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YERSINIA ENTEROCOLITICA INFECTION IN GOAT

A SEROLOGICAL AND BACTERIOLOGICAL INVESTIGATION

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KROGSTAD, OLA: *Yersinia enterocolitica infection in goat. A serological and bacteriological investigation.* Acta vet. scand. 1974, *15*, 597—608. — The distribution of agglutinating antibodies to Yersinia enterocolitica type 2 in sera from goats in an infected herd, and in 190 other animals from different parts of the country was studied. The faecal excretion of Yersinia enterocolitica from the same animals was also examined. Experimental inoculations of two goats were carried out. The serological results indicate that subclinical cases had occurred in the infected herd during the enzooty and during the following months. Faecal excretion of the organism was observed during the first month after the acute phase of the disease. It was found to be difficult to induce the disease experimentally, but clinical signs were observed in 1 goat after injection of live Yersinia enterocolitica intraperitoneally. The Widal-titre rose to 1/1250 during the first 2 weeks after the inoculation and then fell to 1/80 during the next 2 months. The serological results indicate infections with Yersinia enterocolitica, if a Widal-titre of at least 1/80—1/160 in the first month after the acute phase of a disease and a fall to a low level during the following months are found. Among animals from different partst of the country 16.3 % had a Widal-titre which indicated infection with Yersinia enterocolitica during the previous 3—6 months.

Yersinia enterocolitica; serology; goat.

During recent years, infection with Yersinia enterocolitica has been reported with increasing frequency as a cause of disease in man and animals. The occurrence of Y. enterocolitica in man is associated with different types of symptoms such as acute abdominal pain, erythema nodosum and polyarthritis (*Winblad et al.* 1966, *Nilehn & Sjøstrøm* 1967, *Winblad* 1969, *Wauters* 1970, *Mollaret* 1971, 1972, *Lassen* 1972 a, *Rabson & Koornhof* 1972). Cases of septicaemia have also been reported (*Mollaret et al.* 1971, *Zen Yoji & Maruyama* 1972). In animals Y. enterocolitica

has caused enzootics in herds of chinchillas in Europe and America (*Wetzler & Hubbert* 1967, *Mollaret 1972)*, pigs (*Mollaret* 1972) and goats in Norway (*Krogstad et al.* 1972). Infections have also been reported in dogs, sheep, cattle and rabbits (*Langford* 1972). *Dubois et al.* (1972) found that 14 % of the hares in Belgium were carriers of the organism.

Little is known about epidemiology and pathogenesis. There are, however, indications that water may be a reservoir of infection (*Lassen* 1972 b). It is likely that the main route of infection is by ingestion (*Mollaret* 1972, *Lassen* 1972 b, *Gutman et al.* 1973). In connection with the outbreak of disease in goats transmission to man was reported (*Krogstad et al*).

The typing of Y. enterocolitica is based upon somatic O- and flagellar H-antigens (*Winblad* 1967, *Wauters, Wauters et al.* 1972, *Knapp* 1973, *Hurvell* 1973). Y. enterocolitica infection in man and animal is diagnosed by demonstrating agglutinating antibodies in sera or the presence of the organism from pathological materials such as faeces (*Niléhn* 1969, *Wauters, Szita et al.* 1971). In the case of the outbreak in goats (*Krogstad et al.*) Y. enterocolitica type 2 with antigen factors 2 a, 2 b and 3 was demonstrated. This case stimulated to further investigation of the distribution of this organism in goats as reported in the present paper.

Animals in the affected herd were observed closely for clinical signs of illness during the following 12 months. Sera from sick and recovered animals were examined for agglutinating antibodies to Y. enterocolitica type 2 in acute and convalescense phases, and for the presence of Y. enterocolitica in the faeces. An experimental inoculation of goats with live Y. enterocolitica was also carried out. In order to study the distribution of Y. enterocolitica in the goat population in Norway a serological and bacteriological survey was done.

MATERIAL AND METHODS

Sera

1) Sera were obtained from the following sources: 60 samples were obtained from the acute and convalescence phases of sick animals in the infected herd. A total of 134 samples was collected in the autumn, winter and spring from healthy animals in the same herd.

2) Serum samples from 190 healthy female goats from 11 different parts of the country were also obtained. Most of the sera were collected during the spring.

3) Rabbit immune sera were prepared as described by *Winblad et al.* 1966. Rabbits received weekly i.v. injections of 1 ml Y. enterocolitica boiled O-antigen suspension. The rabbits did not show any detectable agglutinating antibodies before immunization. After immunization a titre of 1/1250—1/2500 was found.

Isolation of Yersinia enterocolitica

Faeces from 158 of the animals, 7 faeces samples from the herdsmen and a total of 30 samples from the environment (drinking water, stalls and manure cellar in the infected herd) were cultured on bovine blood and bromthymolbluelactose plates (*Nordic Committee on food analysis* 1969). The plates were incubated at 24°C and at 37°C. Suspected colonies collected after 24, 48 and 72 hrs. incubation were examined biochemically and serologically. Faeces stored at 4°C in broth were subcultured after 2 and 4 weeks (*Ahvonen* 1972).

Experimental inoculation of goats

Two goats (1 of each sex) 9 months old were used. These animals were negative with respect to Y. enterocolitica type 2 agglutinating antibodies, and no faecal secretion of the bacteria was demonstrated. The goats were fed daily for 7 days with approx. 10^9 cells of live S-form of Y. enterocolitica type 2. The same animals received 3 months later about 2.10^9 cells of live S-form of the same organism intraperitoneally. The organism was incubated in broth at 24°C for 24 hrs. Sera were collected once a week from each animal. During the inoculation period and the following 3 weeks bacteriological investigation of faeces was performed daily.

Housing and feeding

During the winter the animals in the herd were placed in stalls with relatively many animals in each, and they were not tied up. The feeding during winter was ensilage and feed concentrate, and the goats were on pasture in the mountains during the summer. The goats experimentally inoculated with Y. enterocolitica were given ensilage and feed concentrate.

Antigen

Antigen preparations of the following strains were used:

Y. enterocolitica type	2	isolated from goat (NVH* 2943)
„	„ 3	(NVH 2971)
„	„ 9	(SIFF** 201)
„	„ 13	(SIFF 553)
„	„ 5	(Pasteur 123)
Brucella abortus		(NVH 344360)

Heat treated O-antigen

Agar plates (1.2 % agar) were inoculated from a typical colony of S-form and incubated for 48 hrs. at 24°C. The colonies were harvested and the cells washed twice in saline and the suspension autoclaved for 1 hr. at 120°C. The suspension was then washed repeatedly and suspended in saline to give an optical density of 0.6 as measured in a Vitatron 20 spectrophotometer***.

Alcohol treated O-antigen

The organisms were prepared as described above, but instead of autoclaving, the sedimented material was suspended in equal volume of 96 % alcohol and saline. The suspension was then stored at 4°C until used. It was then diluted in saline to give an optical density of 0.6.

Agglutination test

The sera were inactivated at 56°C for ½ hr. prior to use. Two fold dilutions in saline of each serum starting with 1/10 were mixed with an equal volume (0.25 ml) of antigen suspension. Preliminary investigations showed that agglutination with alcohol treated O- and heat treated O-antigen gave almost identical results, but using the former O-antigen gave the most reproducible results. The results were also more reproducible on incubating the tubes at 52°C rather than at 37°C. There was no significant difference between reading after 24 and 48 hrs. incubation. The method used for the examinations involved incubating

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* The Culture Collection of the Department of Microbiology and Immunology, Veterinary College of Norway, Oslo, Norway.

** National Institute of Public Health, Oslo, Norway.

*** Dieren, The Netherlands.

the tubes in a water bath at 52°C for 24 hrs. With the aid of a magnifying glass the end point of agglutination was determined as the highest dilution with a finely granular agglutinate (Widal-titre).

RESULTS

Titres of agglutinating antibodies in the acute phase of 10 infected animals and in the herdsman who had been ill are shown in Table 1. The sera were collected up to 2 weeks after the onset of diarrhoea and from 4 animals 3 months later. One goat (368)

Table 1. Yersinia enterocolitica type 2 O-Widal-titre in infected goats which had been ill, and an infected human.

	Titre 1—2 weeks after diarrhoea	Titre 3 months later	Titre 6 months later	Demonstration of Yersinia enterocolitica in faeces*
Goat no. 368	1/80	1/640	killed	—
„ „ 414	1/640	1/80	1/40	+
„ „ 436	1/640	1/160	1/40	+
„ „ 484	1/320	1/40	1/20	+
„ „ 394	—	killed		—
„ „ 443	1/160	„		+
„ „ 452	1/640	„		—
„ „ 454	1/40	„		+
„ „ 471	1/160	„		—
„ „ 474	1/40	„		+
Man	1/1250	1/40	1/40	—

* + Yersinia enterocolitica isolated.
— Yersinia enterocolitica not isolated.

had a titre of 1/640 3 months after the enzooti, the other animals having a lower titre at that time. Y. enterocolitica was demonstrated in the faeces from 6 of the 10 animals in the acute phase of the disease (Table 1). The organism was also demonstrated in the faeces of 5 out of 45 other animals of which some had a period with diarrhoea earlier in the spring (Table 2). Y. enterocolitica could not be demonstrated from faeces of the same animals 3 months later. The animals had then been treated with drugs and had grazed 2 months in the mountains. The distribution of agglutinating antibodies to Y. enterocolitica type 2 O-antigen in sera from the known infected and some control animals in the herd during the 12 months following the enzooti is shown in

Table 2. Distribution of Yersinia enterocolitica type 2 O-agglutinating antibodies and faecal secretion of the organism in the infected herd during 12 months after the enzooty.

Season		Diagnosis	Total number animals	Distribution of Y. enterocolitica type 2 agglutinating antibodies					Isolation attempts	Number of isolations
				0	1/20	1/40	1/80	1/160 and above		
May	1972	diarrhoea	10	1	0	2	1	6	10	6
June	1972	healthy*							45	5
Sept.	1972	healthy	76	32	32	19	1	1	76	0
„	„	were ill during the previous spring	18	6	4	5	1	2	18	0
Febr.	1973	healthy**	35	8	5	15	6	1	10	0
March	1973	diarrhoea	15	5	2	5	2	1	15	0
May	1973	healthy**	40	21	7	11	1		20	0

* Some had had a period with diarrhoea.
** Including remaining earlier infected animals.

Table 2, which also includes the culture examinations. Animals with periodical diarrhoea were closely investigated with respect to yersiniosis. During the 12 months' period 1 goat died of Escherichia coli infection and another 4 goats died of Cl. perfringens type D enterotoxaemia. There were 15 cases with diarrhoea during this period. Y. enterocolitica could not, however, be demonstrated in the faeces from any of these cases. Agglutinating antibodies were demonstrated in serum with a titre of 1/160 from 1 and 1/80 from 2 other goats. Two months later all of these 15 goats had a titre of $\leq$ 1/40. Examinations of the sera from the sick animals against Y. enterocolitica types 5, 9, 13 and Brucella abortus O-antigens gave negative agglutination. When testing the sera with Y. enterocolitica type 3 O-antigen the Widal-titre was almost identical to agglutination to Y. enterocolitica type 2. Samples from the environment in the stable of the infected herd were also examined with respect to Y. enterocolitica with negative results. The antigen was tested each time against homologous rabbit immuneserum.

Feeding the 2 experimental animals with live culture of Y. enterocolitica type 2 did not produce clinical symptoms. However, in 1 (animal A) of 2 goats that received an intraperitoneal

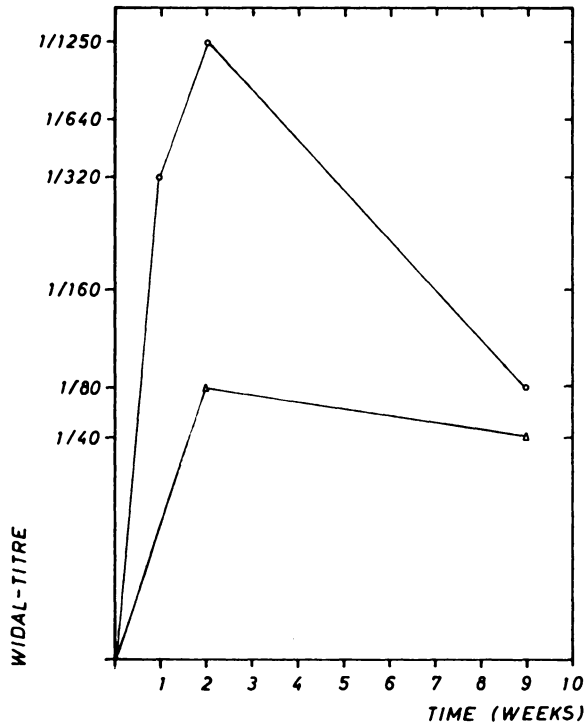

Figure 1. Yersinia enterocolitica type 2 O-Widal-titre in 2 goats which were inoculated intraperitoneally with live bacteria in the S-form.
Animal A ○———○ Male.
„ B △———△ Female.

injection of live S-form of Y. enterocolitica clinical signs were observed 3 days later. The temperature increased slightly and the goat was depressed. The faeces were mucous with some fluid. Y. enterocolitica was isolated from the faeces. The titre of agglutinating antibodies to Y. enterocolitica in the serum was 1/320

Table 3. Distribution of Yersinia enterocolitica type 2 agglutinating antibodies in healthy goats.

	Distribution of Yersinia enterocolitica antibodies					Total
Dilution	0	1/20	1/40	1/80	1/160	
Number of goats	52	36	71	25	6	190
Percentage	27.4	18.9	37.4	13.2	3.1	100

at 1 week and 1/1250 at 2 weeks after the onset of clinical signs (Fig. 1, animal A). After 2 months a titre of 1/40 was found. Clinical signs were not observed in the other inoculated animal (Fig. 1, animal B). The titre of agglutinating antibodies was 1/80 2 weeks after the inoculation.

The distribution of Y. enterocolitica type 2 agglutinating antibodies in sera from goats from different parts of the country is shown in Table 3. Most of the sera were collected during the spring. Agglutinating antibodies were demonstrated in sera collected from each of the 11 parts of Norway. Agglutination to Y. enterocolitica types 5, 9 or 13 O-antigen gave no agglutination. Y. enterocolitica was not demonstrated in any of the 60 animals from which faeces were received.

DISCUSSION

The diagnosis of Y. enterocolitica infection is generally based upon demonstration of agglutinating antibodies in the serum in the acute and convalescence phases and isolation of the organism from pathological material (*Niléhn* 1969, *Wauters* 1970). The present investigation shows that during the first 2 weeks after the acute phase of the disease the titre of antibodies rises rapidly (Table 1 and Fig. 1), and then falls to 1/20—1/80 during the following 3—6 months. This is in accordance with results for humans found by other authors (*Niléhn, Wauters*). One of the animals (Table 1, No. 368) had a Widal-titre of 1/640 3 months after the disease onset, and it is possible that this goat was in constant contact with the organism during the time in question. Humans may have high titres for years without any clinical signs (*Lassen* unpub.).

There are several reports which demonstrate minimum Widal-titres of 1/40—1/160 in humans with yersiniosis in connection with the acute phase (*Winblad et al.* 1966, *Wauters, Szita et al.* 1971, *Ahvonen* 1972). The present investigation shows that a Widal-titre of at least 1/80—1/160 within the first month after the acute phase followed by a fall during the following 3 months to a low level (Tables 1 and 2, Fig. 1) may indicate an infection with Y. enterocolitica. Out of the animals in the infected herd 30.9 % had a titre $\geqq$ 1/40 and 5.3 % $\geqq$ 1/80 3 months after the enzooti. This indicates that subclinical infections have occurred. The titres found in sera from sick and healthy animals during

the following winter would indicate that some animals were infected with the organism. Three of the 15 goats with periodic diarrhoea showed a Widal-titre which indicated infection with Y. enterocolitica. The organism could not be isolated from the faeces of any of these animals. It is, however, difficult to isolate Y. enterocolitica from faeces samples, because the organism is easily dominated by other bacteria such as Escherichia coli. By using a modified selenite broth as a selective medium, isolation of Y. enterocolitica has been facilitated (*Wauters*). In the present investigation the organism was only isolated directly on blood plate.

The non-pathogenicity, under experimental condition, of Y. enterocolitica both for laboratory animals and species susceptible to natural infection blocked pathogenetic and epidemiological studies (*Mollaret* 1972). *Wetzler et al.* (1968), however, induced disease in gerbil by injection of live Y. enterocolitica intraperitoneally. In the present investigation disease could not be provoked by feeding animals with live Y. enterocolitica. Injection of live S-form of the bacterium intraperitoneally gave clinical signs, and the organism was also isolated from faeces. Agglutinating antibodies in the serum against the same organism rose from $< 1/10$ to 1/2500 during the first 2 weeks after the clinical symptoms had been observed. During the following months the titre decreased to a low level. These results may give an idea of the serological development in connection with the disease.

Among the healthy animals from different parts of the country 3.1 % had a titre of 1/160 and 16.3 % a titre $\geqq 1/80$. These results indicate that some of the animals may have been exposed to an infection with Y. enterocolitica during the previous 3—6 months. Of the animals 53.7 % had a titre $\geqq 1/40$. The organism was not isolated from any of the samples of faeces which were collected in these cases. The serological investigation indicates that the organism may be an ubiquitous bacterium in the environment of goats from the whole country. It may, however, induce diarrhoea and death among goats (*Krogstad et al.* 1972), if predisposing factors are present. These factors may include many animals close together in the stable, changes in feeding, poor quality of the fodder or other types of diseases.

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SAMMENDRAG

Yersinia enterocolitica infeksjon hos geit.
En serologisk og bakteriologisk undersøkelse.

En har undersøkt konsentrasjonen av agglutinerende antistoffer mot Yersinia enterocolitica type 2 hos klinisk syke og friske geiter i en infisert besetning. Utskillelsen av bakterien i feces fra de samme dyrene ble også undersøkt. De serologiske resultatene tyder på at subkliniske tilfeller av infeksjon med Yersinia enterocolitica har forekommet i det aktuelle tidsrommet. I den første måneden etter enzootien ble bakterien isolert fra feces fra noen av dyrene, men senere kunne den ikke påvises.

Eksperimentell podning av to dyr per os gav ingen kliniske symptomer, men ved injeksjon av levende bakterier intraperitonealt ble kliniske symptomer observert. Det ene dyr ble noe nedstemt og fikk løs avføring samtidig med at bakterien ble isolert i fra feces. Widaltitret økte fra $< 1/10$ til $1/1250$ i løpet av de første ukene etter at de kliniske symptomer var observert. Konsentrasjonen av agglutinerende antistoffer i serum sank så i løpet av 2 måneder til $1/80$. De serologiske resultatene fra disse to undersøkelsene synes å tyde på at et Widaltiter på minimum $1/80$—$1/160$ i løpet av den første måneden etter inntrådt akutt sykdom, fulgt av en nedgang i løpet av de kommende

måneder, kan betraktes som et indikasjon på infeksjon med Yersinia enterocolitica.

Serum fra geiter fra ulike kanter av Norge blev også undersøkt, og 16,3 % hadde et Widal-titer som tydet på at infeksjon med Yersinia enterocolitica hadde forekommet i løpet av de siste 3—6 månedene. Bakterien ble imidlertid ikke påvist fra feces fra noen av disse dyrene.

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