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YERSINIA ENTEROCOLITICA

EXPERIMENTAL PATHOGENICITY FOR CHINCHILLA

By

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RAEVUORI, M., S. M. HARVEY and M. J. PICKETT: *Yersinia enterocolitica. Experimental pathogenicity for chinchilla.* Acta vet. scand. 1979, *20*, 82—91. — An intragastric inoculation of approx. 2×10^{10} Yersinia enterocolitica cells killed chinchillas in three days in the case of four strains out of six tested. Because of the sensitivity of chinchillas to this bacterium, the test is useful for the evaluation of the virulence and invasiveness of Y. enterocolitica isolates. This animal model could also be used for studies on the mechanism of the infection.

Yersinia enterocolitica; chinchilla; experimental infection; serum titers.

Yersinia enterocolitica is pathogenic to both man and animals (*Bottone* 1977). The organism has been isolated from several animal species (*Langford* 1972, *Kapperud* 1975, *Bottone*). Swine are considered to be the most important animal reservoir of the human infection (*Esseveld & Goudzwaard* 1973, *Toma & Diedrick* 1975, *Pedersen* 1976). Although the disease often shows no clinical symptoms in animals, there are reports of more severe gastrointestinal and generalized infections caused by the organism in dogs (*Farstad et al.* 1976), cats, swine (*Ahvonen et al.* 1973), goats (*Krogstad et al.* 1972) and chinchillas (*Becht* 1962, *Akkermans & Terpstra* 1963, *Daniêls* 1963, *Hubbert* 1972).

Several attempts to establish experimental infections in animals have been made (*Carter et al.* 1973, *Niléhn* 1973, *Rakovsky* 1973, *Quan et al.* 1974, *Alonso et al.* 1975, *Une* 1977 a). Y. enterocolitica was not considered pathogenic to laboratory animals

until recently, when two human isolates were found to cause disease in mice (*Carter et al., Quan et al., Carter* 1975 b). Rabbits have also been infected experimentally (*Une* 1977 a).

The purpose of our study was to construct an animal model for evaluation of the pathogenicity of Y. enterocolitica strains using intragastric application to chinchilla.

MATERIAL AND METHODS

Strains

The following Y. enterocolitica strains from our collection (*Raevuori et al.* 1978) were used: 145, serotype 0:3, isolated from man; 146, 0:4, chinchilla; 151, 0:9, man; 159, 0:17, water; 165, 0:6, man; and ATCC 27729, 0:8, man. Strains 145 and 151 have been in culture collections for years. We received these European strains from the California State Department of Health, Berkeley, who kindly also provided strains 146 and 159 which have been isolated more recently and thus evidently not subcultured as many times as strains 145 and 151. An effort to limit subculturing has been made in the case of the two recent isolates: strain 165, our own isolate (*Greenwood et al.* 1975), and ATCC 27729 (*Carter et al.* 1973).

Test animals

The 60 chinchillas used in the study were donated by a local breeder. All animals, both male and female, were apparently healthy, weighed 450—650 g, and were approx. one year old. None had been previously inoculated with Y. enterocolitica. The animals were kept individually in normal rabbit cages at a room temperature of 20°C. Feed and water were given ad libitum. The feed (Rabbit Chow Checkers, Ralston Purina Co., St. Louis, Missouri, USA) did not contain added antimicrobial agents. Lights in the housing facilities were on during the day (for 10 hrs.) and off at nights. The animals were allowed to adjust themselves to the test conditions for at least three weeks before the experiments were made.

Inoculation

An overnight Y. enterocolitica culture in nutrient broth (Difco) at 27°C was transferred to Nutrient Agar (Difco) plates that were incubated at 27°C for 20 hrs. The culture was diluted, using

Sørensen's phosphate buffer (pH = 7.0), to the density intermediate between McFarland standards 4 and 5. The viable cell count in this solution was determined by plating on Nutrient agar, and ranged from 3.9×10^9 to 5.1×10^9 per ml. The anaesthetized animals (Sodium pentobarbital anaesthetic: Diabutal, Diamond Laboratories Co., Des Moines, Iowa, USA; i.p. injection) that had been without food for 24 hrs. were inoculated intragastrically with 5 ml of the bacterial suspension using Argyle MAR 2604 feeding tubes (Sherwood Medical Industries, St. Louis, Missouri, USA). Five chinchillas plus one control animal (which received only the buffer solution) were used for each strain. The health status of the animals was followed daily.

Samples from the animals

Faeces samples (about 5 g) for the analysis of Y. enterocolitica were collected before the inoculation, and one, seven and 14 days after it. Blood samples (about 3 ml) were taken by intracardial puncture from the anaesthetized animals just before the bacterial inoculation and two weeks after it. The sera were quickly frozen and stored at —20°C for less than a month until the serological tests were made. After killing the animals using overdoses of the anaesthetic liver, spleen and intracardial blood samples were taken for the analysis of Y. enterocolitica.

Detection of Y. enterocolitica

The isolation of the organism from the samples was done using both SS- and MacConkey-agars (Difco) incubated at 35°C for one and two days. No enrichment was used. Potential Y. enterocolitica colonies (small, colourless or slightly reddish) were inoculated into tubes of Klinger iron agar (KIA) medium (Difco) which were read after incubation for 24 hrs. at 35°C. Strains giving Y. enterocolitica-like reactions (slant, alkaline; butt, acid with or without small amounts of gas; no H_2S) were further characterized by ONPG and urease tests. Y. enterocolitica gives a positive reaction in these tests, for which the Key-tablet method (Key Scientific Products Co., Los Angeles, California, USA) was used with incubation for 6 hrs. at 35°C.

Serological methods

The bacterial culture for the preparation of O-antigen was made similarly to the inoculum, except that the final density of

the buffered culture was adjusted to the density intermediate between McFarland standards 0.5 and 1. The viable Y. enterocolitica counts for the six strains were between 6.4×10^8 and 8.6×10^8 per ml. The cultures were autoclaved (121°C, 15 min.), stored in a refrigerator and used within two days. The frozen sera samples were thawed at room temperature and inactivated at 56°C for 30 min. The tube agglutination tests were done using the strain specific antigens. After the dilutions the test was kept for 4 hrs. at 37°C and then for 18 hrs. at 4°C after which the tubes were centrifuged and read.

Additional animal tests

Using strains 159, 165 and ATCC 27729, that were isolated from the livers of chinchillas inoculated in the previous phase of the study, additional tests were made. For each strain two animals were inoculated with 5 ml of the culture prepared as before (buffer dilution of the culture grown on nutrient agar at 27°C for 20 hrs.). Two animals per strain received 5 ml of the culture filtrate (Millipore, 0.45 μm). One control animal was included for each bacterial strain.

RESULTS

Table 1 shows the cumulative number of test animals that died in each group after inoculation. The number of Y. enterocolitica isolated from the faeces samples taken after one and seven days is shown in Table 2. Y. enterocolitica could not be detected in any of the samples taken before and 21 days after

Table 1. Cumulative number of deaths of chinchillas after the intragastric inoculation of Y. enterocolitica. Five animals and one control chinchilla were tested for each strain.

Strain	Cumulative number of deaths after the inoculation			
	1 day	2 days	3 days	4 or more days
ATCC 27729	0	0	4	4
165	0	3	3	3
146	0	0	2	2
159	0	0	2	2
145	0	0	0	0
151	0	0	0	0
Controls	0	0	0	0

Table 2. Number of Y. enterocolitica in faeces after 1 and 7 days, and serum titers 14 days after the intragastric inoculation to chinchilla.

Strain	Y. enterocolitica count per g of faeces after the inoculation		Serum titer
	1 day	7 days	
ATCC 27729	8 000	d	d
	< 100	< 100	1280
	„	d	d
	„	d	d
	„	d	d
165	300 000	< 100	< 20
	8 600	„	20
	500	d	d
	< 100	d	d
	„	d	d
146	800	< 100	80
	500	„	40
	< 100	d	d
	„	< 100	< 20
	„	d	d
159	20 000	d	d
	300	100	40
	100	d	d
	„	< 100	< 20
	< 100	„	„
145	600 000	< 100	20
	90 000	„	< 20
	300	„	„
	< 100	„	„
	„	„	20
151	20 000	400	40
	15 000	< 100	< 20
	10 000	„	„
	800	„	„
	< 100	„	„

d = the animal died.

treatment. Table 2 also shows the serum agglutination titers 14 days after inoculation. Each animal had a titer of < 20 before inoculation.

The most remarkable necropsy finding from the animals in the ATCC 27729 group was an acute ulcerative inflammation of

the ileum, whereas the dead animals in the groups 146, 159, 165 had lesions of more generalized infection: enlarged, degenerated liver and dark, large spleen. Y. enterocolitica was isolated from the liver and spleen of all the dead animals and from some of the blood samples.

The results of the additional inoculation tests with Y. enterocolitica strains isolated from the livers of dead chinchillas were as follows: for the strain 159 one of the two animals died after the third day; for 165, one after the first days; and for ATCC 27729, two after the second day. The inoculations using the filtrates did not alter the health status of the chinchillas.

DISCUSSION

In this study the chinchilla was found to be a useful test animal for evaluation of the virulence of Y. enterocolitica. The pathogenicity of the organism has been tested in different animal species before. In most cases no pathogenicity has been shown except sometimes for certain especially potent strains. Because there are several reports of natural Y. enterocolitica epidemics in chinchilla, the results of the study were not unexpected. The test procedure used is simple and the animals are small and easy to handle. In some locations chinchillas are available cheaply for routine testing.

The four virulent strains (146, 159, 165 and ATCC 27729) killed some of the test animals within three days of inoculation. Y. enterocolitica was detected in the organs of the animals. The chinchillas that survived the treatment did not show any clinical symptoms nor was the organism usually present in their faeces one week after inoculation (Table 2). The differences in the responses of the animals can be partly explained by age: the older animals seemed to be more resistant. Y. enterocolitica counts in faeces after the first day vary remarkably because the samples were collected from the faeces excreted during the first day. Obviously the count would tend to be larger if excretion was later. No loose diarroeal stools were observed for any of the animals in the study. The serum titers of the survivors rose slightly during the study. The rise was significant in the case of strain ATCC 27729, which also produced the most severe symptoms and lesions in the animals. This strain was shown to be especially pathogenic: it has caused infection in mice (*Carter et al.* 1973,

Carter & Collins 1974, *Carter* 1975 a, b). Determinative comparisons of pathogenicity between the strains cannot be made because the isolates were not passed through animals before testing in order to alter any attenuation from in vitro subculturing. In our study, however, serotypes 4, 6, 8 and 17 were shown to be virulent, whereas 3 and 9 were not. *Une* (1977 a), who did not pass the strains through animals either, recently found the Japanese serotypes 3 and 9 to be pathogenic for rabbit using intraduodenal inoculation, whereas some other serotypes were nonvirulent.

In our study the inoculum was grown at 27°C. The properties of the organism depend on the growth temperature (*Bottone* 1977, *Pai & Mors* 1978). Intraperitoneal injections in mice have shown that cultures grown at 25°C are more potent than those grown at 37°C (*Niléhn* 1973). In previous studies of the strain ATCC 27729 lethal effect was obtained with cultures incubated both at 25°C (*Carter* 1975 b) and 37°C (*Carter et al.*) given perorally to mice.

The Sereny-test (*Szita et al.* 1973), rabbit peritoneal macrophages and HeLa-cells (*Une* 1977 b) have been used for evaluation of the invasiveness of Y. enterocolitica. The chinchilla-test has the advantage that the invasion occurs at the natural site, the epithelium of the intestinal wall, and is easy to recognize during necropsy. The culture filtrates made from three of the four virulent strains in our study had no visible effect on the animals tested. *Pai & Mors* from Canada have detected Y. enterocolitica enterotoxin using infant mouse assay, especially in cultures incubated for 48 hrs. at 26 or 28°C (*Pai & Mors*). The toxin titer was significantly less after 24 hrs. than 48 hrs. We used an incubation period of 20 hrs. We have also been able to confirm these results of *Pai & Mors* in our laboratory using the strain ATCC 27729.

The chinchilla-test could be useful for the evaluation of the virulence of isolates of Y. enterocolitica. Because the animal is quite sensitive to the bacterium, it should be possible also to study the pathogenic mechanism of the infection caused by different strains using this animal model.

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SAMMANFATTNING

Yersinia enterocolitica. Experimentell patogenitet för chinchilla.

Av sex testade Yersinia enterocolitica stammar dödade fyra stammar chinchillor inom tre dagar efter en i magsäcken utförd inympning av ungefär 2×10^{10} celler. På grund av detta djurlags känslighet för

Y. enterocolitica är detta test användbar för att bestämma virulensen och invasionsförmågan hos en isolat. Denna djurmodell kan också användas för att undersöka infektionsmekanismen.

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