAYING MOUSE AND FOX MUSCLE TISSUE

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Summary :

The tolerance to degradation processes in meat of nine *Trichinella* genotypes was studied in mouse and fox tissue, respectively. Minced muscle tissue with *Trichinella* larvae of different age was stored at room temperature at 100 % relative humidity. During storage weekly sub samples of the minced meat were digested and released larvae were inoculated in mice to evaluate the Reproductive Capacity Index (RCI). The RCI decreased with the length of storage, but the larvae from older infections appeared better adapted to tolerate the degradation processes. The African species *T. nelsoni* had a relative higher tolerance to elevated temperature during storage and the unencysted species *T. pseudospiralis* was the most vulnerable genotype.

KEY WORDS : *Trichinella*, muscle tissue, foxes, mice, decaying meat, degradation, reproductive capacity.

The nematodes in the genus *Trichinella* has evolved an amazing and complex protection system of interaction with the host muscle cell. The formation of a cyst around the larvae, the so called "nurse cell", with a layer of collagen on the outside protects and maintains the parasite. A set of blood vessels beneath the collagen capsule, the circulatory rete, provides the muscle larvae with nutrients (Despommier, 1998).

The scavenging nature of many *Trichinella* hosts favour the ability of the muscle larvae to survive in the muscle tissue after the death of the host. The carcass will not lay for a long time before the first animals will scavenge on it. Even so the parasite has to be able to withstand to some extent the cadaveric poisons in the meat, in order to survive transmission to a new host. There are investigations regarding the longevity of the muscle larvae in decaying meat, but only a single regarding survival as a function of temperature (Sokolova, 1979; Boev *et al.*, 1979) and differences between the known genotypes. The aim of the present study was to evaluate how long the parasite can survive and withstand the degradation processes in the dead meat. Furthermore genotypic characteristics were studied to identify differences between isolates from the northern hemisphere and tropical isolates.

MATERIALS AND METHODS

Outbreed laboratory mice (Ssc: CF1) and farm bred red foxes (*Vulpes vulpes*) were orally inoculated with nine different *Trichinella* genotypes (doses: 500 larvae/mouse and 10.000 larvae/fox) obtained from the *Trichinella* Reference Centre (Rome) (Pozio *et al.*, 1989). The genotypes and the TCR code were as follows: *T. spiralis* (ISS004), *T. nativa* (ISS042), *T. britovi* (ISS100), three isolates of *T. pseudospiralis* (USSR, ISS013; AUST, ISS141; USA, ISS470), *T. murrelli* (ISS035), *Trichinella* T6 (ISS034) and *T. nelsoni* (ISS037). After five and 37 weeks infection in mice and 10, 20 and 40 weeks in foxes respectively, muscle samples were taken. From mice the whole eviscerated body were taken and from foxes the back muscle (*m. longissimus dorsi*) and upper hind leg muscles (*m. rectus femoris*). The samples, minced separately for each genotype, were divided into 15-20 gr. portions and stored in a Petri dish at room temperature and 100 % relative humidity. Weekly for up to six weeks of storage the muscle larvae (ML) were recovered from the meat samples by digestion according to Gamble (1996). A control sample (day 0) was digested immediately after mincing. The reproductive capacity index (RCI = recovered larvae/ inoculated larvae) of the recovered larvae was evaluated in a maximum of four mice for each sample. An inoculation dose of 500 larvae/mouse was used. If less than 500 larvae were recovered from the rotten meat, only one mouse were inoculated. Storage room temperature were at 22° C ± 1, besides from slightly elevated temperatures in the summer time (27(C (2 in five and ten weeks old infections).

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RESULTS

The RCI and the LPG of the control groups (day 0) of both hosts in all infection groups were high and in accordance with data published by others (Pozio *et al.*, 1992; Raines *et al.*, 1988; Stewart *et al.*, 1990; Webster *et al.*, 1999).

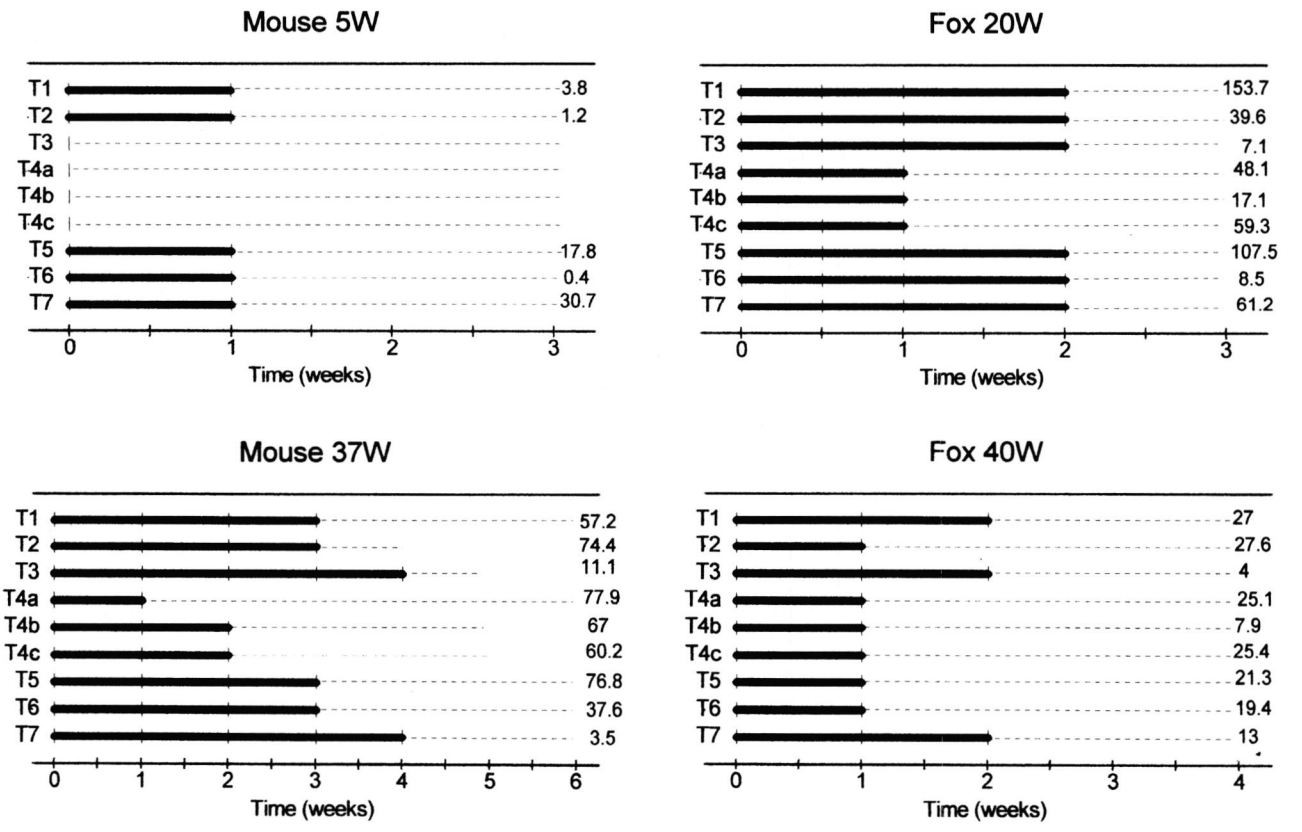
Mouse muscle tissue

The number of muscle larvae in the muscle tissue from five weeks old infection declined rapidly after one week of degradation and the recovered larvae were not able to reproduce in mice (Fig. 1). An exception to this was the African isolate *T. nelsoni*, which managed to survive in the decaying meat at relatively high numbers of larvae for two weeks and also had a high RCI compared to the other isolates after the first week. The RCI of the isolate from the temperate region *T. britovi* and the RCI of the arctic isolate *T. nativa* were very low or zero after one week of storage.

In 37 weeks old infections, the larvae were more resistant to the degradation process and were still infective in the rotten meat up to three-four weeks except from the unencapsulated genotypes of *T. pseudospiralis*, which were the more vulnerable. Only *T. nelsoni* and *T. britovi* were after four weeks still able to establish in mice, but only in small numbers. No significantly differences was shown between the African genotype (*T. nelsoni*) and the genotype from the northern hemisphere (*T. britovi*).

Fox muscle tissue

Larvae from the ten weeks infection were more vulnerable to the decaying process compared to older infections (20 and 40 weeks), and no larvae from any of the nine genotypes were recovered after one week in the rotten meat (figures not shown), except from *T. nelsoni*, which failed to infect a new host. Larvae from 20 and 40 weeks old infection persist for more than three weeks in the decaying tissue and were infec-



T1: *T. spiralis*, T2: *T. nativa*, T3: *T. britovi*, T4a, b, c: *T. pseudospiralis* (Russia), (Australia), (USA), T5: *T. murrelli*, T6: *Trichinella* T6, T7: *T. nelsoni*.
Mouse 5w: T3, T4a, b, c only recovered at day 0 dashed line indicates period of inoculation.

Fig. 1. – The persistence of infectivity of nine *Trichinella* spp. of different ages recovered from decaying mouse and fox meat and corresponding mean RCI for last successful infection.

tive up to two weeks of storage. The three *T. pseudospiralis* isolates were the most vulnerable species of the *Trichinella* genus and failed to establish in mice after one week of storage.
The RCI values for 40 weeks infection compared with 20 weeks infection were quite lower and for some genotypes the ability to infect stopped after one week of storage.

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When comparing 20 weeks and 40 weeks infection in foxes with the 37 weeks infection in mice a difference was the longer persistence of the ML in mice meat. After 21 days of putrefaction the larvae were still infective, while larvae in fox meat stopped to infect mice after 14 days and only a few genotypes in 40 weeks infection were still infective after one week. The three *T. pseudospiralis* isolates were the most vulnerable species and persisted even for a shorter period of time compared to the other genotypes from the same group of infection. The longest persistence of all groups showed *T. nelsoni* and *T. britovi* with four weeks in mice.

DISCUSSION

Our results confirm the hypothesis that the nurse cell enhance the tolerance of the encapsulated species. In both fox and mouse muscle tissue the tolerance to the degradation products of the meat improved with the age of the ML. The coating of the outside of the nurse cell with two types of collagen (IV, VI) on day 10 post invasion (Polvere *et al.*, 1997) is likely to protect the larvae against putrefaction, as well as the host immune system. While the synthesis of Collagen IV ceases after day 15 (20-30 days p.i.), the other type will still be produced for at least another seven months at a low rate (Polvere *et al.*, 1997). This experiment stressed the importance of Collagen VI, as it prolonged the lifetime of the muscle larvae from 20 and 40 weeks infections in foxes and 37 weeks in mice, since larvae of five weeks (35 days) of age in mice and ten weeks in foxes were poorly resistant to the putrefaction process.
Raines & Stewart (1988) investigated the influence of the parasites endogenous food reserves on the length of survival for the ML after the death of the host. This is probably another important factor, which limits the parasite's ability to survive for an even longer time in muscle cell. But still the protection of the nurse cell is the main factor protecting the ML against the process of degradation, since this experiment showed differences in survival between different ages of infection. The improved survival of the African isolate in the decaying meat in five weeks old infection revealed an important biological characteristic of *T. nelsoni*. While the number of ML of the other genotypes decreased very fast during the first two weeks of the experiment, only *T. nelsoni* showed stability in the number of larvae in the decaying meat during this time. Also, in ten weeks old infection in foxes, the larvae of the African genotype survived longest in the decaying meat, but were not infective. Adaptation to the relatively high temperature in his natural environment, appears to give the parasite a better survival in the storage room during the summer time compared to the other isolates. Since it takes between 34 and 60 days p.i. for the ML of the African isolate to establish a nurse cell (Pozio *et al.*, 1992), the protection at this point of infection for most of the *T. nelsoni* ML could only have been poor. Therefore the parasite must have evolved another kind of protection against the degradation process and the elevated temperature than the nurse cell, like for example the expression of host-like proteins for protection of the ML during the migration phase (Stewart, 1989). *Trichinella pseudospiralis* was the most vulnerable species among the other genotypes as investigated before (Raines *et al.*, 1988; Stewart *et al.*, 1990). Although the larvae had prolonged persistence in the decaying meat of older infections. The difference of infectivity of the muscle larvae in the two different hosts are most likely due to the shift of the host. Larvae were maintained in mice, inoculated to the foxes and at last the RCI were tested in mice. Furthermore the larval burden per gram was lower in the foxes. The persistence and infectivity of the muscle larvae in fox meat decline with the age of infection. Larvae from 40 weeks infection were less viable than larvae from the 20 weeks infection in foxes, due to the high age of the ML.
In conclusion, the study has demonstrated that *T. nelsoni* acquires its tolerance to degradation faster than any of the other genotypes, and that the non-encapsulated *T. pseudospiralis* are the less resistant of the genotypes. The latter might imply that the non-encapsulated genotypes are adapted to obligate predators and thereby have a different strategy than the encapsulated species, which might be exposed to scavengers equally well.

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