

## **PERSISTENCE OF *TRICHINELLA SPIRALIS* INFECTIVITY IN FARM ANIMAL FEEDING STUFFS**

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### **Abstract**

*Trichinella spiralis* can infect farm animals via several routes. The infections of swine and horses have been suspected to originate from contaminated feeding stuffs. With this study we wanted to assess how long *T. spiralis* can present a risk for an outbreak in contaminated feeds.

*Trichinella spiralis* -infected rat carcasses were incubated for 6 weeks in several animal feeds. The test feeds were silage, grained barley, and propionic acid -preserved feed; natural pasture conditions were also simulated. Altogether 24 target rats were used; six in each test environment. Samples were collected after one-, two-, four-, and six-week incubations. *Trichinella* larvae were recovered by digestion, and their infectivity was confirmed by inoculating recipient rats with isolated larvae.

Two-week incubation diminished the number of larvae, but larvae were still recovered from all feeds in small numbers after six weeks, except in the environment simulating natural pasture conditions. In sampling after two weeks, the larvae were infective in all test environments. However, after four weeks, they survived only in propionic acid fermented feed and there in small numbers with reduced reproductive capability.

The results indicate relatively long lasting risk to farm animals from rats or other infected material being hazardously mixed with hay or other feed. If silage is stored for at least one month before use, however, the risk from this forage seems to be minimized.

### **INTRODUCTION**

Rodents have been suspected or proven to be a source of trichinellosis for pigs and farmed wild boars. Pork and other pig meat production as well as carnivore game are well recognized potential sources for human trichinellosis. However, herbivores can also transmit the infection to human beings. Since 1975, at least 3 300 people have fallen ill in 13 outbreaks due to horse meat consumption in France and Italy. In addition, China has reported human outbreaks, not only due to pork but also to mutton or beef consumption. All these outbreaks have raised the question of natural transmission of trichinella to horses, or other herbivores. Two hypotheses have been proposed: grazing in pastures contaminated with infected materials, or ingesting infected flesh.

In Finland, sylvatic trichinellosis is highly prevalent, and domestic trichinellosis in pigs and farmed wild boars has been repeatedly reported in meat inspection during recent decades. Moreover, in Finland, trichinella infection is commonly found in rats from dumps. These rats have been infected almost exclusively with *Trichinella spiralis*. Among seven identified trichinella species and two genotypes, *T. spiralis* has been the one most often involved in human outbreaks. It also causes the most serious diseases in man. A real risk exists for forage to become contaminated

during handling and processing at farms. There is, however, insufficient information on the length of time trichinella can survive in infected flesh under natural conditions if mixed into various feedstuffs or left in a pasture. In Finland, fresh-cut hay is used for indoor feeding of cows and horses also in the summer months if the animals are not in pastures. Silage is the common base for cow feed in Finland but is also recommended for sows and used for horses. In swine farming, grain is commonly milled at the farm and mixed with protein concentrates. Alternative fermentation methods for grain, such as preservation with propionic acid additive, have become more popular.

To obtain basic data for exposure assessment, we ran an experiment on the survival of *T. spiralis* in contaminated feeds. The experimental feeds were silage, grained barley, and propionic acid preserved feed, which were compared when mixed with decomposing rat carcasses under natural climate conditions during a Finnish summer.

## **MATERIAL AND METHODS**

24 male Wistar rats (weight ca. 230 g) were each infected with approximately 300 muscle larvae of *T. spiralis* (ISS559) in minced mouse meat. Four weeks post-infection, all target rats were killed and a digestion sample from a left hind leg was taken to confirm the initial intensity of the infection. Six rat carcasses were placed in each of the four test environments; test environments were sampled by tests on three pieces of different infected rats after 1, 2, 4, and 6 weeks' incubation. The infectivity of the larvae found was confirmed by inoculation *per os* of recipient rats.

To simulate the hazard of rodents from the field being mixed in with silage during harvesting, six dead infected target rats were cut in pieces and placed in a large plastic-covered bale of formic acid preserved grass silage on the very same day it was harvested from the field. Each piece of rat was placed in a pouch made from polyamide pantyhose (Anette 40 den, Finnwear Oy, Tornio, Finland) and placed into the bale through a hole made by a sampling drill. The airtight plastic cover of the bale was closed again with adhesive tape and plastic. The bale was stored outside in a similar way as in ordinary farming.

To simulate the hazard of an infected rat's being milled together with barley, the six skinned target rat carcasses were minced in a commercial meat mincer, placed in the same type of polyamide pouch and placed in the grain, which was packed into 100 l plastic containers stored inside a barn. The third environment was propionic acid-preserved feed, a mixture of barley (30%) and oats (70%), not grained but flattened and mixed with commercial preservative solution 10 l/1000 kg (Propcorn7, BP Chemicals, Middlesex, Great Britain). It was infected as above. Both grained barley and propionic acid-fermented feed were stored in the same room in similar plastic containers, side by side.

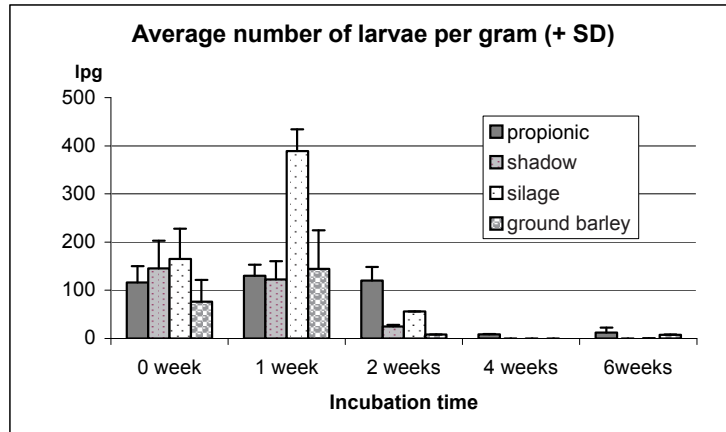
To simulate a situation of rats' having died in the pasture, and also to compare the effect of different forage-processing methods on the survival of *T. spiralis*, one group of six target rat carcasses were placed in a shaded box kept outside close to the silage bale. The box was made from plywood, well ventilated but inaccessible to invasion by any creatures bigger than ants. The carcasses were placed in polyamide pouches but were otherwise intact, save for the removal of the left hind legs for parasitological examination before the incubation.

Intensity of infection was analyzed by artificial digestion by the HCL-pepsin according to recommendations of the International Commission on Trichinellosis (Gamble et al. 2000). The infectivity of the harvested larvae was confirmed by inoculating isolated larvae by stomach tube into the recipient rats. The infection dose was either 300 larvae or fewer, depending on recovery of larvae from samples. Recipient rats were killed after 6 to 8 weeks of follow-up time, and the intensity of infection was analyzed as described above.

**RESULTS**

Per oral feeding of target rats with infected minced mouse flesh yielded variable intensities of infection. When measured in hind leg muscles, the average was  $164 \pm 60$  lpg and from minced meat  $100 \pm 29$  lpg.

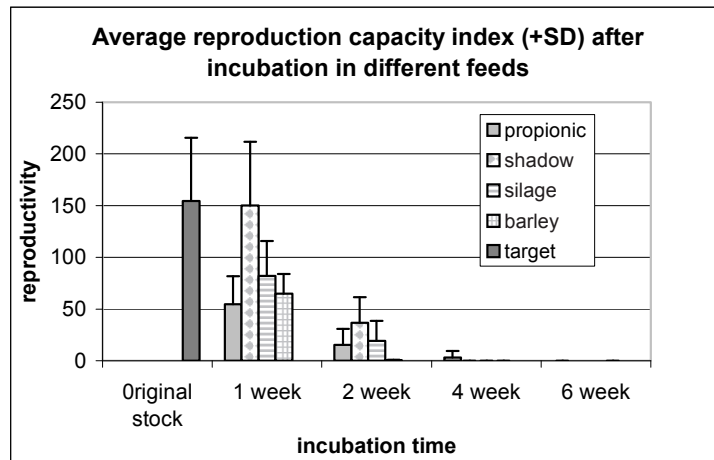
**Figure 1.**



Trichinella recovery from target rats after different incubation periods is presented in Figure 1. Some trichinella were found in all environments until four weeks of incubation, but after two weeks they were found in only small quantities. In six weeks in the shaded box, the fleshy parts of the rats were totally decayed, with no recovery of trichinella. In all other environments, small remnants of flesh with identifiable trichinella were found also after six-weeks' incubation.

Recovery of trichinella after one-week incubation was sufficient to infect four donor rats per each feed with 300 larvae. Later, the number of donor rats and volume of infection dose was justified based on the recovery. Still, after two weeks of incubation, trichinella recovered from all environments were infective (Figure 2); but in four weeks, only parasites from propionic acid-fermented fodder reproduced in recipient rats.

**Figure 2.**



The summer of 2001 was warm in Finland. During the experimental period, the maximum temperature recorded inside the shaded box was 42°C, the minimum 14°C, and the six-week average 23°C. Changes in humidity followed the outside climate. The average humidity was 66% (range: 30% -93%) inside the shaded box. Because grain and propionic acid-fermented feed were kept inside a barn, the daily temperature variation was not as great as in the shaded box, exposed to direct sunlight and night-time temperature drop. The pH decreased in silage from initial 5.1 to 4.5 during the first week and stayed rather constant thereafter. In the propionic acid-fermented feed, the pH stayed between 4.79 and 4.81 in every sampling and in grain 5.8 - 5.9 (Table 2). Moisture of the feeds was also rather constant during the six-week study period.

## DISCUSSION

In an endemic area, rodents can cause a risk for trichinellosis also to indoor animals both by contaminating their feed and because for example pigs scavenge or hunt infective pest animals. Fresh hay is used soon after harvesting. In our experiment, infectivity in the pasture-condition simulation was not at all affected in one week. For this reason, contaminated rat carrion mixed with hay may be the source of an outbreak. The typical management practice of milling the grain at the farm and mixing it with protein concentrate does not include long storage of prepared feed. Two weeks' persistence of infectivity can thus be hazardous if rats have colonized the crop storage. Silage is recommended to be fermented for at least one month before use. In our experiment in summer temperatures, infectivity in silage was minimized by four weeks of incubation. It is worth noting that after four weeks, infective larvae were still found in propionic acid-fermented feed. In endemic areas, rat control, through minimizing direct contact and maintaining feed hygiene, is important in trichinellosis prevention.

## REFERENCES

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