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A SEROLOGICAL STUDY ON THE PREVALENCE OF TOXOPLASMA GONDII IN MEAT-PRODUCING ANIMALS IN SWEDEN

By

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UGGLA, A. and M. HJORT: *A serological study on the prevalence of Toxoplasma gondii in meat-producing animals in Sweden.* Acta vet. scand. 1984, *25*, 567—576. — Sera from 200 sheep, swine and cattle, respectively, collected at slaughter at each of 3 abattoirs situated in the north, middle and south of Sweden were examined for the presence of antibodies to Toxoplasma gondii by the indirect fluorescent antibody test. Seropositive animals (titre $\geq$ 1:20) were found at the following rates from north to south: sheep 60 %, 66 % and 68.5 %, swine 9 %, 2.5 % and 37 %, and cattle 10 %, 6 % and 35 %.

The significance of the serological findings is discussed, and it is concluded that T. gondii infection is common in Swedish farm animals. Thus the meat from particularly swine and sheep may provide a potential source of human toxoplasmosis.

sheep; swine; cattle; survey; fluorescent antibody test.

Toxoplasma gondii is, in most parts of the world, a common intracellular protozoan parasite of man and many other warm-blooded animal species. The domestic cat and other felines act as final hosts, in whose intestine the parasite has a coccidian sexual reproductive cycle resulting in the production of oocysts which are subsequently shed into the environment with the faeces. Most mammals and many birds act as intermediate hosts, in whose tissues the parasite after infection undergoes an asexual intracellular multiplication which finally leads to the forming of microscopic tissue cysts. Such cysts, containing hundreds of organisms, are principally situated in brain, sceletal and cardiac muscle and make a chronically infected animal a potential source of infection through carnivorism. Consequently, both final and

intermediate hosts may contract a Toxoplasma infection in principally two days, either by ingesting oocysts originating from cat's faeces or by consuming meat or organs containing tissue cysts (*Hutchison et al.* 1968).

Human toxoplasmosis is dangerous particularly during pregnancy, as there is a risk of transplacental transmission of the infection to the foetus. This may result in congenital central nervous or ocular lesions. *Huldt et al.* (1979) found, using the dye test, that 60 % of adult women from a Stockholm suburban community had antibodies to Toxoplasma. Infected meat eaten raw or undercooked is regarded as the main source of human toxoplasmosis (*Feldman* 1982), and for this reason numerous studies have been performed in many countries on the prevalence of Toxoplasma in meat animals. However, beside a local survey in pigs (*Hansen et al.* 1977), no such study has previously been conducted in Sweden.

The aim of the present survey was to determine to what extent sheep, swine and cattle from different parts of Sweden may harbour antibodies to Toxoplasma gondii indicating that their meat may serve as a potential source of infection to man.

MATERIALS AND METHODS

Animals

Blood samples were collected from the stick wound at slaughter of 200 sheep, swine and cattle respectively at each of 3 abattoirs in Sweden. These were situated in Skellefteå in the north, Uppsala in central Sweden and Kävlinge in the south. Sheep samples from the central area of Sweden were collected at the abattoir in Västerås (see Fig. 1). The sampling was made at each abattoir over 2 days in October 1982 or in January 1983 (Uppsala).

The animals included in the study were selected randomly from the slaughter line without any knowledge of their origin. Since animals from the same herd are usually culled together, care was taken to avoid sampling of too many sheep and swine in succession. The samples collected at the Skellefteå abattoir represented sheep from 32 farms, swine from 38 farms and cattle from 116 farms. Samples of sheep taken in Västerås were from 40 farms and the swine and cattle at the Uppsala abattoir were from 34 and 62 farms, respectively. Samples from Kävlinge represented sheep from 24 farms, swine from 107 farms and cattle from 121 different farms.

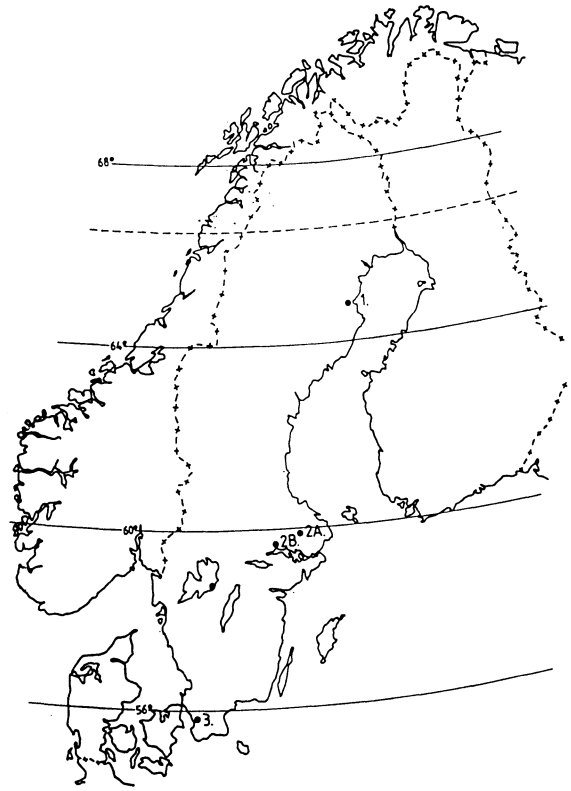

Figure 1. Map of Scandinavia showing the sample localities used in the study. 1. Skellefteå, 2A. Uppsala, 2B. Västerås, 3. Kävlinge. The dashed line indicates the Polar circle.

With respect to age, sex and breeding forms no apparent differences existed between the animal groups from the 3 locations chosen for this study. The sheep groups comprised mainly lambs of both sexes at an average age of approximately 6 months. However, a few older ewes were also included in the material. The swine were fattening pigs, females or male castrates, at 6—7 months of age. The cattle were of various ages, sex and breeding forms, predominantly animals older than 1.5 years.

After preparation, sera were stored at —20°C until tested.

Serological test

Swine and sheep sera were screened by the indirect fluorescent antibody test (IFAT) at dilutions of 1:10 and 1:20 in phosphate buffered saline at pH 7.4 (PBS). One drop from each serum dilu-

tion was added to smears of whole Toxoplasma tachyzoites on microscopic slides (bioMérieux, Charbonnières-les-Bains, France). 1:10 dilutions of sera from known negative and from experimentally infected animals of each species included in the study were used as controls. The slides were incubated in a moist chamber at room temperature for 30 min. After careful washing in PBS the smears were covered for another 30 min with a rabbit-anti-species specific IgG preparation (Dakopatts, Copenhagen, Denmark) at a dilution of 1:40. After final washing in PBS the results were read in a Leitz Dialux fluorescence microscope at a magnification of 600x under oil immersion. A positive result was considered when at least 50 % of the tachyzoites showed a bright complete and unbroken peripheral fluorescence. According to *Boch et al.* (1979) a reaction at the dilution of 1:20 was considered as significantly positive. Cattle sera were inactivated at 56°C for 30 min and subsequently tested at dilutions of 1:10 and 1:20 as described above.

Finally, a maximum of 10 positive sera from each species and geographical area were picked at random and further tested in twofold serial dilutions to their endpoint titres.

RESULTS

The results from the serological screening are presented in Table 1. The frequencies of seropositive sheep (titre $\geq$1:20) were, at the different locations from north to south, 60 % 66 %

Table 1. Serological examination for antibodies to Toxoplasma gondii in shep, swine and cattle from the north, middle and south of Sweden as measured by the indirect fluorescent antibody test.

Animal	Location	Number of sera	Titres			
			$<$ 1:10	1:10	$\geq$ 1:20	$\geq$1:20 (%)
Sheep	Skellefteå	200	36	44	119	60
	Västerås	197	27	40	130	66
	Kävlinge	200	30	33	137	68.5
Swine	Skellefteå	199	173	8	18	9
	Uppsala	200	186	9	5	2.5
	Kävlinge	200	100	26	74	37
Cattle	Skellefteå	200	127	53	20	10
	Uppsala	200	150	38	12	6
	Kävlinge	200	78	52	70	35

and 68.5 %, and swine sera with a positive titre were correspondingly 9 %, 2.5 % and 37 %. Cattle sera inactivated at 56°C for 30 min showing a titre of $\geq$ 1:20 comprised 10 %, 6 % and 35 %, respectively.

The distribution of endpoint titres from 10 positive sera from each species and geographical area are presented in Table 2. Only 5 sera from swine showing a titre $\geq$ 1:20 were available among the 200 samples from Uppsala. The maximum titres obtained were 1:640 (sheep), 1:320 (swine), and 1:160 (cattle).

Table 2. Distribution of IFAT endpoint titres to Toxoplasma gondii in seropositive sheep, swine and cattle from the north, middle and south of Sweden.

Animal	Location	Number of sera	Titres					
			1:20	1:40	1:80	1:160	1:320	1:640
Sheep	Skellefteå	10	2	3	5			
	Västerås	10		1	2	3	3	1
	Kävlinge	10	4	2	3	1		
Swine	Skellefteå	10	2	1	2	3	2	
	Uppsala	5	1	1	2	1		
	Kävlinge	10	6	1	2	1		
Cattle	Skellefteå	10	8	2				
	Uppsala	10	7	3				
	Kävlinge	10	4	4	1	1		

DISCUSSION

Surveys for Toxoplasma infections can be conducted either by direct parasitological or indirect, i.e. serological methods. As there is an adequate correlation between the presence of tissue cysts and the prevalence of antibodies to Toxoplasma in swine and sheep (*Catár et al.* 1969, *Munday & Corbould* 1979, *Boch & Neurohr* 1982), and, furthermore, between tissue cyst loads and antibody levels (*Jacobs et al.* 1963, *Work* 1967), serological surveys have been widely accomplished. The indirect fluorescent antibody test (IFAT) used in this study is sensitive and specific and recommended for serological diagnosis of toxoplasmosis in swine (*Suzuki et al.* 1965), sheep (*Munday & Corbould* 1971), and cattle (*Munday* 1978).

Toxoplasmosis is a common cause of abortion in ewes in many countries (*Hartley & Marshall* 1957, *Linklater* 1979). Reports

from elsewhere have shown that the prevalence of antibodies to Toxoplasma gondii in sheep is often high. Maternally derived antibodies in lambs have usually disappeared at 3 months of age (*Waldeland* 1977), which indicates that the occurrence of antibodies in older lambs and sheep must be regarded as a result of an active infection, though most often subclinical. Sheep are sensitive to Toxoplasma infection for which reason their parasitic loads may well reflect the infection intensity in their environment (*Munday & Corbould* 1979). In this context it is noteworthy that no great differences in the spread of T. gondii in those parts of Sweden chosen for this study were observed. We recorded an overall prevalence of seropositive sheep of 65 % with only slight differences between geographical locations. These results are well in accordance with those from a previous study, where *Uggla et al.* (1983) demonstrated 63 % of 155 Central Swedish ewes to have an IFAT titre of 1:20 or higher. Similar studies in Denmark showed a seropositivity rate of 61 % (*Work* 1967), in Norway 26 % for lambs and 46 % for mature sheep (*Waldeland* 1976), in Germany 55 % (*Boch et al.* 1979), in the Netherlands 30 % (*van Knapen et al.* 1982) and in Scotland 15 % (*McColm et al.* 1981). In such related studies the serological method may differ from one another as may the sampling procedures, which makes a direct comparison difficult. As the prevalence may vary strongly between different flocks even in the same region (*Uggla et al.* 1983), chance may obviously effect the results of serological surveys when using too few samples or when using samples from animals originating from only a few herds. In the present study we have tried to take such factors into consideration.

Large differences were found in the number of seropositive swine from different parts of the country. The range was between 2.5 % in Central Sweden to 37 % in the South and a similar pattern was found for the cattle. Considering the figures recorded for the sheep sera, these differences are probably incidental, despite that it is likely that the milder climate in southern Sweden should favor the conditions for the parasite. In a previous survey from the south-west of Sweden, *Hansen et al.* (1977) found 40 % dye-test positive animals in a material of 67 slaughtered pigs. However, as it is known that hygienic swine breeding conditions work against Toxoplasma infections (*Lubroth et al.* 1982), and as such management practices should be more com-

mon in the major swine producing areas of southern Sweden, the high figures accounted here and from *Hansen et al.* (1977) are somewhat surprising. The figures can be compared to 21 % seropositive pigs found in Denmark (*Work* 1967), 16 % in Norway (*Mohn et al.* 1974), 16 % in Germany (*Boch & Neurohr* 1982), and 0 % in the Netherlands (*van Knapen et al* 1982).

In the present material a large proportion of the cattle sera were initially found to show what was considered as unspecific immunofluorescent stainings at low serum dilutions. These reactions were diminished following heat inactivation of the sera prior to testing. This procedure is however doubtful, as discussed by *Suzuki et al.* (1965), and in our hands it mainly resulted in a diminished sensitivity, giving most sera an overall one or two dilution steps lower titre than if untreated. It should therefore be born in mind that the results for the cattle sera in this study reflect their reaction following inactivation at 56°C for 30 min. With this in mind the frequency of seropositive cattle in southern Sweden reached 35 %, a comparatively high figure. Similar studies in Denmark showed a frequency of 13 % (*Work* 1967), in Germany 21 % (*Boch et al.* 1965), in the Netherlands 22 % (*van Knapen et al.* 1982), and in Scotland 3 % (*McColm et al.* 1981).

In cattle there appears to be no relationship between the prevalence of antibodies to Toxoplasma and the presence of detectable tissue cysts as found for sheep and swine. Most authors have failed to demonstrate parasites in the tissues of naturally infected cattle by means of peptic digestion and mouse inoculation techniques although many of the animals did show positive Toxoplasma serological reactions (*Boch et al.* 1965, *Work* 1967, *Janitschke et al.* 1967). *Jacobs et al.* (1957) detected 1 possibly infected animal among 60 beef cattle parasitologically examined, and exceptionally *Catár et al.* (1969) isolated Toxoplasma from 8 out of 85 muscle samples from cattle. It has been a well established opinion that cattle are resistent to Toxoplasma infection and seem to rapidly eliminate the organism (*Rommel et al.* 1966, *Beverley et al.* 1977, *Munday* 1978). However, *Dubey* (1983) showed that in experimentally infected cattle, encysted Toxoplasma might be found in the tissues, particularly the liver, up to 287 days after inoculation. Still, despite a relatively high frequency of seropositive cattle found in this study, rare beef is not likely to be a major source of human toxoplasmosis in Sweden.

The association between human toxoplasmosis and consumption of infected meat from swine and sheep is well established (*Weinman & Chandler* 1955, *Jacobs et al.* 1957, *Rawal* 1959). However, Toxoplasma cysts in meat and organs loose their infectivity when heated to a temperature of 60°C for 10—15 min or following freezing at —15°C or lower for up to 24 h (*Rawal* 1959, *Jacobs et al.* 1960, *Work* 1968, *Hellesnes & Mohn* 1977). The parasite in meat does not seem to survive drying, normal salting or smoking procedures (*Jacobs et al.* 1960, *Work* 1968). In Sweden the total consumption of lamb and mutton is very small, while pork on the other hand is the prevailing meat eaten. Besides, most lamb and mutton is stored frozen before consumption, which is not the case with pork. The high frequency of infected swine from the main swine breeding districts in the south of Sweden as shown in this study is therefore worth special attention.

The present investigation has shown that a large proportion of sheep and pig carcasses may harbour Toxoplasma cysts. The relatively high titres found (Table 2) also indicate that some carcasses may contain large numbers of cysts. Ingestion and handling of raw fresh meat from sheep and swine raised in Sweden will bring about, therefore, a risk of human infection.

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SAMMANFATTNING

En serologisk studie över förekomsten av Toxoplasma gondii hos köttproducerande djur i Sverige.

Sera från 200 får, svin respektive nötkreatur insamlade vid slakt på vart och ett av tre slakterier belägna i norra, mellersta och södra Sverige undersöktes med indirekt immunofluorescensteknik avseende förekomst av antikroppar mot Toxoplasma gondii. Seropositiva djur (titer $\geq$ 1:20) upptäcktes i en frekvens av från norr till söder för får 60 %, 66 % resp. 68.5 %, svin 9 %, 2.5 % resp. 37 % och nötkreatur 10%, 6 % resp. 35 %.

Betydelsen av fynden diskuteras, och det konstateras att Toxoplasma-infektion är vanligt förekommande hos svenska köttproducerande djur, vilket innebär att konsumtion av rått kött framför allt från svin och får är en potentiell smittkälla för människa.

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