Acta vet. scand. 1979, *20*, 387—395.

From the Department of Microbiology and Immunology,
Veterinary College of Norway, Oslo.

PREVALENCE OF TREPONEMA HYODYSENTERIAE IN HEALTHY PIGS*

By
Eivind Liven

LIVEN, EIVIND: *Prevalence of Treponema hyodysenteriae in healthy pigs.* Acta vet. scand. 1979, *20*, 387—395. — The prevalence of Treponema hyodysenteriae in faecal samples from healthy pigs of various ages in different farrowing units was investigated.

Samples from herds designated as Category I were processed within 2 hrs. of sampling. Samples from herds designated as Category II were transported 2 to 3 days before cultivation procedures started. T. hyodysenteriae was demonstrated in 53.7 % to 93 % of the samples collected from Category I herds. No marked difference in the frequency of positive samples from the various age groups of pigs was found. In Category II herds, the organism was demonstrated in 10 % of the samples.

The degree of beta-haemolysis shown by isolated strains was grouped into 3 groups: weak, moderate and strong. Strongly beta-haemolytic strains, supposedly enteropathogenic, were demonstrated in all Category I herds. Such strains were found in 4.6 % to 25 % of the positive samples in these herds. In Category II herds, 2 out of 17 positive samples harboured strongly beta-haemolytic strains of T. hyodysenteriae.

The amount of growth of T. hyodysenteriae on primary plates inoculated with sample material originating from the 2 categories of herds was mostly moderate or abundant. Strongly beta-haemolytic isolates originating from Category I herds produced abundant growth on primary plates in approx. 60 % of samples harbouring such strains. In samples from Category I herds with strains producing weak or moderate beta-haemolysis sparse and moderate amount of growth of the organism was predominant.

T. hyodysenteriae; prevalence; pig; pathogenicity.

Most countries with modern swine production report the occurrence of swine dysentery. *Doyle* (1944) suggested that Vibrio coli was the causative agent, though later workers, as

* The investigation was made possible by financial support from the Agricultural Research Council of Norway.

reviewed by *Harris & Glock* (1971), failed to reproduce the disease with certainty by the oral administration of this organism.

During the last decade, research on the aetiology of swine dysentery has been concentrated on anaerobic spirochaetes. Using direct FAT, *Terpstra et al.* (1968) demonstrated a spirochaete in the intestinal content from pigs suffering from swine dysentery. This organism was later isolated by *Taylor & Alexander* (1971) and *Harris et al.* (1972), and given the name Treponema hyodysenteriae. Swine dysentery has been successfully reproduced by oral inoculation of susceptible pigs with this organism (*Harris* 1974). Recent reports indicate, however, that the development of swine dysentery might also depend on the presence of other anaerobic organisms in addition to T. hyodysenteriae (*Lysons et al.* 1978).

Epidemiological information on T. hyodysenteriae and swine dysentery is sparse, and it does not seem to have been determined whether or not T. hyodysenteriae is a normal inhabitant of the pig intestine. The present work was therefore carried out to study the distribution of T. hyodysenteriae in healthy pigs originating from herds with no previous history of swine dysentery.

MATERIALS AND METHODS

Faecal samples were collected from 2 categories of herds. Category I comprised 6 different herds in south-eastern Norway. The herds were all farrowing operations either producing weaners for sale or themselves fattening through to slaughter. Standards of management and housing in the herds were high. Samples were collected from pigs belonging to 3 age (size) groups, 20—30 kg (Group I), 50—60 kg (Group II) and 70—90 kg (Group III). Freshly deposited faecal material was collected in sterile glass tubes and processed within 2 hrs. of collection.

Category II herds comprised 95 herds scattered throughout Norway. All were farrowing units producing young pigs for stock breeding purposes. Faecal samples were collected by the herd owners in the manner described above for Category I herds. These were then packed and dispatched to the Department of Microbiology and Immunology, no special thermostatic measures being taken. The period of time elapsing between the collection and processing of samples was 2 to 3 days.

Faecal content was inoculated on TSA-S400*, a selective medium described by *Songer et al.* (1976), by first depositing the faecal material onto the agar surface by means of a swab, and then streaking out the deposit with an inoculating loop. Three parallel cuts were then made in the agar to facilitate growth of the organism. The plates were incubated at 42°C in anaerobic jars with 80 % H_2 and 20 % CO_2 and with a cold palladium catalyst. Plates inoculated with samples from Category I herds were read 3 times at 48 hrs. intervals, while plates inoculated with samples from Category II herds were read just once after 48 hrs. of incubation.

The demonstration of T. hyodysenteriae was based on the characteristic cultural properties and typical appearance on phase microscopy. These criteria were to be satisfied both from primary and secondary agar plates. When T. hyodysenteriae was demonstrated, the degree of beta-haemolysis (weak, moderate, strong) and amount of growth (sparse, moderate, abundant) on the primary plates were recorded. The various degrees of beta-haemolysis were characterized as follows: Strong beta-haemolysis: an intense haemolytic apparent after 48 hrs. incubation with a sharply defined edge to surrounding medium. Moderate beta-haemolysis: a semi-intense haemolysis apparent after 48 hrs. incubation with a more diffuse edge to surrounding medium. Weak beta-haemolysis: a slight haemolysis usually apparent after 48 hrs. incubation with a poorly defined edge to surrounding medium.

RESULTS

The number of samples from the various age (size) groups in Category I herds from which T. hyodysenteriae was isolated is shown in Table 1. The frequency of positive samples found in the herds ranged from 43 out of 80 samples (53.7 %) to 56 out of 60 samples (93 %). The percentage of positive samples in group 1, 2 and 3 pigs was 72, 68.5 and 64.5, respectively.

Of 167 samples collected from Category II herds, 17 were positive with regard to T. hyodysenteriae. These originated from 14 herds.

The degree of beta-haemolysis and amount of growth of T. hyodysenteriae on primary TSA-S400 plates inoculated with

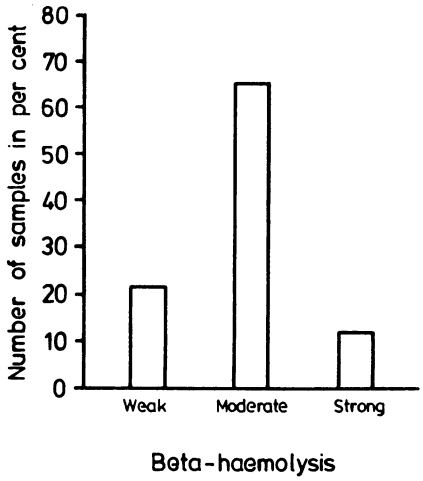
---

* Trypticase-soy-agar containing 400 μg/ml spectinomycin.

Table 1. Demonstration of Treponema hyodysenteriae in faecal samples from pigs in Category I herds.

Herd No.	Number of samples tested	Number of positive samples	Relation between positive and negative samples in the various groups		
			Group 1	Group 2	Group 3
1	80	43	13/25	14/30	16/25
2	85	47	13/20	14/30	17/35
3	60	36	17/25	14/20	5/15
4	60	42	—	16/20	26/40
5	60	55	20/20	18/20	17/20
6	60	56	17/20	20/20	19/20
Total	405	279	80/110	96/140	100/155

samples from Catgory I herds are shown in Fig. 1 and Fig. 2. Most of the strains isolated showed moderate beta-haemolysis, weak and strong beta-haemolysis being found in approx. 22 % and 12 % of the positive samples, respectively. Results varied, however, among the herds of Category I, 4.6 % to 9.5 % of the positive samples from 4 herds showing strong beta-haemolysis, while the corresponding figures for the 2 remaining herds were 16.6 % and 25 %. All 3 degrees of beta-haemolysis were demonstrated in all herds.



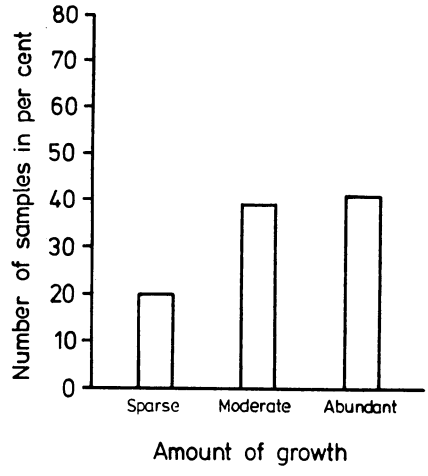


Figure 1. Degree of beta-haemolysis of Treponema hyodysenteriae on primary plates in 279 samples from Category I herds.

Figure 2. Amount of growth of Treponema hyodysenteriae on primary plates in 279 samples from Category I herds.

Of the 17 positive samples from Category II herds in which T. hyodysenteriae was demonstrated, 7 showed strains with weak, 8 with moderate and 2 with strong beta-haemolysis.

Amount of growth of T. hyodysenteriae on primary plates was characterized as sparse, moderate or abundant in approx. 20 %, 40 % and 40 % of the positive samples from Category I herds, respectively. As regards Category II herds, the amount of growth of T. hyodysenteriae was sparse in 2 of the positive samples, moderate in 8 and abundant in 7 samples.

The relationship between degree of beta-haemolysis and amount of growth of T. hyodysenteriae on the primary plates as regards samples from Category I herds is presented in Fig. 3. Samples from which strains with weak beta-haemolysis were isolated showed sparse, moderate and abundant growth of the organism on primary plates in about the same proportions. Most samples harbouring strains of T. hyodysenteriae producing moderate beta-haemolysis showed moderate or abundant amount of growth of the organism, while the percentage of samples with strongly beta-haemolytic strains producing sparse, moderate and abundant amount of growth was approx. 20 %, 24 % and 56 %,

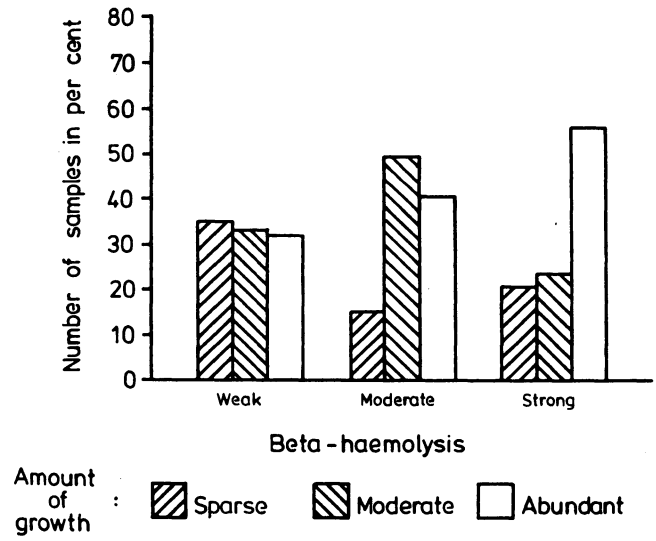


Figure 3. Relationship between degree of beta-haemolysis and amount of growth of Treponema hyodysenteriae in 279 samples from Category I herds.

respectively. Due to the few positive samples found in herds belonging to Category II, the relationship between degree of beta-haemolysis and amount of growth of T. hyodysenteriae on the primary plates was not considered.

DISCUSSION

In the present study, T. hyodysenteriae was demonstrated in all investigated herds of Category I. The frequency of positive samples in the different herds ranged from approx. 50 % to approx. 90 % of all samples tested. The amount of growth of T. hyodysenteriae from positive samples was moderate or abundant on the primary plates in most cases. Strains showing the different degrees of beta-haemolysis were demonstrated in all herds, 12 % of the positive samples producing strongly beta-haemolytic strains. As regards Category II herds, T. hyodysenteriae was demonstrated in 10 % of the samples.

Kinyon et al. (1977) stressed that enteropathogenic and non-enteropathogenic strains of T. hyodysenteriae can be differentiated on the basis of different patterns of beta-haemolysis. According to their terms, non-enteropathogenic strains produce "weak beta-haemolysis", while enteropathogenic strains produce "beta-haemolysis". In the present study, strains of T. hyodysenteriae showing strong beta-haemolysis were found in pigs from all Category I herds. None of these pigs showed symptoms on swine dysentery, although samples from Category I herds from which strongly beta-haemolytic strains of T. hyodysenteriae were isolated comprised 4.6 % to 25 % of all positive samples, in the various herds.

Harris et al. (1978) tested over 2000 samples taken from 10 herds and found that the average herd frequency of samples containing enteropathogenic (beta-haemolytic) strains of T. hyodysenteriae was 4.63 %.

The disagreement, regarding the prevalence of probable enteropathogenic strains of T. hyodysenteriae might reflect the lack of objectivity in characterizing the beta-haemolysis produced. The relatively large number of samples with strains producing a beta-haemolysis which could be characterized neither as weak nor strong indicates the difficulty in applying this criterium when evaluating the pathogenicity of the strains. It is therefore necessary to search for more objective methods to

determine enteropathogenicity of this organism. *Whipp et al.* (1978) recently published a colonic loop test in swine which might prove to be reliable in demonstrating enteropathogenicity of T. hyodysenteriae isolates.

No exact quantitation of T. hyodysenteriae organism was performed in the present investigation. However, the amount of growth of the organism on the primary plates indicated that in most cases the organism was an established part of the faecal flora in herds belonging to Category I. The fact that T. hyodysenteriae was isolated from far fewer samples from Category II herds as compared with Category I herds probably reflects the much longer time elapsing between collection and processing of the samples. *Chia & Taylor* (1978) found that survival of T. hyodysenteriae was greatly reduced at 25°C compared with temperatures of between 0°C and 10°C. Examining the plates only once after 48 hrs. of incubation also led to fewer positive samples being registered than would have been the case if additional readings had been taken after a further 2 and 4 days.

Swine dysentery seems to have been occasionally responsible for losses in individual Norwegian herds, though the disease has not so far been considered as being widespread. The present investigation suggests, however, that strongly beta-haemolytic, and thus possibly enteropathogenic strains of T. hyodysenteriae, are commonly present in most herds in south-eastern Norway. It is therefore likely that environmental factors such as housing and management play a significant role in the development of the disease.

The results presented by *Teige et al.* (1978) where the influence of nutritional factors like vitamin E and selenium on the development of swine dysentery was documented support this assumption.

REFERENCES

Chia, P. S. & D. J. Taylor: Factors affecting the survival of Treponema hyodysenteriae in dysenteric pig faeces. Vet. Rec. 1978, *103*, 68—70.

Doyle, L. P.: A vibrio associated with swine dysentery. Amer. J. vet. Res. 1944, *5*, 3—5.

Harris, D. L.: Current status of research on swine dysentery. J. Amer. vet. med. Ass. 1974, *164*, 809—812.

Harris, D. L. & R. D. Glock: Swine dysentery: A review. Iowa State Univ. Vet. 1971, no. 1, 4—11.

Harris, D. L., R. D. Glock, C. R. Christensen & J. M. Kinyon: Swine dysentery. I. Inoculation of pigs with Treponema hyodysenteriae (new species) and reproduction of the disease. Vet. Med. 1972, *67*, 61—64.

Harris, D. L., J. M. Kinyon, R. D. Glock, J. G. Songer & I. T. Egan: Cultural detection of swine infected with pathogenic Treponema hyodysenteriae. Proc. 5th Wld Int. Pig Vet. Soc. Congr., Zagreb 1978.

Kinyon, J. M., D. L. Harris & R. D. Glock: Enteropathogenicity of various isolates of Treponema hyodysenteriae. Infect. Immun. 1977, *15*, 638—646.

Lysons, R. J., G. A. Hall, T. J. L. Alexander, J. Bew & A. P. Bland: Aetiological agent and pathogenesis of swine dysentery. Proc. 5th Wld Int. Pig Vet. Soc. Congr., Zagreb 1978.

Songer, J. G., J. M. Kinyon & D. L. Harris: Selective medium for isolation of Treponema hyodysenteriae. J. clin. Microbiol. 1976, *4*, 57—71.

Taylor, D. J. & T. J. L. Alexander: The production of dysentery in swine by feeding cultures containing a spirochaete. Brit. vet. J. 1971, *127*, lviii—lxi.

Teige, J. jr., F. Saxegaard & A. Frøslie: Influence of diet on experimental swine dysentery. 2. Effects of a vitamin E and selenium deficient diet supplemented with 3 % cod liver oil, vitamin E or selenium. Acta vet. scand. 1978, *19*, 133—146.

Terpstra, J. I., J. P. W. M. Akkermans & H. Ouwerkerk: Investigation into the etiology of vibrionic dysentery (Doyle) in pigs. Neth. J. vet. Sci. 1968, *1*, 5—13.

Whipp, S. C., D. L. Harris, J. M. Kinyon, J. G. Songer & R. D. Glock: Enteropathogenicity testing of Treponema hyodysenteriae in ligated colonic loops of swine. Amer. J. vet. Res. 1978, *39*, 1293—1296.

SAMMENDRAG

Forekomst av Treponema hyodysenteriae hos friske griser.

Forekomsten av Treponema hyodysenteriae ble undersøkt i fecesprøver fra friske griser i ulike aldersgrupper og fra ulike besetninger. Prøvene fra kategori I besetninger ble undersøkt innen 2 timer etter prøveuttak mens prøver fra kategori II besetninger ble transportert 2 til 3 dager før undersøkelsen ble påbegynt. T. hyodysenteriae ble påvist i fra 53,7 % til 93 % av alle prøver innsamlet fra kategori I besetninger. Det kunne ikke påvises forskjell i frekvens av positive prøver i de ulike aldersgrupper. Fra kategori II besetninger ble T. hyodysenteriae påvist i 10 % av prøvene. De isolerte stammene produserte forskjellig grad av beta-hemolyse. Denne ble inndelt i 3 kategorier: svak, moderat og sterk. Sterkt beta-hemolytiske stammer, som antas å være enteropatogene, ble påvist i alle besetningene tilhørende kategori I. Slike stammer ble funnet i fra 4,6 % til 25 % av de positive

prøver fra disse besetninger. Fra besetninger tilhørende kategori II ble sterkt beta-hemolytiske stammer av T. hyodysenteriae påvist i 2 av 17 positive prøver.

Fra prøver fra begge kategorier besetninger var veksttetthet av T. hyodysenteriae på primærskålene oftest moderat eller rikelig. I ca. 60 % av prøvene fra kategori I besetningene med sterkt beta-hemolytiske isolater forekom rikelig veksttetthet på primærskålene. I prøver fra kategori I besetninger med stammer som ga svak eller moderat beta-hemolyse var sparsom og moderat veksttetthet oftest forekommende.

(Received December 12, 1978).

Reprints may be requested from: Eivind Liven, the Department of Microbiology and Immunology, Veterinary College of Norway, Postbox 8146, Dep, Oslo 1, Norway.