

Persistence of *Trichinella spiralis* in Rat Carcasses Experimentally Mixed in Different Feed

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Oivanen L, Mikkonen T, Haltia L, Karhula H, Saloniemi H, Sukura A: Persistence of *Trichinella spiralis* in rat carcasses experimentally mixed in different feed. Acta vet. scand. 2002, 43, 203-210. – *Trichinella spiralis* infected rat carcasses were incubated for 6 weeks in several animal feeds to assess how long *Trichinella* can present a risk for an outbreak in contaminated feeds. In groups of 6, 24 infected target rats were placed in silage, grained barley, propionic acid-preserved feed, and also into simulated pasture conditions. Test environments were sampled after one-, 2-, 4-, and 6-week-incubations. *Trichinella* larvae were recovered by digestion, and their infectivity was evaluated in rats. A two-week incubation reduced the number of recovered larvae, but still after 6 weeks low numbers were isolated from all feeds except from the experimental group simulating pasture conditions. After 2 weeks storage, the larvae were infective in all storage environments. However, up to 4 weeks, they survived only in the propionic acid-fermented feed and there in small numbers with reduced reproductive capability. This indicates the possibility of farm animals to get infection 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 appears to be minimized.

Trichinellosis; domestic cycle; exposure assessment; feed hazard; animal hygiene; zoonosis; disease control; rat; feed contamination; carrion.

Introduction

Rodents have been suspected or proven to be a source of trichinellosis for pigs and farmed wild boars (Schad *et al.* 1987, Smith & Kay 1987, Leiby *et al.* 1990, Gamble *et al.* 1999, Oivanen *et al.* 2000). Pork and other pig meat products as well as carnivore/omnivore game are well recognized potential sources for human trichinellosis. However, herbivores can also transmit the infection to human beings. Since 1975, at least 3300 people have fallen ill in 13 outbreaks due to horse meat consumption in France and Italy (Boireau *et al.* 2000, Touratier 2001). In addition, China has reported human outbreaks, not only due to pork but also to mutton or beef consumption (Murrell 1994, Wang

& Cui 2001). 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 rodent carcasses or feeding with infected flesh from pigs or wildlife (Pozio *et al.* 2001).

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 (Oivanen *et al.* 2000, Sukura *et al.* 2001, Oivanen *et al.* 2002). Moreover, in Finland, *Trichinella* infection is commonly found in rats from dumps (Mikkonen 1998, Mikkonen *et al.*

unpublished). These rats have been infected almost exclusively with *Trichinella spiralis* (Oivanen et al. 2002). Among *Trichinella* genotypes, *T. spiralis* has been the one most often involved in human outbreaks (Capó & Despommier 1996). A real risk exists for forage to become contaminated during handling and processing at farms. Fitzgerald & Prakasam (1978) tested *T. spiralis* survival in sewage sludge. The encysted larvae survived no longer than 96 h. Thus, such sludge seems not to offer much of a risk for pasture and field contamination. Von Köller et al. (2001) demonstrated that under laboratory condition, in steady room temperature, some species of *Trichinella* can survive several weeks in decaying meat and that host species and the age of infection influence on this survival. On the contrary, no information is available of longevity of *Trichinella* under natural conditions in different feeds or in the pasture. Data on parasite survival in feeds is needed for proper risk assessment for herbivore or omnivore domestic animals. 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 (Suomi 1999) and used for horses. In swine farming, grain is commonly milled at the farm and mixed with protein concentrates. Alternative fermentation methods for grain have become more popular, such as wet preservation with acidic additives.

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

Materials and methods

Study design

Twentyfour male Wistar rats served as target rats in 4 test environments and 39 served as recipient rats to confirm the infectivity and reproduction capacity index (RCI) of isolated larvae. At the start of the experiment, these rats on average weighed 233 g (14 g; standard deviation, SD). Target rats were each infected with approximately 300 muscle larvae of *T. spiralis* (ISS559, code at the International Trichinella Reference Center, Rome) in minced mouse meat. This *T. spiralis* strain had originally been isolated from a natural infection of a Finnish pig and maintained in laboratory mice for 8 generations before the experiment. Five weeks after passage mice were euthanized and eviscerated skinned carcasses were minced for inoculum for target rats. Four weeks post-infection, all target rats were anesthetized with CO₂ and euthanized by decapitation. 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 test environment. The environments were sampled by tests on 3 pieces of different infected rats after 1, 2, 4, and 6 weeks' incubation. The infectivity of the larvae found were confirmed by inoculation per os of recipient rats. The committee on animal experiments of the University of Helsinki has approved the study (D. no: 354/2001).

Test environments

Silage. Six dead infected target rats were placed in one large plastic-covered bale of silage of approximately 750 kg on the day of harvest. During silage processing, shredded hay was mixed with a formic acid-based preservation solution 5l/1000 kg of cut hay (AIV-2000, Kemira Agro Oy, Oulu, Finland), but the natural fermentation decreases the pH further. A shredder-baling machine cuts hay at the shortest into 4-cm pieces. Therefore, the dead rats

were cut into similar pieces. 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 air-tight 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.

Grained barley. To simulate the hazard of an infected rat being milled together with barley, the 6 skinned target rat carcasses were minced in a commercial meat mincer (LM-5, Koneteollisuus OY, Nurmijärvi, Finland), placed in the same type of polyamide pouch and placed in the grain, which was stored in 100 l plastic container inside a barn.

Propionic acid-preserved feed. By this method, seeds are not grained but flattened and mixed with commercial preservative solution 10 l/1000 kg (Propcorn7, BP Chemicals, Middlesex, Great Britain). A mixture of barley (30%) and oats (70%) was purchased as ready mixed feed. The 6 skinned target rat carcasses were minced and handled as above. Both grained barley and propionic acid-fermented feed were stored in the same room in similar plastic containers, side by side.

Pasture simulation. 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 6 target rat carcasses was 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 except, for the left hind legs having been removed for parasitological examination before the incubation.

Environmental factors analyzed

Outside temperature was recorded both near the shaded box and the silage bale and inside the barn close to the grain containers. Inside temperature and humidity in the shaded box were recorded by a computer based monitor (Tinytag temperature/humidity logger, Gemini Data Loggers LTD, Chichester, England). The pH was monitored in each feed, both with indicator paper and by pH meter in the liquid phase after over-night incubation in a refrigerator with distilled water added equal to 50% of the volume. The content of dry matter was also recorded after overnight drying in the incubator at 105°C, but was reported as moisture (100% – dry matter content %). At the end of the experiment, pH and moisture were monitored in the remaining target rats as well as in a fresh minced rat carcass.

Parasitological examinations

Intensity of infection was analyzed by artificial digestion by the HCL-pepsin method shaking either with a Jumbomix (Interscience, Saint Nom, France) or a magnetic stirrer according to recommendations of the International Commission on Trichinellosis (Gamble *et al.* 2000). Data from minced meat were used for those rats which were minced for the purpose of simulating a particular feed-processing (target rats in ground barley or propionic acid-fermented feed). For other target rats (shaded box or rats in silage) results from the left hind leg muscles were used. 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. To calculate the reproduction capacity index (Dick 1983, Kapel *et al.* 2000), the total number of

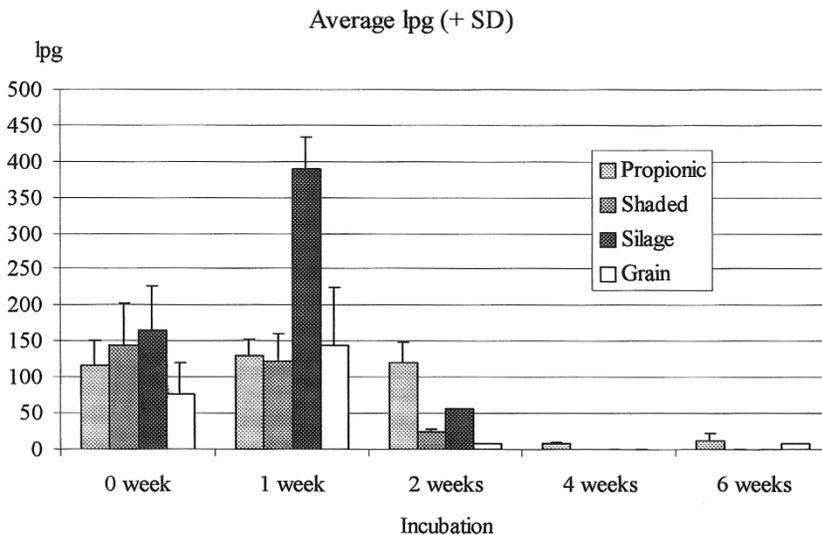


Figure 1. Average lpg with standard deviation in rats incubated in different feeds.

Trichinella in each rat was estimated by multiplying larvae per gram of muscle (lpg) by the animal's total weight and dividing this arbitrary value by infection dose. Data are presented as average and standard deviation.

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 (60, SD) lpg and from minced meat 100 (29) lpg.

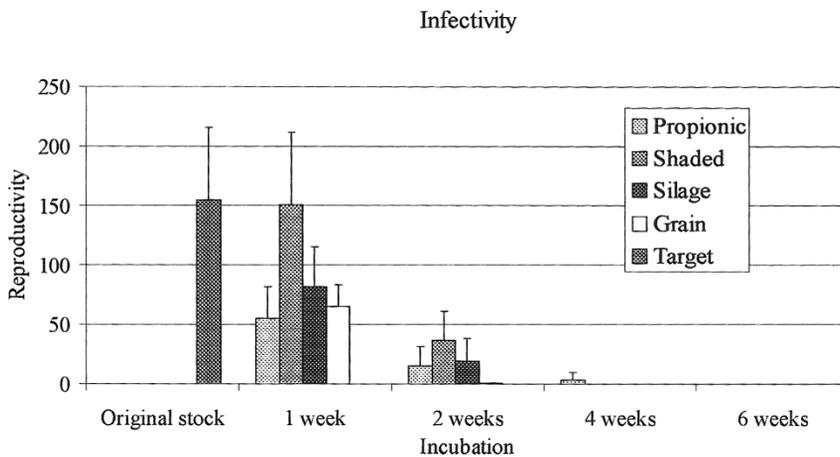


Figure 2. Average reproduction capacity index with standard deviation after incubation.

Table 1. Effect of 6 weeks' incubation on target rat carcasses in different environments.

	pH	moisture (%)
Fresh rat	6.1	62.2
Propionic	7.0	33.1
Shaded	7.4	19.8
Silage	7.4	67.0
Grain	6.5	21.1

Propionic = propionic acid-fermented feed.

Shaded = shaded box simulating pasture conditions.

Grain = grained barley.

Trichinella recovery from target rats after different incubation periods is presented in Fig. 1. Some *Trichinella* were found in all environments until 4 weeks of incubation, but after 2 weeks they were found in only small quantities. After 6 weeks in the shaded box, the fleshy parts of the rats were totally decayed, with no recovery of *Trichinella*. In all other environments than the shaded box, small remnants of flesh with identifiable *Trichinella* were found also after 6 weeks of incubation.

Recovery of *Trichinella* after a one-week incubation was sufficient to infect 4 donor rats each inoculated with 300 larvae. Later, the number of donor rats and volume of infection dose was justified based on the recovery. Still, after 2 weeks of incubation, *Trichinella* recovered from all environments were infective (Fig. 2) but in 4 weeks only parasites from propionic acid-fermented fodder reproduced in recipient rats. By 6 weeks, no parasites were found to be infective. Original stock infectivity was calcu-

lated from initial inoculation of target rats with 300 larvae in minced mouse meat (Fig. 2).

The target rats were badly decomposed after 6 weeks of incubation. In silage, the fleshy parts were liquefied, and only bones and hairs were left in the polyamide pouches. In grained barley, the rat carcasses were mummified, and in propionic acid-fermented feed, large moldy feed clumps surrounded the rat carcasses. In the shaded box, maggots had consumed the carcasses by 6 weeks. Maggots were found even in one-week samples, and the breeding of maggots and fur beetles was the main decaying factor in otherwise mummifying rat carcasses. Moisture had increased in carcasses incubated in silage (Table 1), but target rats in other environments were desiccated. The pH in all incubated carcasses was higher than in a fresh minced rat carcass (Table 1).

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 (weekly average range: 18.5-25.5°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, which was exposed to direct sunlight and nighttime temperature drop. The pH decreased in silage from initial 5.1 to 4.5 during the first week and stayed rather constant thereafter (Table 2). In the propionic acid-fermented feed,

Table 2. pH and moisture (%) of feeds at different sampling times (weeks of incubation).

Weeks	0		1		2		4		6	
	pH	%								
Propionic	4.9	19.6	4.8	19.9	4.8	20.2	4.8	20.9	4.8	21.4
Silage	5.1	71.6	4.5	74.8	4.7	75.1	4.5	72.8	4.7	73.8
Grain	5.8	12.1	5.9	12.0	5.8	12.9	5.8	13.5	5.8	13.4

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 6 week study period (Table 2).

Discussion

Initial analyses of target rats showed lower lpg yield in minced meat than in muscles of the hind leg. It is a well-known fact that *Trichinella* larvae are not evenly distributed throughout the skeletal musculature (Alkarmi et al. 1990, Kapel et al. 1994, Pozio et al. 1999, Oksanen et al. 2000, Mikkonen et al. 2001). Obviously, hind leg muscles are the predilection of trichinellosis in rats, and the lower lpg yield in minced meat indicates the dilution effect of other musculature and tissue.

One-week recovery yields (lpg) were on the same level or even a bit higher than in the initial samples. The small increase detected in infection intensity can be explained by the decreased moisture of the rat carcasses which caused relatively higher figures per weight. However, bigger increases may be explained by the fact that the larvae are not evenly distributed in rat bodies; there is always sample-to-sample variation in the same bodies and even in the same muscle. In the samples incubated longer than one week, the larval recovery in propionic acid-fermented feed tended to be higher than in other environments. Unlike in natural conditions, rats in the shaded box were not exposed to rain. Therefore, the carcasses appeared to dry and mummify if not consumed by colonizing maggots.

The effect of proteolytic putrefaction seen as an increase in pH was strongest in silage and mildest in grain. In silage, the humid environment increased the moisture of the carcasses, but other environments dried them up. The drying effect was highest in the hot shaded box, but dry grain also took up much of the water from rat carcasses.

Intriguingly, infectivity was least affected in those target rat carcasses kept in the shaded box, but because the flesh was devoured up by maggots, no larvae could be recovered, and the RCI was not confirmed after 4 weeks. The different feed processing methods all seemed to have a negative effect on the reproduction performance of *Trichinella* seen at the 2 weeks' sampling. Maroli & Pozio (2000) showed that *Trichinella* larvae can survive and be infective when ingested by maggots. Their survival in maggots depended on time and environmental temperature, but was not longer than 5 days.

Silage packed in bales is often stored outdoors until used, also in wintertime; freezing does not spoil the feed. Those *Trichinella* species resistant to freezing can survive in contaminated fodders even during the winters of northern Europe. Stewart et al. (1990), studying the persistence of *T. pseudospiralis* in mouse carcasses, found them were infective for only 2 weeks when kept in 24°C, but in those mouse carcasses kept at 4°C, infectivity was preserved up to 30 days. A lower environmental temperature may thus prolong the persistence of infectivity in feeds. In pork buried in the ground *T. spiralis* survived infective at least for 90 days (Jovic et al. 2001). The ability of *Trichinella* to be infective also in different feeds for some weeks can be the explanation for unexpected herbivore hosts known to be sources of human outbreaks (Boireau et al. 2000, Touratier 2001).

In an endemic area, rodents can cause a risk for trichinellosis also to indoor animals both by contaminating their feed and because these animals (such as pig) scavenge or hunt infective pest animals (Schad et al. 1987, Murrell et al. 1987). 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 4 weeks' incubation. It is worth noting, that after 4 weeks, infective larvae were still found in propionic acid-fermented feed. In endemic areas, rat control is important to prevent trichinellosis. Methods are minimizing direct contact and maintaining feed hygiene.

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Sammanfattning

Trichinella spiralis hållbarhet i rått-kadaver som experimentellt blandats i olika foder.

Döda råttor infekterade med *Trichinella spiralis* inkuberades under 6 veckor i olika djurfoder för att uppskatta hur länge *Trichinella* utgör en smittorisk i kontaminerat foder. 24 infekterade råttor, i grupper på 6 djur, placerades i silage, säd, foder konserverat med propionsyra och på simulerat naturligt grönbete. Efter en, 2, 4 och 6 veckors inkubering togs prov från alla experimentella omgivningar. *Trichinella* larver återvanns ur proven genom digestion och larvernars infektivitet beprövades genom inokulering i mottagliga råttor. Två veckors inkubering minskade antalet larver, men efter 6 veckors inkubering kunde fortfarande ett litet antal larver isoleras ur alla fodertyper med undantag av den lagringsform som simulerade naturligt grönbete. Vid provtagningen efter 2 veckors inkubation var larverna infektiiva i alla foder. Men efter 4 veckors lagring fanns infektiiva larver endast i fodret som konserverats med propionsyra och här i ett litet antal och med reducerad förökningsförmåga. Härur slutleds att risken att råttor eller annat infekterat material blandas i hö eller annat foder kan utgöra en fara för boskapsdjur. Om silage lagras åtminstone en månad innan användning minimeras risken i detta foder.

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