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ISOLATIONS OF HAEMOLYTIC ESCHERICHIA COLI FROM DISEASED AND HEALTHY ANIMALS ON A PARTICULAR PREMISES[1])

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The reports by *Schofield and Davis* (1955) and by *Gregory* in the same year that in pigs with "oedema disease" a heavy growth of haemolytic E. coli could be obtained from the faeces stimulated extensive study of the occurrence and significance of these bacteria in pigs. The strains of haemolytic E. coli which were isolated represented only a few serotypes belonging to the O-groups 138, 139 and 141 (*Sojka et al.* 1957, *Ewing et al.* 1958, *Rees* 1959). The serotypes O138:K81(B):H14 and O141:K85(B):H4 are often associated with gastroenteritis, especially in recently weaned pigs (among others *Sojka et al.* 1960, *Månsson* 1962) but can also be encountered in the intestinal tract of healthy pigs (among others *Campbell* 1959). In healthy pigs, however, these serotypes are usually only sparsely represented in the intestinal flora (*Sojka et al.* 1960) but can sometimes be demonstrated in practically pure culture. For the occurrence of disease, more than a mere quantitative increase seems to be necessary. Changes in the environment, for example a change of feed, can lead to an increase in the number of E. coli in the faeces from 6.2 to 63.9 per cent without pigs necessarily showing signs of disease (*Buxton and Thomlinson* 1961). Yet it is in conjunction with weaning that most deaths occur, just when one could expect a relative increase in haemolytic E. coli in the intestinal contents because of the change in diet. In pigs dying at this time growth

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of haemolytic E. coli is profuse in cultures from the intestinal contents and often in cultures from the viscera as well.

Serotypes other than those mentioned here are usually encountered in younger piglets with enteritis. *Saunders et al.* (1960), however, recovered a strain belonging to O-group 138 in one of 23 piglets examined during the first week after birth. There was no bacteraemia.

The following account is based upon observations made on a particular premises in which the serotype O138:K81(B):H14 was repeatedly isolated during the course of several years from pigs with haemorrhagic enteritis and septicaemia. Apart from a description of the disease pattern during these years, mention will also be made of the environmental factors which could have contributed towards the occurrence of the overt disease and some experiments along these lines will also be described.

The animals belonged to the Department of Genetics and Animal Husbandry at the Veterinary College. From 4 to 8 breeding sows, a few market pigs, and some 20 head of cattle, half of them cows, are kept in one building and cared for by the same staff. The floors and fixtures were cleaned at short and regular intervals. The bacteriological observations recorded here were carried out between 1953 and 1961. During this period the pigs have been used for a number of experiments. The most important of these in this connection were trials of different milk substitutes for piglets which were not allowed to nurse their dams and the experiments on market pigs to ascertain the effects of different rations on the composition of the intestinal flora (*Månsson and Olsson* 1961). The piglet experiments, described by *Dyrendahl et al.* (1953 and 1958), were carried out between 1952 and 1955. The piglets were taken from their dams immediately after birth before they received colostrum. Losses due to infectious disease were often heavy in these litters. Many of the piglets were autopsied in the Department of Pathology at the College and samples taken for bacteriological examination. Studies on the effects of different rations upon the composition of the intestinal flora were carried out from 1957.

DISEASE PATTERN

1953.

In one experiment, trial of a substitute for sow milk, 6 piglets in the litter died, one shortly after birth and the other 5, which had developed severe diarrhoea, at one to three weeks of age. The new-

born piglet was anaemic (and bacteriologically negative). The other pigs had enteritis and signs of septicaemia and one of them necrotic gastritis as well. Four of the five piglets were examined bacteriologically; profuse growth of haemolytic E. coli was obtained from the liver, spleen, and intestinal contents.

Mortality was also high in a second experiment. Sixteen piglets were autopsied. They died between one and three weeks of age, all with acute enteritis or gastroenteritis with signs of septicaemia. For ten of these piglets an abundant growth of haemolytic E. coli was obtained from viscera and intestinal contents. Three piglets were bacteriologically negative and three were not examined bacteriologically.

The bodyweights of the autopsied piglets were less than expected for the age. At three weeks of age weights hovered about 2 kg.

1954.

Experiments with milk substitutes for piglets were repeated and several animals died but only a few were autopsied. Seven piglets in one litter died after diarrhoea and treatment with sulphonamides. These piglets were bacteriologically negative. A 16-days-old pig from another experiment and which weighed only 2.5 kg. had lesions of enteritis with septicaemia; profuse growth of haemolytic E. coli was obtained from the viscera and intestinal contents.

1955.

Heavy mortality also occurred this year. Fourteen piglets which died under much the same circumstances as in previous years were autopsied. All these piglets had acute gastroenteritis with signs of septicaemia; abundant growth of haemolytic E. coli was obtained from the viscera and intestinal contents. Two other piglets, bacteriologically negative, were anaemic. Deaths occurred at much the same age as previously and body weights were low — piglets which survived 3 or 4 weeks weighed only 2 to 4 kg. Another pig, 7 weeks old and weighing 15.8 kg., died unexpectedly shortly after being taken from the sow (conventional rearing). The autopsy diagnosis was gastroenteritis with signs of septicaemia; again an abundant growth of haemolytic E. coli was obtained from the viscera and intestinal contents.

1956.

Fewer piglets died this year; six were autopsied and found to have enteritis. Four of these piglets had signs of septicaemia and yielded an abundant growth of haemolytic E. coli in cultures from viscera and intestinal contents. The other two piglets were bacteriologically negative. All these piglets had remained with their dam but were much smaller, weighing only about 7 kg. each at the time of death, than their surviving littermates.

1957.

Mortality among the animals was low this year; a piglet and two young calves were autopsied. Waxy degeneration of the skeletal

musculature was seen in the piglet and bacteriological examination gave negative results. Septicaemia with β-streptococci was diagnosed in one calf. The other calf, which was one month old and had developed diarrhoea a few days before death, had signs of septicaemia at autopsy; haemolytic E. coli was recovered from the organs.

During the years 1953 to 1957, haemolytic E. coli was also isolated from the faeces of pigs which were clinically healthy. Isolations were made from the faeces of surviving littermates to the dead pigs and from the faeces of animals in litters in which the mortality was low. All the pigs in litters with a high mortality usually yielded haemolytic E. coli, sometimes practically in pure culture. Among other litters about 60 per cent of the pigs had haemolytic E. coli in their faeces, usually in relatively small numbers. After the death of the calf in septicaemia in 1957, faecal samples from all the cattle on the premises were examined for the presence of haemolytic E. coli but with negative results.

During the period 1958 to 1961, no deaths occurred which could be associated with haemolytic E. coli. On the other hand, several experiments were carried out on pigs in order to study the effect of different diets on the composition of the intestinal flora. Faecal samples from these pigs were examined at weekly intervals from weaning until slaughter. Haemolytic E. coli was repeatedly demonstrated in the course of each experiment. One or two pigs out of 15 have usually yielded this organism on each occasion samples were taken and a particular pig could shed this organism in its faeces for up to ten consecutive weeks. The relative numbers of haemolytic E. coli have varied. Growth was usually sparse but in some samples was abundant (20,000 per g. moist faces). Variations in the protein content of the diet had no effect on the relative numbers of haemolytic E. coli isolated.

SEROLOGICAL STUDIES

The serotype O138:K81(B):H14 was recovered from dead animals. In addition to this serotype, the serotypes O141:K85(B):H4 and O139:K82(B):H1 as well as a number of strains which could not be typed with the sera used were isolated from the faecal samples. Serological typing was carried out in the manner described in an earlier paper (*Månsson* 1962).

The clinical course for the pigs which died was brief — the pigs usually died within a day or so. Pigs which died at that time of weaning usually did so without showing any premonitory signs (*Månsson* 1962). Because of this, there was no possibility of using the serum antibody titre as the basis for an opinion on the significance of haemolytic E. coli as an infectious agent. Since the serotype which was isolated from dead pigs could also be isolated from the faeces of clinically healthy pigs it is obviously

of interest to know whether or not the haemolytic E. coli incited a demonstrable antibody titre in the healthy pigs. Only a few serological studies of this type have been reported. *Gregory* (1958) was able to demonstrate specific K agglutinins in blood serum samples taken from surviving pigs three weeks after an outbreak of "oedema disease". Specific O agglutinins, on the other hand, could not be demonstrated.

Coli enteritis in human beings is followed by the appearance of specific agglutinins in about ⅓ of the cases and infection with certain serotypes leads to a positive Widal test in about ½ the cases (*Braun et al.* 1954). An O-titre of 1:20 is considered specific. Agglutitinins for B- and H-antigens did not occur. The O-titres were greatest during the second and third weeks and then decreased. There was no correlation between the magnitude of the O-titre and the clinical severity of the disease. These agglutinations were performed by the centrifugation method, a method which is more sensitive than conventional methods (*Kauffmann and Ørskov* 1956), especially if inactivated serum is used (*Clement et al.* 1953).

A Widal test was performed on several pigs using the centrifugation technique described by *Braun et al.* The results for two groups of pigs will be reported here; in both instances O138:K81(B):H14 was used as antigen.

The first group comprised seven pigs of which six had been included in three of the feeding experiments conducted during 1958. These pigs belonged to different litters, listed in Table 1

Table 1.
O-titres in serum samples from different pigs when agglutinated with serotype O138:K81(B):H14.

Pig no.	Litter no. (see text)	Serum dilutions 1/20	1/40	1/80	1/160	1/320	From feeding experiment no.
936	I	+++	+++	+	—	—	I
940	I	+++	+++	+	—	—	I
963	II	+++	+++	++	—	—	II
966	II	+++	+++	+++	++	—	II
967	II	++	—	—	—	—	II
975	III	+++	+++	+	—	—	III
134		—	—	—	—	—	

+++: distinct agglutination
++ : less distinct but unequivocal agglutination
+ : weak agglutination
— : no agglutination

as I, II and III. The serotype 0138:K81(B):H14 had been isolated from the faeces, usually in relatively small numbers on several occasions up to the time the Widal tests were performed. These pigs had shown no clinical signs of disease which could be associated with haemolytic E. coli and were four months old at the time the serum samples were taken. Pig no 134 was a boar, 1½ years old, which had not been in contact with the other pigs.

In 1961 similar serological tests were made on six pigs, also used in a feeding experiment but purchased from another farm. Faecal cultures from the pigs yielded O141-strains in pigs no. 257, 259 and 262. No O138-strains were found. The pigs were four months old when examined and had never shown clinical signs which could be attributed to haemolytic E. coli. The O-titres obtained are listed in Table 2.

Table 2.

O-titres in serum samples from different pigs when agglutinated with serotype O138:K81(B):H14.

Pig no.	Serum dilution 1/20	1/40	1/80
253	—	—	—
257	+	—	—
259	++	—	—
260	+	—	—
262	++	+	—
264	++	+	—

With the exception of one pig in each of the groups listed in tables 1 and 2, agglutination was obtained in the 1/20 serum dilutions. Agglutination was more pronounced, and the titre higher, for most of the pigs in Table 1, i. e. the pigs with the agglutinating serotype in the faeces. Even if the titre 1/20 can only be accepted with reservation as a specific antibody response until further trials can be carried out, it can be seen from the tables that the serotype O138:K81(B):H14 when present in the intestinal flora was able to elicite an antibody response in the host animal. Since it can be assumed that an O-titre in pigs, as is the case in human beings (*Braun et al.*), regresses after a time if the antigenic stimulus is reduced or eliminated, it is conceivable that higher titres than those recorded here could have been present earlier, particularly 2 or 3 weeks after weaning

which is the time the animals seem most prone to develop the enteritis and septicaemia which is often associated with haemolytic E. coli. The serum samples examined here were taken some 8 to 10 weeks after this time but still showed distinct O-titres. B-agglutinins could not be demonstrated in any of the serum samples examined. The results obtained here are compatible with those reported by *Braun et al.* for coli enteritis in human beings. *Gregory* found B-agglutinins in his pig sera but these could not be detected in the present series. Since the relative numbers of haemolytic E. coli in faeces can vary widely (*Buxton and Thomlinson*), and the organism may be intermittently present in faeces, the Widal test — as can be seen from Table 2 — may prove to be valuable complement for epizootological studies.

An idea of the sensitivity of the centrifugation method compared with that of the conventional method, water bath at 50°C for 18 hours, can be obtained from examining particular serum samples by both methods. The O-titres given in Tables 1 and 2 were the values obtained by the centrifugation method. When samples were read after 18 hours at 50°C without centrifugation, a weak agglutination was obtained in 1/20 dilutions of serum samples from pigs 940 and 936. The other serum samples included in Table 1 were negative. All the serum samples in Table 2 were negative in a 1/20 dilution. The centrifugation method, even without the use of inactivated sera, was clearly much more sensitive.

DISCUSSION

The results show that haemolytic E. coli, with serotype O138: K81(B):H14 of special interest, was present on these particular premises through the whole period of observation, 9 years. During the first half of this period several deaths occurred, especially among piglets and among older pigs shortly after weaning. The clinical course was brief. Many of the pigs died without premonitory clinical signs while others, and particularly the piglets, had diarrhoea for some days before dying. The autopsy changes were those of enteritis, usually with signs of septicaemia. When examined bacteriologically, a profuse growth of haemolytic E. coli was obtained from the viscera and intestinal contents in some 80 per cent of the animals examined. Most of the animals which were bacteriologically negative had been treated with sulphon-

amides. Apart from haemolytic E. coli, no other bacteria have been isolated which could conceivably account for the signs of infection. The serotype recovered (O138:K81(B):H14) from the autopsy material, which also includes a calf dead of septicaemia, has also been isolated from the faeces of many clinically healthy animals on the premises. Deaths among the pigs occurred in conjunction with changes in the diet and during trials of milk substitutes for piglets which were not allowed to nurse. Most of the pigs in the autopsy material had a relatively low body weight for their age.

The stage seems to set for septicaemia when certain serotypes of haemolytic E. coli are present in the intestinal tract of pigs which are underweight and are exposed, for example, to changes in diet. The mere presence of haemolytic E. coli in the intestinal contents is not sufficient to elicite disease, nor is solely an increase in their relative numbers in the intestinal contents. To demonstrate this point three healthy pigs weighing between 12 and 15 kg. were given 400 ml. broth culture of strain O138:K81 (B):H14 daily for 4 days. Blood serum from these pigs did not contain a demonstrable antibody titre against the serotype given. A profuse growth of the strain appeared in the faeces after 24 hours but no clinical signs of disease were observed, apparently because of absence of other prerequisites for the occurrence of disease. Similar negative results have been reported by *Hess and Suter* (1959) and *Sojka et al.* (1960). Two healthy pigs, weighing 18 and 20 kg., were given an intravenous injection of 11 ml. of a 16-hours broth culture of strain O138:K81(B):H14. After 48 hours one pig had a temperature of 39.8°C and refused to eat. By another 24 hours the temperature was 40.4°C. The pig then gradually returned to normal during the next two days. No signs referrable to the gastrointestinal tract were noticed. The other pig remained clinically healthy. Nor did broth cultures given by other parenteral routes induce disease. Neither the oral nor the parenteral administration of haemolytic E. coli has resulted in disease, an indication that other factors in the environment and in the state of the host animals are decisive for the occurrence of the gastrointestinal disorders with which haemolytic E. coli is associated. *Buxton and Thomlinson* have proposed that a sudden increase in the numbers of haemolytic E. coli in the intestinal contents sensitises the animals and elicites an anaphylactic shock. The lesions of "oedema disease" and haemorrhagic

enteritis are presumed to be an expression of this shock. Both these diseases have been associated with the serotypes encountered in the present material. Other prerequisites in addition to those required for enteritis with septicaemia would seem to enter into the aetiological background of "oedema disease" since the latter has never been encountered during the observation period.

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SUMMARY

Haemolytic E. coli, and particularly the serotype O138:K81(B):H14, was present among the animals kept on a particular premises during an observation period of 9 years. During the first half of this period mortality was heavy among young piglets (enteritis with signs of septicaemia). Profuse growth of haemolytic E. coli was obtained from the viscera and intestinal contents. During the latter half of the period mortality was much less but the same serotype was repeatedly isolated from the faeces of clinically healthy animals. The serotype O138:K81(B):H14 was widely distributed among the animals and, apart from the pig isolations, was present in large numbers and pure culture in several organs from a calf which died of septicaemia. A positive Widal test was obtained for healthy pigs with this serotype in the faeces (Table 1). Environmental factors in the development of overt disease are discussed. The oral and parenteral administration did not elicite gastrointestinal signs. "Oedema disease" was never observed during the period covered by these studies.

ZUSAMMENFASSUNG

Hämolytische Escherichia Coli Stämme bei gesunden und kranken Tieren in einem Tierbestand.

Häm. E. Coli, in erster Linie der Serotyp O138:K81(B):H14, konnte in einer Periode von 9 Jahren bei einem Tierbestand nachgewiesen werden. In der ersten Hälfte der Observationszeit trafen mehrere Todesfälle unter Spanferkeln ein, bei denen oft eine septische Enteritis festgestellt wurde. Bei Züchtungen vom Darminhalt und Eingeweiden wurde sehr reichliches Wachstum von häm. E. Coli beobachtet. In dem späteren Teil der Beobachtungszeit nahm die Anzahl der Todesfälle bedeutend ab, jedoch kam derselbe Serotyp im Faeces von gesunden Tieren weiterhin vor. Der Serotyp O138:K81(B):H14 war stark im Tierbestand verbreitet und kam ausser bei Schweinen auch reichlich und in Reinkultur in verschiedenen Organen bei einem Kalb vor, mit der Sektionsdiagnose Sepsis. Positiver Widal-Test wurde erhalten, bei Schweinen die diesen Serotyp im Darmkanal hatten (Tabelle 1) aber keine Krankheitszeichen zeigten. Zu Krankheitsfällen beitragende Millieuursachen werden diskutiert. Zufuhr von häm. E. Coli per os oder parenteralt ergaben keine gastroenteritische Krankheitszeichen. Die Oedem-Krankheit wurde bei keinem Fall während der Beobachtungszeit festgestellt.

SAMMANFATTNING

Hämolytisk Escherichia coli hos sjuka och friska djur i en besättning.

Häm. E. coli, i första hand serotypen O138:K81(B):H14, påvisades i en besättning under 9 års tid. Under första hälften av observa-

tionstiden inträffade ett flertal dödsfall bland spädgrisar, hos vilka en septisk enterit ofta konstaterades. Vid odling från tarminnehåll och viscera iakttogs mycket rikligt med häm. E. coli. Under senare delen av observationstiden minskade dödligheten högst betydligt, men ofta förekom samma serotyp fortfarande i faeces från friska djur. Serotypen O138:K81(B):H14 hade stor utbredning i besättningen och förutom hos gris förekom den rikligt och i renkultur i ett flertal organ hos en kalv med sektionsdiagnos sepsis. Positiv Widal-test erhölls, då grisarna haft denna serotyp i tarmkanalen (Tabell 1) men ej visat sjukdomstecken. Bidragande miljöorsaker till sjukdomsfallen diskuteras. Tillförsel av häm. E. coli per os eller parenteralt gav inga gastrointestinala sjukdomstecken. Oedema disease iakttogs ej vid något tillfälle under observationstiden.

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