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A Study on the Survival of *Taenia saginata* Eggs on Soil in Denmark

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Ilsøe, B., N. C. Kyvsgaard, P. Nansen and S. Aa. Henriksen: A study on the survival of *Taenia saginata* eggs on soil in Denmark. Acta vet. scand. 1990, 31, 153–158. – The infectivity of *Taenia saginata* eggs exposed to environmental conditions on a natural soil surface in Denmark was studied by feeding the eggs to susceptible calves, followed by determination of the number of cysts developed. The results indicated that a small proportion of the eggs remained infective for 6 1/2 months, but not for 9 1/2 months when deposited in May 1986, and for 5 1/2 months but not for 8 1/2 months when desposited in September 1987. Viability of eggs was tested in vitro and compared with infectivity obtained in calves.

Cysticercus bovis; egg survival; infectivity; viability; soil surface; climate.

Introduction

Several factors influence the availability and the infectivity of taeniid eggs in the environment; e.g. natural ageing and the exposure to adverse physical and biological factors (*Lawson & Gemmell* 1983). Low to moderate temperatures and high humidity are considered to favour long-term survival of taeniid eggs (*Gemmell et al.* 1983).

Under laboratory conditions *Penfold et al.* (1937) demonstrated that a small proportion of a batch of *T. saginata* eggs stored in saline at 2–5°C maintained infectivity through 95 days but not through 116 days. The survival of infective eggs of *T. saginata* on pasture has been studied in different parts of the world, as thoroughly reviewed by *Gemmell* (1978), *Wilkens* (1981), *Bürger* (1983), *Gemmell et al.* (1983) and *Lawson & Gemmell* (1983).

In Denmark *Jepsen & Roth* (1952) demonstrated that eggs of *T. saginata* remained infective after about 5 1/2 months (159 days) at outdoor exposure in a situation where faeces containing tapeworm segments were deposited on grass in winter (February). Yet the maximum duration of survival of eggs was not demonstrated. Under climatic conditions roughly comparable with those in Denmark, *Wilkens* (1981) in Braunschweig, Western Germany, demonstrated that *T. saginata* eggs applied onto pasture by wastewater irrigation were infective to tracer calves at least 4 months (119 days) after the application in spring (May). However, neither in this study the maximum survival was determined.

It is well documented that the agricultural use of sewage sludge implies a risk of spreading infective *T. saginata* eggs to cattle (*Haugaard* 1984, *Holt* 1985, *Nansen & Henriksen* 1986). According to new Danish regulations on the agricultural use of sewage sludge, spreading is allowed only on arable land which over the subsequent 12 months is used for grain or seed crops before it is used for pasture or green-fodder crops (*Anon.* 1985).

As part of a survey evaluating the effectiveness of these new Danish regulations in preventing the spreading of bovine cysticercosis (*Ilsøe & Kyvsgaard* 1988), attempts were made to determine the maximal survival time of infective *T. saginata* eggs deposited on the soil surface in spring and in autumn in Denmark.

Material and methods

Experimental design

Identical batches of *T. saginata* eggs were deposited in small bags on the soil surface. On day 0 and subsequently at intervals of 3 months the infectivity was assessed by inoculating young calves, serologically negative with regard to *C. bovis,* followed by determination of the number of cysts developed in them. Two experiments were carried out. One started in May 1986, the other one in September 1987. The experiments were terminated when inoculation of calves no more led to development of cysts.

Parasite material

T. saginata eggs were obtained from freshly voided segments. In the first experiment, all eggs used were isolated from spontaneously voided segments. In the second experiment, the majority of eggs were obtained from the posterior segments of a tapeworm expelled after treatment with niclosamide. The eggs were suspended in 0.9 % saline and allocated in portions of 11,500 eggs (± 500). Only eggs that appeared morphologically fully developed were included in the counting. After mixing with 2–3 grams of a structural material (Vermiculite), the eggs were wrapped in small bags made of nylon mesh (Monodur®, pore size 22.4 µm). The bags were sealed by melting with hot iron, and their impermeability for eggs was finally tested. The egg bags were then deposited on the soil surface on a site with half-shade.

Infectivity tests

At intervals of 3 months, 3 egg bags were removed from the soil site. The bags were cut in small pieces, and mesh plus contents were suspended in water and dosed orally to 3 young calves from farms with no history of bovine cysticercosis. Prior to the experiment, these calves were found to be serologically negative with regard to *C. bovis* (*Kyvsgaard et al.* in press).

The calves were slaughtered 10 weeks post infection. The number of cysts detected by careful slicing into 5 mm pieces of the heart, liver, kidneys, oesophagus, lungs, spleen, masseters, diaphragm and tongue was recorded. The number of cysts in the forelimbs, hindlimbs and truncus muscles was calculated by doubling the number detected by likewise slicing of the musculature of the left or right half of the carcass. If no cysts were found by this procedure, the remaining musculature was sliced as well. When no cysticerci could be demonstrated in any of the 3 calves in a group, dosing of new calves was discontinued.

Viability tests

In the second experiment, supplementary in vitro viability testing was carried out on eggs from the batch for the in vivo infectivity tests. Portions of 16,000 eggs were kept in test tubes with saline next to the egg bags. This procedure was used, as observations indicated that the admixture of structural material incriminated the viability testing procedure.

The viability analysis comprised a hatching procedure in sodium hypochlorite followed by an activation test in trypsine/bicarbonate/bile (modified after *Stevenson* (1983)), as described by *Ilsøe & Kyvsgaard* (1988) and *Brødsgaard* (1989).

Table 1. Numbers of *Cysticercus bovis* in calves dosed with eggs of *Taenia saginata* exposed on the soil for different periods.

PART I:

Duration of exposure:	No. of cysts detected:	Mean (% of day 0)
0	1683	
	1061	1144
	688	(100 %)
3 months	580	
(13 weeks)	496	439
May–July	240	(38.4 %)
6 1/2 months	26	
(29 weeks)	22	17
May–November	2	(1.4 %)
9 1/2 months	0	
(43 weeks)	0	0
May–February	0	

PART II:

Duration of exposure:	No. of cysts detected:	Mean (% of day 0)	% infective of 11,500:	% viable of 16,000:
0	55			
	154	141		
	213	(100 %)	1.2 %	1.7 %
3 months	43			
(12 weeks)	74	94		
Sept.–Dec.	164	(66.7 %)	0.8 %	0.6 %
5 1/2 months	66			
(24 weeks)	52	45		
Sept.–March	16	(31.9 %)	0.4 %	0
8 1/2 months	0			
(36 weeks)	0	0	0	0
Sept.–May	0			

Results

The number of cysts developed in the experimentally infected calves in relation to the duration of exposure of the eggs is shown in Table 1 and Fig. 1. The ratio of live to degenerated cysts did not decline with increasing egg age.

The infectivity on day 0, when measured as the mean number of cysts developed from the 2 egg batches used in each part of the experiment, differed markedly.

In the first experiment, the shape of the die-off curve of eggs, as drawn between the mean numbers of cysts, was neither linear nor exponential (Fig. 1). A minor number (mean 17) of these eggs remained infective after 29 weeks of exposure (May-November). However, no eggs were found to be infective after 43 weeks (May-February). This experiment included a summer period with temperature up to 30°C and no rainfall for

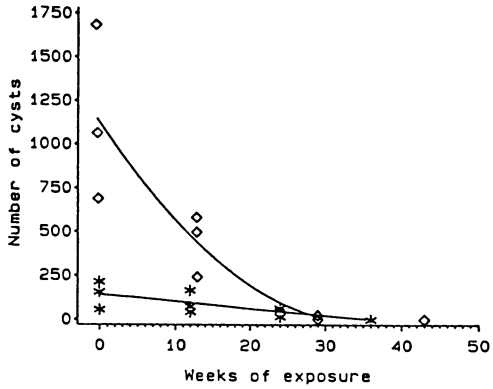

Figure 1. Numbers of *C. bovis* in calves dosed with eggs of *T. saginata* in relation to duration of exposure on the soil:
◇): Calves dosed with eggs deposited on the soil in May 1986.
*): Calves dosed with eggs deposited on the soil in September 1987.
Note: The marks at 36 weeks (*) and 43 weeks (◇) each represents *three* calves all found not to harbour cysts.

several weeks, followed by a winter period with average (24 h) temperatures below zero in January and February, where the temperature occasionally reached – 18°C.

In the second experiment, the die-off curve of eggs approximated that of a linear function (Fig. 1). A number (mean 45) of eggs remained infective for 24 weeks (September-March), but none were found to be infective after 36 weeks (September-May). This experiment was carried out during a winter which was unusually mild for Danish conditions, with average (24 h) temperatures above zero except for one week in January, where the temperature occasionally reached – 10°C.

In the second experiment, the percentage of eggs in saline recorded as viable by the hatching and activation tests was roughly in accordance with the percentage of eggs found to be infective from the bags, except for the eggs exposed for 24 weeks. None of the eggs kept in saline through that period were able to hatch and activate, although the infectivity to calves still turned out to be relatively high (Table 1).

Discussion

The main purpose of the described experiments was to define the rate of decline of infectivity and the maximal duration for *T. saginata* eggs remaining infective under conditions prevailing at the surface of Danish soil.

The egg portions used in the second experiment showed a markedly lower infectivity on day 0 as compared with the eggs used in the first experiment. This may possibly be ascribed to strain differences between individual *T. saginata* worms (*Froyd* 1962, *Wouters et al.* 1987). The fact that the majority of the eggs of the second experiment originated from segments expelled after anthelmintic treatment may not be decisive, since *Gemmell et al.* (1983) states that cestocides have no ovicidal effect. However, it seems likely that the batch of eggs may have contained a certain proportion of juvenile eggs, which, even though they appeared morphologically fully developed, were not yet infective on day 0. Such eggs may reach maturity during the exposure on the soil (*Gemmell* 1978, *Lawson & Gemmell* 1983), thus explaining the slower decline in infectivity of this batch of eggs, as compared to the eggs included in the first experiment (Fig. 1).

The shapes of the die-off curves are likely to be influenced by a multitude of determinants. Apart from genetic and age-related factors, the climatic conditions will also influence the rate of decline of the infectivity, and thereby perhaps explain some of the differences in the shape of the curves from the two experiments. However, the exact die-off pattern of *T. saginata* eggs will demand further studies of the infectivity decline under

controlled conditions. One should be well aware that such studies are extremely resource requiring.

In previous reports no clear correlation between the viability and the infectivity of *Taenia* eggs has been recorded (*Gemmell* 1978, *Hughes et al.* 1985, *Pike* 1988). In the present experiment, some accordance between the demonstrated viability and infectivity of eggs seems to have been demonstrated, at least in the initial stages of the trial. However, at the last date (24 weeks exposure), when a small proportion of eggs still were infective, they did not show any viability by the in vitro test. These results therefore suggest that the in vitro viability testing cannot substitute the in vivo infectivity testing. The in vitro viability testing may, however, be useful when estimating the size of the egg doses in an infectivity trial.

In conclusion, the present studies have demonstrated that the survival of infective eggs of *T. saginata* exposed to the climatic conditions prevailing at the soil surface in Denmark, may exceed some 6 1/2 months from spring to winter and some 5 1/2 months from autumn to spring, but not 9 1/2 and 8 1/2 months respectively. Thus, under practical conditions the legislatory resting interval of 1 year between sludge application and the use of the area for pasture or harvest of fodder crops seems to be sufficient to prevent infection with bovine cysticercosis from *T. saginata* eggs from sludge applied onto farmland.

In a WHO report on the prevention and control of cysticercosis, it is recommended to wait at least 6 months after sludge application before using the area for pasture. However, it is indicated that it cannot be ruled out that cattle may become infected with cysticercosis on sludge treated pasture even after this time, especially if it covers a winter period (*Gemmell et al.* 1983). Actually, the present results suggest that the resting period recommended by WHO is too short, at least under Danish, and possibly also under other Northern European conditions.

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Sammendrag

En undersøgelse af Taenia saginata æg's overlevelsesevne på jordoverfladen i Danmark.

T. saginata æg, der havde været udsat for miljøforholdene på jordoverfladen i forskellige tidsintervaller, blev infektivitetstestet ved at indgive æggene oralt i modtagelige kalve. Efter slagtning blev antallet af udviklede cysticercer i hver kalv opgjort. Resultaterne viste, at en lille del af æggene havde bevaret infektiviteten i 6 1/2 måned men ikke i 9 1/2 måned, når de blev udlagt i maj, og i 5 1/2 måned men ikke i 8 1/2 måned, når de blev udlagt i september. Desuden blev æggenes viabilitet, undersøgt in vitro, sammenlignet med infektiviteten.

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