

A case-control study of risk factors in light *Taenia saginata* cysticercosis in Danish cattle

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Kyvsgaard, N. Chr., B. Ilsøe, P. Willeberg, P. Nansen and Sv. Aa. Henriksen: A case-control study of risk factors in light *Taenia saginata* cysticercosis in Danish cattle. Acta vet. scand. 1991, 32, 243-252. – Risk factors in light *T. saginata* cysticercosis in cattle herds were investigated in a case-control study. The case group consisted of farms from which cattle with low-grade cysticercosis had been detected at slaughter. The control group consisted of farms where no cases were detected during the same period. The major risk factor identified was allowing cattle access to drink from streams carrying effluent from sewage treatment plants (odds ratio = 3.6). Spreading of septic tank sludge through deliberate or accidental mixing with animal slurry, deposition of sewage sludge, and proximity to railways, camping sites or sewage treatment plants seemed to be of minor importance.

epidemiology; streams; sewage; effluent.

Introduction

In Danish cattle, the prevalence at slaughter of *Taenia saginata* cysticercosis declined from a maximum of 1.35% in 1952 to the present estimate of 0.10% (Danish Veterinary Services 1988). The prevalence in Denmark is lower than in countries such as Germany, where the prevalence was 0.7% in 1982 (Zimmermann 1985). Within Denmark the highest prevalence seems to be in South Jutland, where a figure of 0.7% was recorded in one large abattoir in 1986 (Ilsøe & Kyvsgaard 1988). Only a few of the cysticercosis cases are classified as massive with resulting condemnation of carcasses (Hansen 1973, Biering-Sørensen 1978).

The present incidence of human taeniasis in Denmark is low. Based on the sale of cestocides the incidence rate was estimated to 17 persons infected per 100,000 inhabitants per year (Ilsøe et al. 1990), whereas the corre-

sponding figure was 87 in Germany (Zimmermann 1985).

The routes of transmission in massive outbreaks, referred to as »cysticercosis storms«, have been studied in a number of investigations. In North America cysticercosis storms in feedlot cattle have often been ascribed to dispersal of eggs by infected persons working within the herd or in the cattle feed industry (McAninch 1974, Slonka et al. 1975, Hancock et al. 1989). Recently, infection routes were identified in Danish herds with cases of massive cysticercosis (Nansen & Henriksen 1986, Ilsøe et al. 1990). Here the most common infection source was septic tank sludge. Contamination of pasture or fodder frequently occurred when contractors emptied septic tanks immediately before handling liquid manure with the same machinery. In addition, cattle became heavily infected by grazing close to sewage treat-

ment plants and/or by drinking at a short distance downstream from the outlet of effluent. Sludge from sewage treatment plants deposited on farmland was found to be an infection source by *Nansen & Henriksen* in 1986 but not by *Ilsoe et al.* in 1990 after more restrictive rules for sludge usage had been implemented.

The transmission routes in light infections are less well-known but are considered to be more indirect than in outbreaks of massive cysticercosis. An indication of the significance of water streams was given by *Pawlowski* (1982), who found that in a region close to a river receiving sewage from a city, all »localities« located downstreams had one or more cases of cysticercosis compared to 73% upstreams. In New Zealand it has been demonstrated that eggs of *T. hydatigena* and *T. ovis* could be spread over long distances by flies (*Lawson & Gemmell* 1983, 1985). However, such dispersal could not be demonstrated in a preliminary Danish study (*Kyvsgaard et al.* 1988). Also birds may contribute to the spreading of *T. saginata* eggs. In Denmark, *Guildal* (1956) found *Taenia* eggs in the intestines of 9.5% of seagulls shot near a sewage treatment plant.

The relative importance of the various possible transmission routes is, however, largely unknown (*Gemmell* 1986). The present case-control study was therefore set up in an attempt to find a quantitative relationship between low grade cysticercosis and the presence of a number of possible transmission factors.

Materials and methods

The study was geographically limited to an area of South Jutland located between the German border and a line running east-west north of the city of Åbenrå. The case group consisted of farms from which cattle with bovine cysticercosis had been detected at

slaughter. The recordings were made from February 1986 until May 1987 in 3 abattoirs (referred to as B, T, and R) which slaughtered approximately 120,000 head of cattle in 1986. The recordings included date of slaughter, age (abattoir B) and sex of the animal, together with name, address and telephone number of the owner.

The present study was limited to light cysticercosis infections, i.e. when 10 or less cysts were found by thorough slicing of the heart, masseters, diaphragm and tongue. Three farms within the study area had a total of 10 animals condemned due to massive cysticercosis during the study period. These farms were included in a countrywide survey of massive cysticercosis (*Ilsoe et al.* 1990) and they were therefore excluded from the present study.

The control herds were selected by simple random sampling from a file of all cattle herds in the area described above. No matching with case herds was carried out.

Telephone interviews of the herd owners in the case-group were carried out during the summer and autumn of 1987, and of those in the control group during the summer of 1988. Questions were asked about herd size, general feeding practices, contact with water streams, possible contamination of these streams with sewage effluent, liquid manure practices including the hiring of contractors to spread the manure, sewage sludge spreading, and proximity to railways, camping sites or sewage treatment plants. Finally it was asked if tapeworm infection had been diagnosed among the farmers family or persons working on the farm.

From the initial list of 321 case farms 274 interview forms were completed (85%). The success rate of interviews in the control group was 125 of 165 (76%). The most common reasons for failure of owners to comply were: Herd owners could not be reached by

telephone, some farms in the control group had ceased farming cattle before the start of the study period, the infected animal had been purchased immediately before slaughter in some case herds, and a few farmers did not want to participate in the interview. Initially, 182 farms had been selected for the control group, but 17 of these were already included in the case group corresponding to 9.3% of the herds.

Data processing

The unit of analysis in this study was the herd. No attempt was made to evaluate risk

factors of cysticercosis in individual animals.

Statistical analysis was carried out using the PC version of SAS (SAS Institute Inc. 1985). Chi-square (χ^2) test and the Mann-Whitney test were used as statistical tests (Snedecor & Cochran 1967). Statistical significance is assigned with one, two, or three asterisks for the 5, 1 and 0.1 per cent significance levels respectively. Odds ratio (OR) was used as a measure of association and the estimated population attributable fraction (estimated PAF) was used as an estimate of the proportion of all cases attributable to the particular

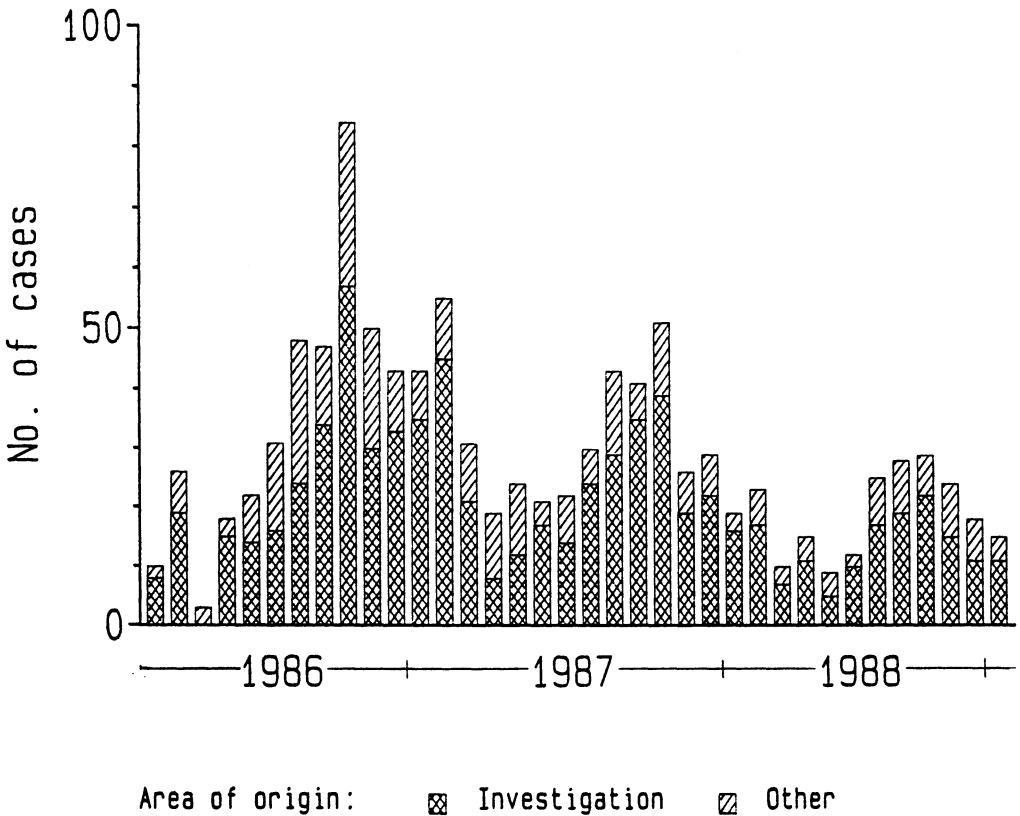


Figure 1. The monthly number of light (non-condemned) cysticercosis cases at 3 abattoirs in South Jutland. Cases originating from farms within the investigation area are indicated separately. The slaughter on abattoir R ceased in 1987.

infection source. The Mantel-Haenszel method was used to control for confounding (Kleinbaum et al. 1982, Martin et al. 1987).

Results

Reported cases

In Fig. 1 the monthly number of cattle with *T. saginata* cysticercosis on the 3 abattoirs is indicated for a 3 year period. It is apparent that there is a distinct seasonal variation with the highest prevalence recorded in the autumn. The age and sex distribution of the infected cattle detected at abattoir B is given in Fig. 2.

In 232 of the 274 case herds only one animal was found infected with cysticercosis within the study period. Thirtyone herds had 2 infections detected, 8 had 3, and 2 herds had 4

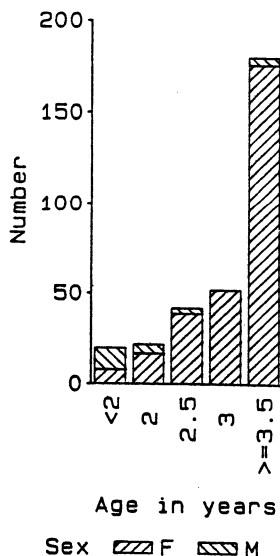


Figure 2. The age and sex distribution of cysticercosis cases originating from the investigation area and slaughtered at abattoir B from February 1986 to May 1987. The abattoir slaughtered approximately 55,000 head of cattle per year. Fifty percent of these were »oxen«, mostly culled cows, and 50% were »calves« mostly young fattening bulls.

cases. The highest number was found in one herd where 5 cases were reported. There was no distinct tendency towards geographical clustering of the case-herds.

Herd size and herd type

The median number of animal in the case herds (100, range 2 to 514) was higher than in the control group (80, range 6 to 330). The difference was found significant ($p < 0.01$) in a Mann-Whitney test after ranking the herd sizes.

Of the case herds 91% were dairy herds compared to 83% of the control herds. In the remaining herds beef cattle were kept, young bulls were fattened for slaughter or heifers were bought, raised and finally sold at calving. The mean size of the dairy herds was larger than that of the other herds.

As some of the management factors investigated were strongly associated with herd size and type, confounding was controlled by adjusting for herd size and/or the analysis was restricted to one homogenous herd group.

Grazing practice and harvest of grass for fresh feed

This part of the analysis was limited to dairy herds. Most of these farms had cows (93% of case herds and 94% of control herds) and heifers (98% and 97%) on pasture at least part of the summer. Male animals, mostly steers, grazed in a few herds (6% and 11% respectively). The number of herds where grass was harvested and given as fresh fodder to cows in the stable was significantly higher in the case group than in the control group (OR = 2.0*, Table 1). As the rate of this practice increased with herd size, confounding from the difference in herd size, between the case and control group was suspected. However, a significant difference remained after correction for confounding (OR = 1.8*).

Table 1. Relationship between the feeding of freshly harvested grass on stable to cows and the finding of cysticercosis at slaughter. The table is restricted to dairy herds. Analysis is carried out for all dairy herds combined (top) as well as stratified, controlling for the effect of herd size.

Fresh grass fed on stable to cows in dairy herds:	Case herds	Control herds	Odds ratio (OR)
Yes	92	24	2.0*
No	156	80	1
Total	248	104	

Stratified by herdsize:

1 - 60 animals:	Yes	4	4	0.67
	No	33	22	1
61 - 120 animals:	Yes	48	10	2.2
	No	71	32	1
> = 121 animals:	Yes	40	10	2.0
	No	52	26	1

Mantel-Haenszel procedure: 1.8*

Breslow-Day test for homogeneity of the OR: 2.1 (NS)

Contact to streams carrying sewage effluent

Watering cattle from streams carrying the effluent from sewage treatment plants involved a substantial risk of acquiring cysticercosis. Cattle watered from these streams in 29% of the case herds compared to 13% of the control herds (Table 2, OR = 3.5***). A smaller risk was associated with watering cattle from streams with outlet of septic tank overflow from single houses (OR = 2.2*). Cattle grazing next to these streams without having drinking access were not at increased risk, although there was a possibility of temporary flooding of the pasture.

The proportion of cases that were caused by drinking from sewage contaminated streams was estimated at 28%. This estimate was cal-

Table 2. Relationship between various degrees of contact to sewage polluted streams and the finding of cysticercosis at slaughter. Herds with contact to more than one stream are categorized after the most polluted.

Contact to sewage polluted streams:	Case herds	Control herds	Odds ratio (OR)
Watering from streams carrying effluent from sewage treatment plants	79	16	3.5 ***
Watering from streams with single toilet outlets	47	15	2.2 *
Grazing next to (without drinking from) streams with sewage effluent	22	13	1.2
Grazing next to (without drinking from) streams with single outlets	24	7	2.4
Grazing next to and/or watering from clean streams	40	30	0.95
No contact to streams	62	44	1
Total	274	125	

culated as the estimated PAF, using the data from Table 2. Herds where cattle drank from streams carrying effluent from sewage treatment plants or sewage from single households constituted the exposed group and all other herds the non-exposed group.

As the rate of watering cattle from streams carrying effluent from sewage treatment plants increased with herd size, analysis was carried out controlling for the effect of herd size (Table 3). However, the adjusted odds ratio (3.3***) does not differ from the unadjusted.

Interaction between the contact with effluent carrying streams and geographical localization was found. Separate analysis of data from the Eastern hilly part of the area and the Western flat lowland showed that the risk was higher in the Western region (Table 3). The localization of the farm was

Table 3. The effect of watering cattle from streams with effluent (»Effluent«) from sewage treatment plants analysed for different strata of herdsize (top) and geography (bottom). The non-exposed group (»No contact«) is formed by herds with no contact to streams or with contact to clean streams only.

Watering from streams with effluents		Case herds	Control herds	Odds ratio (OR)
<i>Stratified by herdsize:</i>				
1 - 60 animals:	Effluent	11	1	12.7
	No contact	32	37	1
61 - 120 animals:	Effluent	26	5	2.5
	No contact	47	23	1
> 121 animals:	Effluent	42	10	2.6
	No contact	23	14	1
Mantel-Haenszel procedure:				3.3***
Breslow-Day test for homogeneity of the OR: 2.3 (NS)				
<i>Stratified by geography:</i>				
East:	Effluent	64	11	4.8
	No contact	41	34	1
West:	Effluent	15	5	2.0
	No contact	61	40	1
Mantel-Haenszel procedure:				3.5***
Breslow-Day test for homogeneity of the OR: 1.7 (NS)				

used for the stratification. This localization does not necessarily correspond to the area of grazing, as some animals might have been grazing outside the farm, mostly in the marshlands of the Western region.

Irrigation of fields was a common practice in parts of the study area but in most instances the water came from wells. Eleven case herds compared to 2 control herds reported that they were using surface water from streams.

However, the OR of 2.5 was not significantly different from 1.

Liquid manure handling

Transmission of *T. saginata* eggs was considered to be a possibility when contractors were hired for the spreading of liquid manure. The machinery used by the contractors was always of suction type and could therefore be used for emptying septic tanks. However, no significant differences were found between the case and the control group concerning the frequency of hiring contractors for the spreading of at least part of the liquid manure (Table 4). For the systems where the urine was separated from the solid manure the odds ratio was 0.88. For farms with integrated slurry systems the odds ratio was 0.72.

Significantly more owners of case herds (44%) than of control herds (34%, OR = 1.7*) reported that machinery used for the spreading of liquid animal manure also had been used for emptying septic tanks. The difference was more pronounced in the herds where the spreading at least partially was carried out by contractors. Seventythree per cent of 144 case herds compared with 51% of 78 control herds reported of possible contamination (OR = 2.6**).

Deposition of sewage sludge, and proximity to sewage treatment plants, railways or camping sites

Deposition of sludge from sewage treatment plants either on own property or on neighbouring land, and distance of less than 100 m from fields used for grazing or fodder production to sewage treatment plants, railways or camping sites were all factors with a low prevalence. No definite risk could be demonstrated for any of these factors (Table 5).

Table 4. Relationship between spreading practices of liquid animal manure (urine or slurry) and the finding of cysticercosis at slaughter. Some farms had both types of manure systems.

Spreading of animal urine	Case herds	Control herds	Odds ratio
Contractors ¹⁾	89	56	0.88
Own machinery only	45	25	1
Total	134	81	

Spreading of slurry	Case herds	Control herds	Odds ratio
Contractors ¹⁾	74	30	0.72
Own machinery only	92	27	1
Total	166	57	

Human tapeworm carriers on the farm

Three herd owners from the case group but none from the control group reported that helminth infections consistent with *T. saginata* had occurred in the family in the period where the infected animal was raised.

Discussion

A number of biases may occur in this case-control study. One of these might be due to low sensitivity (false negative classification) as only part of cattle with low grade cysticercosis is detected by meat inspection (Kyvsgaard *et al.* 1990). Therefore some of the control herds might actually have delivered cattle harbouring cysts to slaughter during the study period. Such misclassification would influence the results of the study in a conservative direction, i.e. the real difference between the case group and the control group for a given factor would be higher than the one actually found.

As reply rates of at least 70-80% are normally considered necessary to avoid bias caused

1) Exclusively or partially

Table 5. Relationship between spreading of sewage sludge, proximity to sewage treatment plants, railways or camping sites and the finding of cysticercosis at slaughter. The χ^2 results are placed in parenthesis when expected cells numbers are below 5.

Sewage sludge spread on:	Case herds	Control herds	Odds ratio
Own land	7	2	1.6
Neighbouring land only	10	10	0.44
No spreading	257	113	1
Total	274	125	

Distance to sewage treatment plant:	Case herds	Control herds	Odds ratio
< = 100 m	4	6	0.29 (*)
> 100 m	270	119	1
Total	274	125	

Distance to railway:	Case herds	Control herds	Odds ratio
< = 100 m	14	13	0.46
> 100 m	260	112	1
Total	274	125	

Distance to camping site:	Case herds	Control herds	Odds ratio
< = 100 m	10	0	>4.7(*)
> 100 m	264	125	1
Total	274	125	

by replies from non-representative groups (Martin *et al.* 1987), the reply rates found in this study seem satisfactory both for the case group (85%) and for the control group (76%). The difference in reply rates between the two groups might be associated with the smaller herd size in the control group compared with the case group.

The difference in herd size found between the case and the control group might be ascribed to a higher risk of infection in the larger herds. Such an increased risk could be

caused by a factor associated with large herd size as e.g. watering of cattle from streams carrying the effluent from sewage treatment plants. Another explanation might be that even with an equal within-herd prevalence rate of cysticercosis, it is more likely that the larger herd will have at least one animal detected at meat inspection. In contrast, the control herds were selected independently of herd size.

Drinking contact to water streams carrying the effluent from sewage treatment plants was found to be an important risk factor (OR = 3.5***). A smaller risk was associated with cattle drinking from streams with sewage outlets from single households. Herds with cattle grazing next to these streams but without access to drink were not found to have elevated risk. Similarly, *Pawlowski* (1982) found that the frequency of cysticercosis was higher downstream from a sewage outlet than upstreams.

The observed risk was interpreted as increased transmission of *T. saginata* eggs. There is evidence that retention times in sewage treatment plants are insufficient for sedimentation of *T. saginata* eggs (*Arundel & Adolph* 1980) and these will consequently pass with the effluent to the streams. The concentration of eggs in the stream depends of the distance from the effluent outlet. In investigations where sewage effluent possibly had caused massive outbreaks, the cattle had been drinking close to the sewage outlet (*Holt* 1985, *Ilsøe et al.* 1990). When the effluent is diluted, the eggs from even a single tapeworm can be spread to a large number of cattle resulting in geographically widespread but usually low-grade infections.

The seasonal variation in occurrence of cysticercosis might indicate that the animals are infected during the summer, which is also in accordance with the transmission by streams. The seasonal pattern could, how-

ever, be influenced by the fact that a relatively high proportion of the cattle slaughtered in the autumn are culled cows (*Skamling* 1989), the group representing the highest number of cysticercosis cases.

The risk of cattle acquiring cysticercosis from contact to water streams was higher in the Western part of the area than in the Eastern part. The watershed in South Jutland is located far to the East and consequently the streams running from East to West will have a longer course and thus receive waste water from more cities than the streams in the Eastern part. Although the population density in the Eastern part is higher, the large cities in that area discharge wastewater effluent directly into the sea.

The employment of contractors for the spreading of liquid manure could not be demonstrated to involve any increased infection risk compared to farms using only their own machinery. This is in contrast to the findings in massive cysticercosis outbreaks described by *Nansen & Henriksen* (1986) and *Ilsøe et al.* (1990), where infections could be ascribed to contractors that had emptied septic tanks immediately before the spreading of liquid manure with the same machinery. As the human prevalence of *T. saginata* is low, contamination of pasture with septic tank sludge might have occurred on the farms in the present study without leading to infection.

A significantly higher proportion of herd owners in the case group compared with the control group reported that machinery used for the spreading of liquid manure also was used for emptying of septic tanks. This difference was highest in herds where contractors had been employed (OR = 2.6**). However, these reports were often based on subjective estimates, and the difference may reflect that owners of cysticercosis-downgraded cattle had speculated about the

source of infection. A true difference would have resulted in a difference between the case group and the control group in the frequency of hiring contractors.

A small but significant risk was associated with the harvest of fresh grass for feeding cattle in the stable. The explanation of this difference is difficult but it could be associated with the mechanical harvest preventing the cattle from their normal selective grazing behaviour.

No risk was demonstrated by spreading of sewage sludge or by proximity to railways, camping grounds or sewage treatment plants. These risk factors were uncommon in both groups. Consequently a case-control study might not be the optimal design to determine the risk (Martin *et al.* 1987).

There was no indication in the present study that any significant proportion of the cysticercosis cases were caused by tapeworm infected persons working on the farms. This is in contrast to reports of cysticercosis storms in American feedlots (McAninch 1974, Slonka 1975).

This screening of suspected risk factors has revealed that transmission routes leading to light cysticercosis infections mainly consist of infected water streams. The study was not designed to reveal transmission by e.g. imported feed concentrates or by vectors over long distances. Evaluation of these factors would need additional studies designed differently.

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Sammendrag

»Case-control« undersøgelse af risikofaktorer for lavgradige *Taenia saginata* cysticercoseinfektioner i dansk kvæg.

Risikofaktorer for lavgradige *Taenia saginata* cysticercoseinfektioner blev belyst i en »case-control« undersøgelse. Case-gruppen bestod af besætninger, hvorfra der var fundet slagtedyder med op til 10 tinter, og kontrolgruppen omfattede besætninger uden tinfund i dyr, slagtet indenfor samme tidsrum.

Den største enkelte risikofaktor ved lavgradig cysticercoseinfektion var adgang til at drikke fra vandløb, der førte urensset spildevand og effluent fra spildevandsrensningsanlæg (Odds Ratio 3,6). Spredning af spildevandsslam, evt. efter opblanding i husdyrgylle, og græsning op til jernbane, campingpladser eller rensningsanlæg synes derimod at spille en mindre væsentlig rolle for udbredningen af lavgradige *T. saginata* cysticercoseinfektioner.

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