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ONCHOCERCA GUTTUROSA IN DANISH CATTLE

PREVALENCE, GEOGRAPHIC DISTRIBUTION AND HOST-VECTOR RELATIONSHIPS *

By

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KOLSTRUP, NILS: *Onchocerca gutturosa in Danish cattle. Prevalence, geographic distribution and host-vector relationships.* Acta vet. scand. 1975, *16*, 1—13. —

1. Onchocerca gutturosa is reported in Danish cattle for the first time. Microfilariae were found in 38 (9.4 %) of 406 cows that were 2 years or older.
2. Microfilariae were not randomly distributed throughout the skin but were concentrated in the umbilical area.
3. No difference in prevalence was observed between breeds of cattle.
4. Most of the infected cows had grazed on fields close to streams that contained Simulium ornatum, the vector of O. gutturosa.
5. Differences in preferred biting sites of S. ornatum from cow to cow were correlated with the arrangement of hair.

Onchocerca gutturosa; prevalence; vector; (Simulium ornatum).

Onchocercid nematodes are cosmopolitan parasites of vertebrates and are transmitted by nematoceran flies. A well-known member of the Onchocercidae, Onchocerca volvulus Leuckart 1893, causes eye and skin disease in man (*Nelson* 1970, *Duke* 1972, *Duke & Anderson* 1972). This species is host specific to man and the chimpanzee and is limited in geographic distribution to tropical Africa and Central and South America. The geographical localization and the narrow host specificity are

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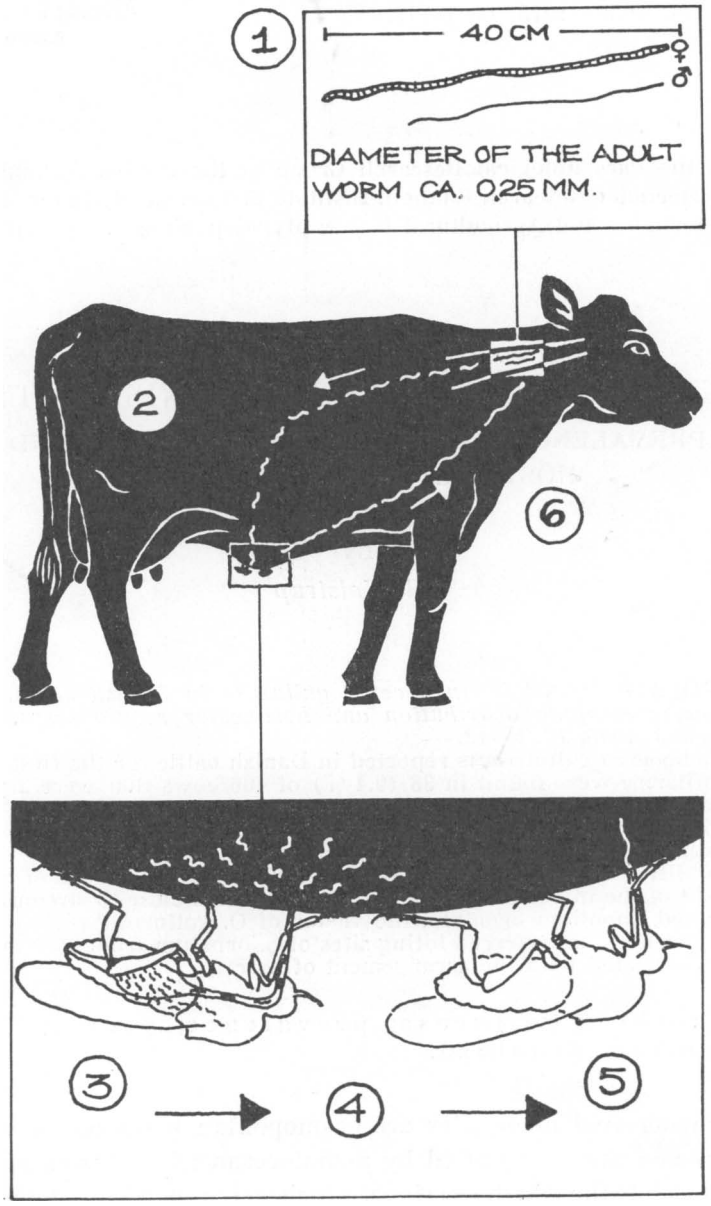


Figure 1. Lifecycle of Onchocerca gutturosa.

1. The adults mate and the female sheds microfilariae.
2. The microfilariae migrate out into the umbilical skin.
3. The microfilariae are taken up by the biting Simulium ornatum.
4. After ingestion of blood and microfilariae Simulium ornatum flies away and the microfilariae develop to infective larvae in 2—3 weeks.
5. Infective larvae migrate into the cow when the black fly takes a subsequent blood meal.
6. Infective larvae migrate up in the ligamentum nuchae and develop to adults.

reasons for the rather limited research on O. volvulus. Therefore, an increasing interest has been focused on Onchocerca infections in domestic animals in temperate regions with the hope that they might mimic the human disease (*Nelson et al.* 1966, *Eichler* 1971, 1973 a, b, *Eichler & Nelson* 1971, *Mellor* 1973).

The life cycle of Onchocerca spp. is heteroxenous with vertebrates acting as definitive hosts and blood sucking Diptera serving as vectors. In the vertebrate host the adult nematode produces embryos called microfilariae which inhabit the skin. When susceptible flies bite an infected host, microfilariae are ingested with the blood meal and migrate to the thoracic muscles where they develop as intracellular parasites. After moulting twice to the third, or infective, stage the larvae migrate from the fly to the final host when the fly takes a subsequent blood meal. The nematodes mature in the vertebrate. After mating the female sheds microfilariae.

The adult stage of Onchocerca gutturosa Neumann 1910 is commonly found in cattle in the ligamentum nuchae and less frequently in the connective tissue surrounding other organs such as the spleen and rumen, the tibiofemoral ligaments, stifle joints, scapular cartilage and humoral trochanter. A diagram of the life cycle of O. gutturosa is shown in Fig. 1. Infection with O. gutturosa is not considered of economic importance and consequently it has received little attention in the veterinary literature.

Although *Stiles* (1892) included a worm, probably O. gutturosa, in a check list of cattle parasites under the name O. lienalis, O. gutturosa was first described in detail from North Africa by *Neumann* (1910). In Europe it has been reported from France (*Popescu-Baran* 1939), Rumania (*Oltaneu & Georgescu* 1965), USSR (*Gnedina* 1950), Ukraine (*Mikhailyuk* 1967), Austria (*Supperer* 1952) and England (*Steward* 1937, *Webber et al.* 1957, *Venkataratnam & Kershaw* 1961, *Eichler & Nelson*).

There are no previous reports of O. gutturosa occurring in Denmark.

MATERIALS AND METHODS

From February to May 1973 cattle from Jutland, Denmark, were examined at the incinerating plant "Kronjyden" and the abattoirs "Skive Andelsslagteri", "Tulip-Vejle" and "Tulip-

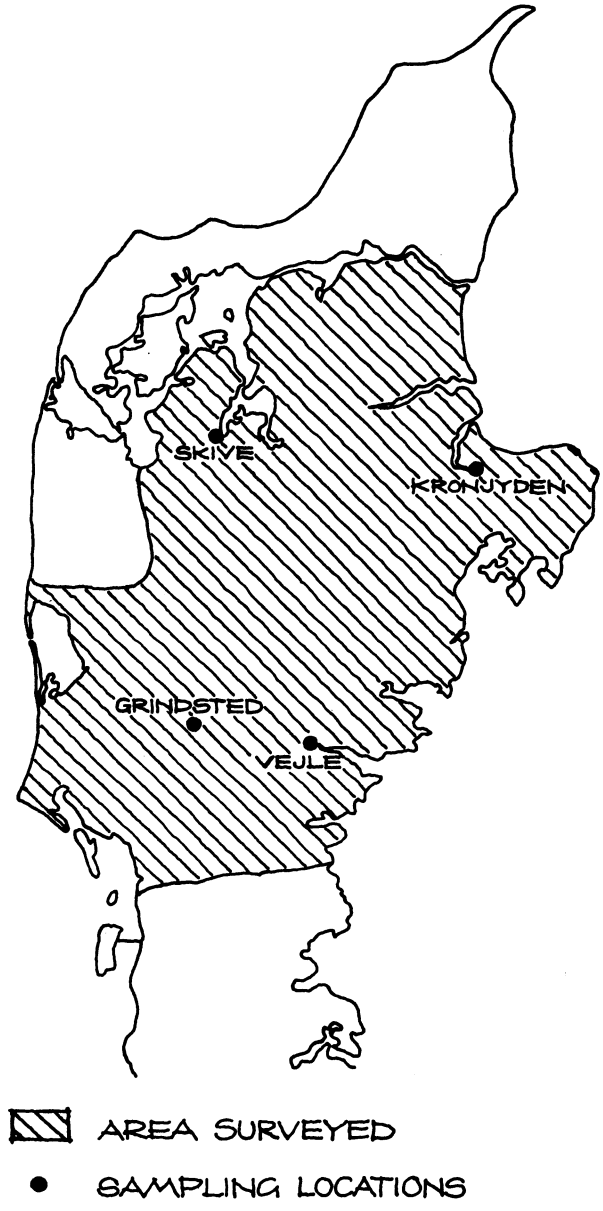


Figure 2. Sampling area.

Grindsted". Fig. 2 shows the location of these collecting sites and the area from which the examined cattle originated.

Cattle older than 2 years were examined. The diagnosis was made by cutting pieces of skin measuring approx. 5 × 10 cm from the umbilical area with subsequent examination for microfilariae using the following technique modified after *Eichler & Nelson* (1971).

After shaving the skin, 3 pieces of tissue measuring 4 mm in diameter were taken at random with a circular leather punch. The subcutaneous tissue was removed from the skin punches, macerated and placed into bags made from surgical gauze (Fig. 3). The bags were then suspended in a 5 ml syringe filled with 75 % Tyrode's solution, 25 % human or pig serum and penicillin. The syringe was held in a vertical position at room temperature for 4 to 18 hrs. before the medium was ejected into a centrifuge tube. Following centrifugation for 15 min. at 2000 r.p.m., the supernatant was decanted and the remaining 0.1 to 2.1 ml was examined for microfilariae under the compound microscope at 100 ×. This extraction technique was used also when the horizontal distribution of microfilariae throughout the skin was determined.

A separate search for adult worms was conducted at "Kronjyden". The ligamentum nuchae of infected cattle was removed from the cranium to the 8th or 10th thoracic vertebra and exa-

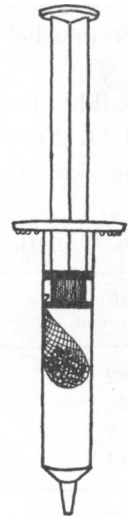


Figure 3. Five-ml syringe with suspended bag.

mined thoroughly for adult worms. In addition the gastrospleenic region and the tibiofemoral condyle were examined.

To determine the filarial species, extracted microfilariae were suspended in serum from which thick smears were made. The smears were stained with either Giemsa or Haematoxylin. Living infected cattle were found by collecting wild simuliids that fed around the umbilical region and examining their blood meal for microfilariae under the compound microscope. Cows I, II, III and IV were found to be infected in this way. All of these 4 cows were Red Danish Milk Breed and originated from the Gjern area.

Laboratory reared simuliids were fed on infected cattle according to the method of *Eichler* (1973 a). The laboratory reared and naturally occurring simuliids were dissected and examined for developing parasite stages in the midgut, thorax and head.

RESULTS

Out of a total of 406 cows, 38 (9.4 %) were found to be infected with O. gutturosa. The prevalences of infection at the incinerating plant and the abattoirs are presented in Table 1.

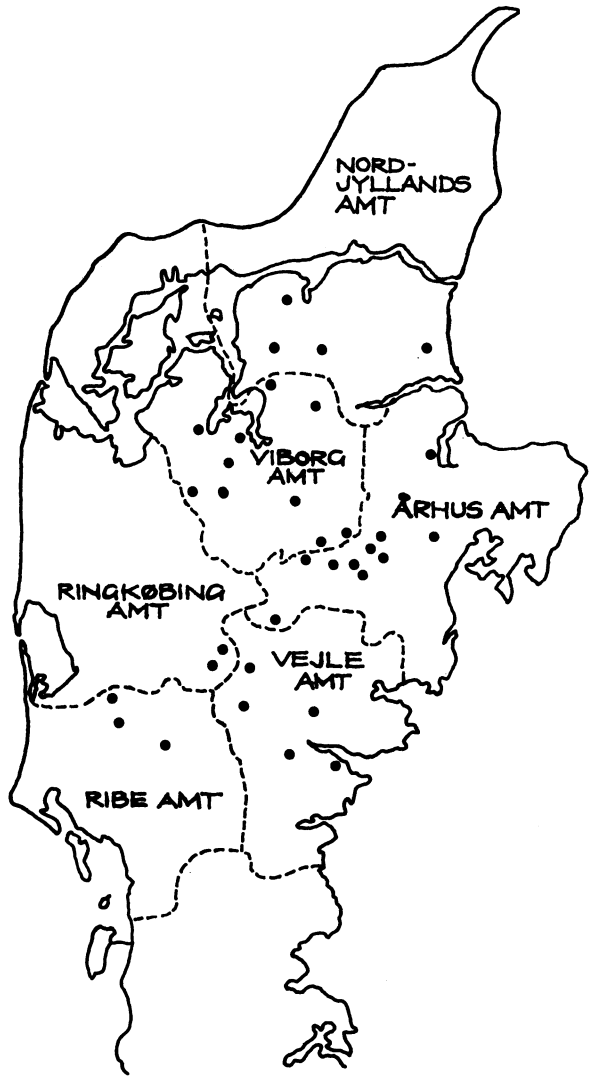
To assess the geographical distribution, the addresses of 34 owners of infected cattle were obtained. The results are shown in Fig. 4.

The infection frequency of different breeds is shown in Table 2. The results in Table 2 were tested using a chi-square test. This revealed no significant difference between the breeds ($0.05 < P < 0.1$).

In order to elucidate possible strain differences between the British and Danish strain of O. gutturosa, the horizontal distribution of microfilariae in the skin was investigated. The results

Table 1. Infection rates of cattle from various localities.

Incinerating place or abattoir	Number of animals examined	Number of animals infected	Per cent infected
"Skive"	92	8	8.7
"Kronjyden"	240	19	7.9
"Tulip-Vejle"	58	7	12.1
"Tulip-Grindsted"	16	4	25.0
Total	406	38	9.4



F i g u r e 4. Places wherefrom infected cows originated. (Corresponds to Table 2).

shown in Fig. 5 are based on examination of 6 cows. Skin snips were examined from the places indicated by arrows. The results correspond to those of *Eichler & Nelson* (1971).

Ten ligamenta nuchae, 7 gastrospleenic regions and 2 tibiofemoral ligaments of infected cattle were examined for adult stages of O. gutturosa but none was found.

Table 2. Frequency of infection in relation to breed.

Breed	Number of animals examined	Number of animals infected	Per cent infected
Black Pied Friesian Cattle	138	11	8.0
Red Danish Milk Breed	112	13	11.6
Jersey	59	4	6.8
Others	5	2	—
Total	314	30	9.6

In May 1973, 4 species of Diptera were caught when biting cattle infected with O. gutturosa. Eighty-three Simulium ornatum Meigen (sensu *Davies* 1968), 1 Simulium erythrocephalum Degeer and 1 Culicoides sp. were caught on the ventral part of abdomen while 17 Simulium equinum were caught in the ear. The flies were dissected and 12 S. ornatum contained filarial

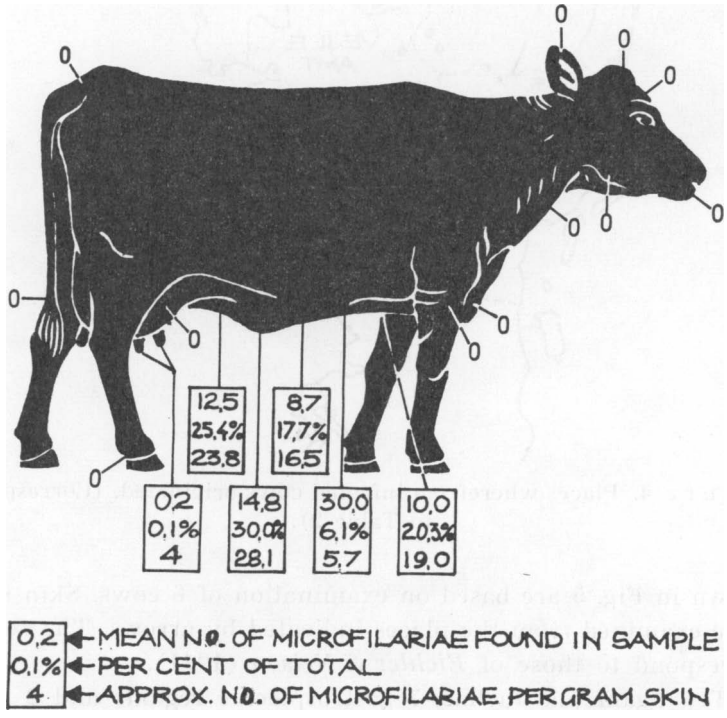


Figure 5. Horizontal distribution of microfilariae in skin of cows (mean of 6 cows).

larvae, morphologically identical with O. gutturosa. Of 16 laboratory reared S. ornatum fed on infected cows, 2 contained infective larval stages of O. gutturosa. Thus, S. ornatum can act as a vector of O. gutturosa in Denmark.

Eichler (1971) stated "The biting behaviour of S. ornatum is affected by the arrangement of the hair in relation to the skin surface. The umbilicus is preferred because here the hairs are so arranged that the flies readily gain access to the skin". To determine whether this also occurred when the Danish S. ornatum strain took a blood meal, detailed observations were carried out on the 4 infected cows of the Red Danish Milk Breed.

Cow I was dark red to brown with a thick hair cover on the ventral side. It was not often bitten as the simuliids were not able to penetrate the thick hair cover and were severely disturbed by some hair approx. 4 cm long that hung from the area between the umbilicus and the udder. The cow was bitten on the ventral parts of the udder, teats, on the umbilicus and in a whorl about 30 cm in front of the umbilicus.

Cow II was yellowish red with a very protruded umbilicus. It had a thin hair cover and did not possess the long hair between the umbilicus and the udder. The whorls and the midventral line were much broader than on cow I and left areas of bare skin. The black flies heavily attacked the area 10 cm behind the umbilicus, but were less interested in the umbilicus and the udder. On 1 occasion with moderate attack, red blood marks due to recent bitings were counted. After 2 hrs. exposure there were 21 marks on the umbilicus, between the umbilicus and the udder 32 marks, on the udder 12, and 4 marks were counted in a whorl 15 cm in front of the umbilicus.

Cow III had the same colour as cow I and had an umbilicus that did not protrude very much. A whorl just in front of the umbilicus and expecially the udder were attacked whereas the umbilicus was bitten less often.

Cow IV was the most attacked. Its colour was the same as cow II, but the hair cover was thinner and with a broad band of skin left naked on the mid ventral line and umbilicus. The breadth of this band was about 1 cm except for the umbilicus, where it was approx. 4 cm. The umbilicus was protruding. Cow IV was heavily attacked over an area from 1 m to 75 cm in front of, and 25 cm behind, the umbilicus.

A horse with a very thin hair cover was also observed. S. ornatum attacked a much broader area, and although the simuliids still concentrated in the area around the umbilicus, they accumulated in a rectangular area which stretched from 20 cm in front of the umbilicus to the udder with a breadth of approx. 40 cm.

DISCUSSION

Different infection rates of cattle with O. gutturosa have been reported from Europe. *Popescu-Baran* (1939) found that 56 out of 60 cows in France harboured O. gutturosa. In England, *Webber et al.* (1957) found that 32 % of 164 cows had microfilariae in the skin, while *Eichler & Nelson* (1971) reported that at least 50 % of all cows older than 4 years were infected. The frequency in Jutland is low compared to other European areas. This is perhaps due to the northern location of Denmark and the occurrence of few areas where the vector can attack without disturbance from the wind. The differences in the investigated age groups also have a profound effect on the infection frequency.

In general, infected cows came from farms situated close to rivers. This is not surprising as the development of simuliids is closely dependent on rivers, where dense populations of adults are likely to be found.

Twenty years ago it was thought that Onchocerca microfilariae were randomly distributed in the skin. But in 1954 it was shown that O. volvulus microfilariae in humans were distributed in the skin in high densities near the nodules caused by the adult worms and the sites most often bitten by the vector (*Kershaw et al.* 1954). *Venkataratnam & Kershaw* (1961) examined the ears, arms, briskets, abdomens and thighs of 20 cows infected with O. gutturosa and found the microfilariae only in the skin near that part of the body infected with adult worms — the ligamentum nuchae, gastrospleenic region or both. This has not been confirmed by *Eichler & Nelson* who examined hides in great detail and showed that the density of microfilariae was highest at the biting sites of the vector, S. ornatum. The results here show that the microfilarial distribution of the Danish strain of O. gutturosa is similar to that found by *Eichler & Nelson.* The microfilariae were concentrated on the ventral side of cattle with the highest density around the umbilicus and the mid ventral line, and this coincided with biting sites of S. ornatum.

While in England the author easily found adults of O. gutturosa in the ligamentum nuchae, the failure to find adult O. gutturosa in Danish cattle may have resulted from the small sample number or different location of the adult worms, as recorded by *Bwangamoi* (1970) in Uganda. It is possible that the Danish strain of O. gutturosa is also not located in the host in the same places as the British strain. Confirmation of this hypothesis will only come from further investigations and the recovery of the adult stages.

S. ornatum is a vector of O. gutturosa throughout Europe (*Steward* 1937, *Gnedina* 1950, *Supperer* 1952, *Eichler* 1971). The present study shows that S. ornatum also acts as a vector of O. gutturosa in Denmark. Only 1 Culicoides sp. and 1 S. erythrocephalum were found. These and other biting insects emerging later in the season may also act as vectors, but this was not demonstrated in the present study.

There are no reports on host selection of cattle-biting simuliids or on differences in the preferred biting sites on individual cows. The comparison with the biting habits on a horse, where the simuliids can easily penetrate the hair cover over the whole ventral side, disclosed that S. ornatum was attracted towards the ventral side of the attacked animals. The hair cover is an important factor that determines where the flies bite when they arrive. This observation agrees with observations of *Davies* (1957) and *Eichler,* but there are more places where the black flies can gain access to the skin of the Red Danish Milk Breed. It would be most interesting to know if these individual differences were repeated for the distribution of O. gutturosa in the host. In this way it might be determined if the O. gutturosa microfilariae could actively seek out the places which were bitten most frequently.

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SAMMENDRAG

Onchocerca gutturosa i dansk kvæg. Prævalens, geografisk udbredelse og vektor-værtsforhold.

1. Onchocerca gutturosa er for første gang beskrevet i dansk kvæg. Mikrofilarier blev fundet i 38 (9,4 %) af 406 undersøgte køer i alderen over 2 år.
2. Mikrofilarierne var ikke jævnt fordelt i huden af køerne, men var koncentreret i navleområdet.
3. Der kunne ikke konstateres forskelle i infektionsfrekvensen mellem de forskellige kvægracer.
4. De fleste inficerede køer havde græsset på marker tæt ved åer, som indeholdt larver af kvægmyggen Simulium ornatum, vektoren for O. gutturosa.
5. Forskelle i kvægmyggenes foretrukne bidesteder kunne forklares ved hårenes arrangement.

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