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CALF PARATYPHOID I.—A GENERAL DISCUSSION OF THE DISEASE IN RELATION TO ANIMALS AND MAN.

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CONTENTS.

HISTORICAL.

Various forms of epizootic diarrhoea or septicaemia of calves have been known to exist in different parts of the world for very many years. But, as these were generally described merely as "calf scours" or "Kälberruhr", the nature and type of disease alluded to is not always clear. Excellent reviews of the history of calf scours and of various theories formulated from time to time to explain its etiology were given by Jensen (1893, 1913). To these, reference should be made for details. Jensen stated that some of the accounts of "calf scours" given more than a century ago fully described the symptoms of the disease of his day. According to him the description given by Tolnay in 1799 corresponded to the condition reported by him.

Obich (1865) was the first to incriminate an infectious agent as the cause, and he suggested that the infection was spread by means of the faecal discharges of affected calves. Obich's theory, however, did not receive much support until Franck (1876) published his handbook on obstetrical aids. In this book he insisted that the disease was caused by an infection which arose like a miasma from the stable floors. Dieckerhoff (1891) believed that the calf became infected when fed on milk contaminated with facultative parasites occurring in dirty stables, where they persisted for many years. Nocard (1886), on the other hand, associated calf scours with contagious abortion (Brucellosis) and suggested infection *in utero*. Mørkeberg (1887) also believed that infection was prenatal and, therefore, advised the isolation of pregnant cows in clean breeding boxes. But Friedberger and Fröhner (1887) thought that the calf became infected at birth during its passage through the genital tract of a cow suffering from a catarrh of the vagina.

In South Africa Hutcheon (1893) was the first to report a disease of calves that can be regarded as paratyphoid. This disease was apparently identical with

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the condition described as *Lewersiekte* (liver disease) or " yellow liver " by Otto Henning (1894). Hutcheon thought that infection was spread from farm to farm by means of the faeces of infected calves. Later Henning (1932) reported that he had studied a similar disease in Natal during 1920. The etiology of this disease was elucidated by Viljoen and Martinaglia (1928) and Henning (1939).

Jensen (1893) incriminated an oval coliform bacillus present in the internal organs of affected calves as the cause of " Kälberruhr ". He found this organism extremely pathogenic for healthy newly-born calves and readily reproduced the disease with milk to which cultures of the organism had been added. Morphologically and culturally Jensen was unable to distinguish this bacillus from the *Bact. coli* which were commonly found in the intestinal tract of normal healthy calves, but which he found to be entirely innocuous for calves.

Thomassen (1897) reported a septicaemic disease of calves in Holland which was often associated with diarrhoea. From the internal organs of affected calves he isolated a " Gaertner-like " organism resembling *Bact. coli* and *S. typhi,* and named it " pseudo-typhoid bacillus ". He found that this organism failed to produce indol or to ferment lactose. Poels (1899) described this bacterium as " pseudo-coli bacillus ". In 1903 Jensen (1913) introduced the name " *Bacillus paracoli* " for the pathogen of calves and showed that this organism was related to " *S. paratyphi-B* " and " Gaertner's bacillus ". Langer (1904) studied cultures of bacteria obtained from calf livers disseminated with greyish-yellow, necrotic nodules of paratyphoid and found that these formed H_2S but failed to produce indol, and that suspensions of the organism were agglutinated by *S. typhi* serum. Schmidt (1908) also isolated a " Gaertner-like " bacterium from the organs of calves affected with septicaemia, diarrhoea, and pneumonia, and suggested that this disease was identical with " pseudo-coli-bacillosis " of Poels and " para-coli-bacillosis " of Jensen. Titze and Weichsel (1908) examined 28 strains of the so-called " paracolon bacillus " and were unable to differentiate 23 of them from " Gaertner's bacillus ". Moreover, Uhlenhuth and Hubener (1913) reported that most of the strains labelled *B. paracoli* by Jensen should be regarded as organisms of the Gaertner group.

Further outbreaks of disease in calves caused by Gaertner's bacillus or *Bacterium* (*Salmonella*) *enteritidis* were reported by Miessner and Kohlstock (1912), Luxwolda (1913), Christiansen (1914), Warnecke (1914), Douma (1916), Meyer, Traum and Roadhouse (1916) and several others.

As the identification of the organisms associated with calf typhoid and some forms of calf scours generally was not based on their serological characters, the nomenclature used by many of the earlier workers is not reliable. It was only after Bruce White (1929) had differentiated the strain *Salmonella dublin* from *S. enteritidis* serologically and had given it the status of an independent species, that the position was clarified. Some old laboratory strains obtained from outbreaks of food poisoning, septicaemia, and meningitis, labelled *Bact. enteritidis* Gaertner were re-examined by Smith and Scott (1930), and found to be serologically identical with *S. dublin* of Bruce White. Six strains of *B. paracoli* isolated from cases of calf paratyphoid in Denmark were also examined and all six were identified as *S. dublin.* Smith and Scott (1930) concluded that this organism had a special association with cattle and that cow's milk was the common vehicle of human infection. Finally Bartel (1938) and Henning (1939) showed that *S. dublin* was by far the most common cause of paratyphoid in calves, and Field (1948) reported that this organism was responsible for the vast majority of cases of paratyphoid in adult cattle.

DISTRIBUTION.

Calf paratyphoid has a world-wide distribution. It was reported by Viljoen and Martinaglia (1926, 1928) and Henning (1939, 1944) in South Africa; by Daubney (1927) in East Africa; by Strozzi (1934) in Italy; by Bosworth and Lovell (1931), Craig, Davies, and Massey (1941) and Field (1948) in England; by Clarenburg (1933) in Holland; by Karsten (1921, 1929, 1933), Pröscholdt (1931), Miessner and Koebe (1931) and Bartel (1938) in Germany; by Olson (1939) in Sweden; by Christiansen (1923) and Hansen (1947) in Denmark; by Rosati (1938) in Sicily; by Gallo (1939) in Venezuela; by Guerrero (1943) in Colombia and Ecuador; by Stewart and Hayston (1941) in Australia; and by Shirlaw (1935) in India. In addition the author has identified *S. dublin* as the cause of calf paratyphoid in other parts of Africa, viz. Southern Rhodesia, South-West Africa, Nigeria, the Belgian Congo, and Portuguese East Africa. It is significant, however, that outbreaks of paratyphoid are extremely rare in North America. Although it is highly probable that the epizootic reported by Meyer, Traum and Roadhouse (1916) was due to *S. dublin,* Bruner and Moran (1949) believe that infections due to this organism are confined almost exclusively to cattle and foxes raised west of the Rocky Mountains. In a study of 3,090 cultures of Salmonella from 2,285 outbreaks of infection in man and animals in the United States Edwards and Bruner (1943) were able to identify only 25 strains as *S. dublin.* Of these only two were isolated from ruminants; one was isolated from a fowl, and 22 from carnivores. Josland (1950) reported that *S. dublin* had not been identified in New Zealand, but he recorded the isolation of 80 strains of *S. typhi-murium* from material obtained from cattle.

PATHOGENICITY.

Different strains of Salmonella may vary markedly in their pathogenicity when allowed to spread either naturally or under experimental conditions among the members of a susceptible herd.

According to information obtained by Topley, Greenwood, Wilson and Newbold (1928) strains of *Salmonella typhi-murium* that cause rapidly spreading and highly fatal epizoötics among mice are extremely virulent when inoculated intraperitoneally, whereas strains of low virulence do not readily spread among a herd and are incapable of setting up severe epizoötics. These strains, however, may persist for long periods in the tissues of infected mice and cause an occasional death in normal mice when exposed to the infection. An outbreak of the disease is usually initiated only after the introduction of an epidemic strain of the organism.

It is well-known that the pathogenicity of any strain of Salmonella is not strictly specific. There is much evidence to show, however, that different types of the group produce specific pathological changes in selected hosts.

The most cosmopolitan of all types is *S. typhi-murium,* which may infect any mammal or bird. It commonly causes septicaemia in animals and birds, and gastro-enteritis in man.

Although the different strains may exhibit slight differences in their serological and biochemical behaviour, it is usually extremely difficult to establish host reservoirs.

Species like *S. typhi, S. paratyphi A, B and C* are confined almost exclusively to man, whereas *S. pullorum* and *S. gallinarum* generally are pathogenic only for poultry.

S. cholerae-suis, which is by far the most common Salmonella found in the pig, may also cause infection of man and other animals.

S. dublin, although sometimes the cause of gastro-enteritis and prolonged fever in man as well as septicaemia in animals, is pathogenic mainly for cattle, particularly calves (Bosworth and Lovell, 1931; Henning, 1939; Hohn and Hermann, 1934). Out of 102 Salmonella strains isolated by Henning (1939) from outbreaks of calf paratyphoid in South Africa 97 were identified as *S. dublin,* two as *S. enteritidis,* and three as *S. typhi-murium.*

The Incidence of Salmonella Infection in Cattle.

In a further study during the period 1939 to 1950 an additional 507 strains of Salmonella were isolated by the author from outbreaks of calf paratyphoid in South Africa. Of these 491 were identified by him as *S. dublin,* eleven as *S. typhi-murium,* four as *S. enteritidis* and one as *S. bovis-morbificans.* Twelve further strains were isolated from adult cattle. Two of these were from young adult bovines that became sick after transportation by rail; and one was isolated from the faeces of a cow that was responsible for an outbreak of food infection traced to milk by Pullinger and Scott-Millar (1945). The remaining nine strains were isolated from the faeces of apparently healthy adult carriers in herds badly infected with calf paratyphoid.

The single strain of *S. bovis morbificans* was isolated from an outbreak involving a number of calves that developed paratyphoid in spite of repeated inoculation with routine *S. dublin* vaccine. It is of interest to note that within two weeks of isolating this organism from the calves three more strains of the same organism were isolated from widely separated areas. One was obtained from brawn that had been responsible for an outbreak of gastro-enteritis in human beings; one from the stools of a patient suffering from food poisoning; the third from suspected food and the evacuations of patients involved in an outbreak of food infection on a large passenger ship.

In many of the outbreaks studied it was possible to carry out ante- and post mortem examinations of the affected animals, but in several cases organs only were submitted for examination. When paratyphoid was suspected farmers were trained to submit fresh pieces of liver and spleen to the laboratory in 50 per cent. glycerine for bacteriological examination, or in 10 per cent. formalin for pathological study.

When glycerine was not available farmers were advised either to pack suspected organs in dry salt, or to make thick smears on glass slides from the bile of suspected animals.

Portions of the suspected liver and spleen submitted were cultivated on MacConkey's bile-salt agar and on an enrichment medium, such as tetrathionate broth. When bile smears were sent the dry bile was carefully washed off the slides into some tetrathionate broth, incubated for 24 hours and then subcultured on MacConkey's bile salt agar. In infected cases *S. dublin* could be isolated readily either from the primary culture on MacConkey or from a sub-culture on the same medium made after enrichment. Hardly without exception the histological examination confirmed the bacteriological investigations.

Although a large number of positive cultures were obtained from suspected faeces, *S. dublin* was much more readily and far more frequently cultured from suspected organs. In many cases where *S. dublin* could not be isolated from faeces or blood cultures these organisms were readily obtained in cultures made from the liver and the spleen. Negative faeces cultures, therefore, could not be regarded as conclusive proof of the non-existence of *S. dublin* infection.

In this way an early diagnosis of outbreaks of calf paratyphoid could be made from areas not readily accessible to the laboratory worker, and the timely application of prophylactic measures could be carried out.

In a study of 70 outbreaks of Salmonella infection in bovines, involving mostly adult cattle, Field (1948) found *S. dublin* responsible for 66 and *S. typhi-murium* for four only. Later he (Field, 1949b) reported the isolation of 257 strains of *S. dublin* and nine of *S. typhi-murium* from cattle.

During the course of a bacteriological examination of meat from the abattoirs at Leipzig, Knoth (1936) isolated 536 Salmonella strains from calves, and of these 506 were *S. dublin*. From adult cattle he obtained 18 strains, of which 17 were *S. dublin*. Bartel (1938) isolated 1690 strains of Salmonella from domestic animals commonly used for food. These included 1324 strains (78·37 per cent.) of *S. dublin*, of which 1,027 were derived from calves, 253 from adult cattle, 28 from pigs, ten from horses, and six from sheep. He further reported that *S. dublin* was the cause of 90 per cent., and S. *typhi-murium* (Breslau) of 8·6 per cent., of all outbreaks of calf paratyphoid. During a period of ten years Baars and Gleisch (1939) isolated 1,222 strains of Salmonella from animals used for food, and identified 88·8 per cent. as "*S. enteritidis*" (*S. dublin*?) and 6·2 per cent. as *S. typhi-murium*. "*S. enteritidis*" was obtained six times as often from calves as from adult cattle. Lutje (1939), on examining 461 Salmonella strains isolated from cattle, identified 404 of these as *S. dublin*.

In a survey of the incidence of Salmonella infection of domestic animals in the Rhineland, Rohrer and Wienand (1941) found that *S. dublin* was by far the most important pathogen. They obtained this organism from 121 calves, 23 adult cattle, eight horses, and three pigs, and also from the udders of two cows.

In a bacteriological study of 2,552 samples of bovine bile Field (1949) isolated 276 Salmonella strains, which included 257 *dublin*, nine *typhi-murium*, eight *derby*, one *enteritidis*, and one *broncaster*.

Olson (1939) examined material from 2,485 calves in Sweden and isolated Salmonella strains (presumably *S. dublin*) from 78. While he was making a routine study of material from 7,800 calves in Copenhagen, Hansen (1947) recovered *S. dublin* from 383 of these and *S. typhi-murium* from only one.

During the course of an extensive study of the causes of the mortality of calves in Denmark, Christiansen (1923) found that, out of a total of 1,535 deaths, "Gaertner bacillus" was responsible for 399 (26 per cent.).

Several outbreaks of Salmonella infection have also been reported in adult cattle. Mohler and Buckley (1902) described an outbreak involving seven animals in which "*S. enteritidis*" was incriminated. Miessner and Kohlstock (1912) reported an epizootic which started among cattle kept at pasture; but later it spread to stabled animals. Bugge and Dierks (1922) isolated "*S. enteritidis*" from the udder of a cow that had died from septicaemia and enteritis. Lutje (1926) recovered the same type of organism from the internal organs and faeces of four cows which had also died from septicaemia and enteritis. During an investigation of an epizootic of paratyphoid involving a number of adult cattle, Lehr (1927) isolated "Gaertner bacilli" from the faeces, urine, and milk of some of the cows, and Rudolf (1928) incriminated this bacterium as one of the causes of mastitis in cows. Standfuss, Wilken and Sorrensen (1932) isolated "Gaertner" bacilli from the faeces, and in some cases also from the internal organs of apparently healthy carriers, as well as from obviously sick cattle. They further found that calves

might become infected when allowed to suck from carrier cows. Judging from our present knowledge of the antigenic structure of Salmonella strains there is probably very little doubt that *S. dublin* was the cause of most of these outbreaks.

More recently several epizootics of Salmonella infection in adult cattle were reported in which reliable methods of antigenic assignment were employed. In these *S. dublin* proved to be the predominant pathogen. Thus, Olson (1939) described epizootics of *S. dublin* in cattle in Sweden. Bythell (1946) reported an outbreak which involved a herd consisting of heifers and cows. There were twelve deaths. The infection was apparently introduced by pregnant heifers and the disease occurred in pastured animals which had been watered from a stagnant pool. Grunsell and Osborne (1948) also studied several cases of *S. dublin* infection in cows during a period when they were not housed but while they were kept at pasture and milked out in the field. John (1946) described four cases of *S. dublin* infection in a herd harbouring an apparently healthy carrier as well as in a number of other animals with an abnormally high agglutination titre. Field (1948) encountered this organism as the cause of infection in both adult and young adult cattle on 64 farms in West Wales. Barron and Scott (1949) reported twelve outbreaks affecting 18 adult animals, of which nine died.

The only previous report of *S. dublin* infection of adult cattle in South Africa is that of Bishop, Schatz and Canham (1943). These workers incriminated *S. dublin* as the cause of abortion in first-calf heifers and succeeded in isolating the organism from the organs of one of the aborted foetuses.

Many other workers have described abortion in cattle due to infection with *S. dublin.* Bert (1943) found that no less than three out of five carrier-cows, that were actively discharging *S. dublin* in the faeces, had recently aborted. In an extensive study of paratyphoid in cattle Olson (1939) reported that many pregnant cows infected with *S. dublin* might abort. Field (1948) noticed an extremely high abortion rate in herds harbouring infected animals. Grunsell and Osborne (1948) believed that the abortion might occur either at the height of the disease or after the recovery from clinical symptoms. Cases of abortion in cattle due to *S. dublin* have also been reported by Lutje (1939), Bythell (1946), and John (1946).

Salmonella Dublin Infection of Furbearing Animals and Fowls.

Apart from bovines *S. dublin* is pathogenic for other animals and man. During a period of 15 years, Lutje (1938, 1939) isolated 235 Salmonella strains from pigs. These included 187 strains *cholerae-suis,* 12 *voldagsen,* 26 *typhi-murium,* nine *dublin,* and one *danysz.* In material obtained from 2,300 furbearing animals Hansen (1947) isolated *dublin* from 101 (foxes), *typhi-murium* from 28, and other Salmonella strains from three.

Outbreaks of *Salmonella dublin* infection have been reported in fox-breeding establishments by Wramby (1937), Olson (1939), Olson (1940), Momberg-Jörgensen (1942), Roemmele (1944), and Bruner and Moran (1949). In most of these epizootics the disease was largely confined to puppies, and there was a mortality rate as high as 88 per cent. on some farms. In some cases there was evidence to show that the source of infection was food stored at room temperature during warm summer weather. In other cases rats were incriminated as carriers and disseminators. When the food was kept in cold storage, when provision was made for proper sanitary conditions, when rat infestation was prevented, and when young puppies were immunized with a formalinized vaccine, the incidence of the disease decreased markedly.

Salmonella dublin has also been reported in the fowl. Lutje (1937) described an outbreak of acute diarrhoea in chickens housed in premises previously occupied by calves which had been suffering from paratyphoid. He found that *S. dublin* was the cause of the diarrhoea. Olson (1939) and Kauker (1943) also reported *S. dublin* infection in fowls which were kept on farms where paratyphoid had raged among the local calves.

Salmonella Infection of Reptiles and Arthropods.

Salmonella strains have been isolated not only from mammals and birds but also from reptiles and arthropods. Bruner and Moran (1949) have reported 14 cases of Salmonella infection in snakes and other reptiles. Mackerras and Mackerras (1948) as well as Bruner and Moran (1949) have also recovered Salmonella strains from cockroaches. Parker and Steinhaus (1943) have succeeded in infecting ticks (*Dermacentor andersoni*) with *S. enteritidis,* and Vorele and Olerte (1946) were able to transmit this organism to *Pulex irritans* and *Ctenocephalus canis.* Moreover, Ostrolenk and Welch (1942) have demonstrated that the common housefly (*Musca domestica*) when fed on food contaminated with *S. enteritidis* is capable of spreading this bacterium to other flies as well as to clean food and to miscellaneous surfaces with which it has been in contact.

Carriers.

Although a small proportion of apparently healthy animals are known to harbour Salmonella strains, Savage (1940) believes that no evidence has so far been obtained to prove that members of the Salmonella group of bacteria occur as normal habitants of the animal body. He regards the presence of these bacilli in a small percentage of animals as an indication that the animals have survived an apparent or inapparent infection and that they merely serve as examples of the carrier state.

There are several ways in which *S. dublin* infection is maintained and spread in a herd. Of these the carrier-animal is probably one of the most important. Olson (1939) and Field (1948) believe that calves generally obtain the infection from adult carrier-cattle. According to Karsten (1929, 1933) and Field (1948) adult cattle, unlike calves, remain carriers of infection after they have recovered from the disease, and these animals are usually responsible for the introduction of the disease into a clean herd. Karsten believes that subclinical cases of " Gaertner " infection in calves are not readily recognized during life, but that they may be detected in the abattoir from the necrotic miliary nodules in the liver, and that a bacteriological examination of the bile of suspected animals will often reveal the presence of carriers. Miessner and Köbe (1929, 1931), have found that recovered adult animals may remain carriers and may continue to discharge *S. dublin* indefinitely in the faeces. According to them, clinically healthy carrier-animals may be detected in some infected herds but not in others. Standfuss, Wilken and Sorrensen (1932) have reported that carriers which have never shown any clinical evidence of infection may yield " Gaertner " bacilli in cultures made from the bile, from the liver and from other organs, and that carrier nursing-cows may infect their calves. According to Rievel (1933) the carrier state in the living animal can be recognised by means of the agglutination test as well as by the bacteriological examination of the faeces, but in the dead animal bile cultures are the most reliable method of making a diagnosis. Rievel has recovered " Gaertner bacilli " irregularly from the faeces of carrier-animals for a period of 18 months.

Field (1949) has also shown that the presence of Salmonella infection can be readily determined by an examination of the bile. By means of the agglutination test Lutje (1940) and Bythell (1946) have detected positive cases, of which some were discharging *S. dublin* in the faeces. In order to detect carriers in a suspected herd Rankin and Slavin (1947) have carried out the agglutination test with the whey; and *S. dublin* in an almost pure culture was recovered from the faeces of one of the animals which gave a positive whey test.

In the course of a study of an outbreak of *S. dublin* infection in a herd John (1946) has observed an abnormally high agglutination titre in some of its members, and from the faeces of one of these he recovered *S. dublin*. He also believes that all recovered adult animals may become carriers and may discharge *S. dublin* in the faeces for long periods. In the same way Field (1948) has demonstrated that apparently healthy carriers in infected herds may discharge the infection regularly or irregularly in the faeces for a long time. Craig, Davies and Massey (1941), however, have been unable to detect any carriers in a herd kept on a farm where very severe losses were sustained among hand-raised calves, and the source of infection has remained obscure.

On examining a herd with a high abortion rate owing to *S. dublin* Bert (1943) found five cows and three calves that were excreting *S. dublin* in the faeces. The agglutination titre of the positive carriers varied from 1:400 to 1:6,400; but seven animals with negative faecal examinations had an equally high agglutination titre.

During the course of a study of calf paratyphoid in South Africa the author examined several infected and uninfected herds serologically and cultured the faeces of animals whose sera showed an "H" agglutination titre of not less than 1:200. The "H" agglutination titre of the sera of animals from non-infected herds seldom exceeded 1:25, and "O" agglutinins were usually not evident at dilutions of 1:5. In infected herds it was frequently possible to detect a variable number of animals whose "H" titre exceeded 1:200; "O" agglutination, however, remained significantly low even in animals that proved to be carriers by discharging *S. dublin* in the faeces. But it was, on the whole, exceedingly difficult to detect *S. dublin* in the faeces of animals from infected herds; for even the faeces of cattle whose sera had a titre of 1:200 or more could not always be relied upon to yield *S. dublin* on culture. In some undoubtedly infected herds faeces cultures remained consistently negative, although several members of these herds had an "H" titre of more than 1:200. In this respect our observations agree with those of Craig, Davies and Massey (1941) who were unable to find carrier-animals in a herd among which the disease had existed enzootically for a number of years.

On rare occasions *S. dublin* could be isolated from the faeces of animals with an "H" titre of less than 1:200. But by far the greatest number of positive cultures were obtained from animals with a high agglutination titre. For the detection of carriers in infected herds, therefore, serological and also cultural methods should be applied. But it should be pointed out that, in *S. dublin* infection in cattle, far more reliance should be placed on "H" than on "O" agglutination. In actively infected as well as in carrier-animals the agglutinogenic response appears to be confined largely to the production of "H" agglutinins, as the "O" agglutinin titre of the serum is seldom raised above the normal level, which rarely exceeds 1:5. In this respect the animal's response to *S. dublin* infections differs from its reaction to infection with *S. typhi-murium* and *S. abortus-equi*. Henning and Haig (1939) and Henning (1946) have shown that for the detection of the carrier state in birds infected with *S. typhi-murium* and in equines infected with *S. abortus-equi* "O" agglutination alone is significant as "H" agglutinins are either entirely

absent or occur in such small amounts that their presence can hardly be demonstarted. When susceptible equines, however, are actively infected with *S. abortus-equi* or inoculated with *S. abortus-equi* vaccine, both "H" and "O" agglutinins are formed (Henning, 1946; Henning and McIntosh, 1946).

When animals are actively immunized with *S. dublin* vaccine the agglutinogenic response also results largely in the production of "H" agglutinins; and it is only in some cases that a significant amount of "O" agglutinin is produced (see Henning, 1952a).

Karsten (1929) and Field (1948) reported that adult cattle remain carriers of the disease after a clinical recovery but that calves lose the infection. As far as adult cattle are concerned our observations are in full agreement with these findings. We have found that adult cattle that have recovered clinically from an apparent or an inapparent natural or artificial infection usually remain persistent carriers and continue to discharge the organisms either regularly or intermittently for an indefinite period. Our observations on actively infected calves have shown that even when typical clinical symptoms are manifested faeces cultures are negative during the first week or two. Faeces cultures frequently become positive after two or three weeks and may remain regularly or intermittently positive for several weeks, sometimes indefinitely (see Henning, 1952a, calf 5075). There seems, therefore, to be little doubt that the infection is spread and maintained in a herd by means of clinically recovered calves as well as adult cattle.

According to our observations, clinically recovered carrier-animals discharge the organisms more consistently in the faeces than those actively infected and, consequently, should be regarded as at least as dangerous as animals showing clinical symptoms of the disease.

In a previous study Henning (1939) reported that Salmonella infected faeces may remain viable after drying in the incubator for more than 900 days. Premises in which infected animals or carrier-animals have been housed, therefore, should be regarded as a potential source of danger for at least 900 days, unless these premises have been properly disinfected meanwhile.

The Role Played by Rodents and the so-called Rat-"Viruses" in the Dissemination of Salmonella Infection.

It has been established conclusively that rats and other rodents may harbour organisms of the Salmonella group, particularly *S. enteritidis* and *S. typhi-murium*, and that they frequently excrete these bacteria with the faeces. Rodents may, therefore, play a rôle in the dissemination of bacteria that cause Salmonella infection. Bainbridge and Boycott (1909) and Boycott (1911) reported spontaneous outbreaks of *S. enteritidis* infection in rats. But Savage and White (1923) did not regard Salmonella strains as natural inhabitants of the intestinal tract of rats, although they realized that these organisms might be present in some animals. They succeeded in isolating *S. enteritidis* from the intestinal tract of six out of 96 rats caught in slaughter-houses. Meyer and Matsumura (1927) examined 775 wild rats in San Francisco and recovered Salmonella strains from the intestinal contents and internal organs of 58: *S. enteritidis* from 28, and *S. typhi-murium* from 30. Many of these animals were shedding the organisms in the faeces. Verder (1927) examined 100 wild rats and demonstrated paratyphoid-enteritidis bacteria in the internal organs of six. Of 100 rats trapped in Aberdeen Kerrin (1928) found eleven infected with *S. enteritidis*. In a study of 750 field rats trapped in Liverpool Khalil (1938) isolated 89 Salmonella strains from 55 (7·3 per cent.) of the rats. Of these 45 were *S. enteritidis*, 40 were *typhi-murium*, and three were *newport*.

Welch, Ostrolenk and Bartram (1941), however, believe that the comparatively high incidence of Salmonella strains reported in rodents should be attributed to the restricted areas from which the material was obtained. In a study of rat and mice excreta collected in areas from all over the United States they could find barely a 1·2 per cent. contamination. Nevertheless, serious outbreaks of Salmonella infection have been traced to rodent secreta by other workers. Thus, Selthe and Krumwiede (1924) described an epidemic in New York, involving 59 persons. The vehicle of infection was cream cakes and éclairs, and a strain of Salmonella was isolated from the leftovers of the food. Rodent pellets deposited on the shelf where the cake had been kept were examined and found to harbour the same strain of Salmonella that had contaminated the cake. Another outbreak, involving 208 persons, of whom three died was reported by Staff and Glover (1936). *S. enteritidis* was found to be the cause of infection, and the vehicle was cream cake-filling. An investigation revealed that infected rat faeces had apparently contaminated the cake-filling used for the bakery products. Jones and Wright (1936) described an outbreak of food infection, involving five members of a family. There was one death, and *S. typhi-murium* was incriminated. Milk contaminated with mouse pellets, infected with *S. typhi-murium,* was found to have been the vehicle of infection.

But rodents are also responsible for spreading Salmonella infection in animals. Thus, Nordlund (1938) described four outbreaks of scours in farm animals due to *S. dublin,* and rats were incriminated as the source of infection. *S. dublin* was isolated from the grain feed of the animals which was contaminated with rat droppings, and also from a number of rats caught on the premises. Moreover, Field (1948) was able to recover *S. dublin* from six rats caught on farms where paratyphoid had raged in both adult cattle and calves.

Some years ago the author investigated an extremely virulent outbreak of paratyphoid infection in an aviary of very valuable canaries and found *S. typhi-murium* to be the cause. The outbreak occurred soon after the introduction of a new bag of mixed grain for feed. Several rodent pellets were found mixed with the grain, and from these a strain of *S. typhi-murium* similar to the one isolated from the dead canaries was recovered.

If it is conceded that rodents may play a rôle in the dissemination of Salmonella infection the indiscriminate use of commercial cultures of Salmonella for the eradication of rats must be regarded as dangerous and should not be allowed. Although the author is not aware of any cases of natural infection of domestic animals with rat " virus ", a number of outbreaks of food infection in man have been reported by different workers. Shibayama (1907) and Bainbridge (1912) described epidemics of gastro-enteritis in man where commercial rat " virus " had been incriminated. Langer and Thomann (1914) reported an outbreak in which there was one death. Wrenscher (1921) described three outbreaks in which food contaminated with rat " virus " was the cause. Deaths were recorded in two of the outbreaks. Spray (1926) studied an epidemic involving 135 persons in a University Hostel and suggested that a commercial rat " virus " placed in the serving room had been responsible for the contamination of the food which caused the infection. A strain of *S. enteritidis,* identical to the rat " virus " used, was isolated from 14 of the patients. Kristensen and Bojlen (1931) described ten outbreaks of acute gastro-enteritis in Denmark, involving 52 persons. In two of these the evidence incriminating rat " virus " was conclusive, and it was clear that the infection had followed the careless handling of " virus ". There was one death. In the remaining eight outbreaks the evidence implicating rat " virus ", though very strong, was inconclusive. More recently Kokko (1947) reported an outbreak of Salmonella

food infection in a hospital at Helsinki, involving 430 persons. Milk was found to have been the vehicle, and the only two persons in the hospital who escaped infection had not partaken of the milk. The milk incriminated came from a farm where a rat " virus " had been used.

Moreover, Tiede (1931) showed that the use of bacterial preparations for rodent destruction might lead to infection of game animals, like hares and hamsters. Owing to the danger of contaminating vegetables and plants used as food for man and animals, he advocated legislation devised to prevent the employment of rat " virus " for rodent extermination. If game animals can become infected under natural conditions, there is no reason to assume that domestic animals would be immune.

Public Health.

Since Gaertner (1888) described an epidemic of food infection at Frankenhaus due to "*S. enteritidis*", and involving 57 persons, many outbreaks of Salmonella food infection have been reported in different parts of the world.

In a report on 112 outbreaks of food-poisoning Savage (1920) showed that Salmonella strains were responsible for 41 of these. In 20 of the epidemics, including 15 caused by "*S. enteritidis*", the source of infection was traced to cattle, and a frequent vehicle of infection was found to be milk obtained from cows suffering from acute diarrhoea. Although the majority of outbreaks of Salmonella food-infection in man described by Savage and White (1925) were due to *S. typhimurium*, "*S. enteritidis*" predominated in cases where the vehicle of infection was of bovine origin.

In Germany, Meyer (1930) described 281 epidemics during the period 1923 to 1928. Of these 168 were traced to cattle, 60 to pigs, 49 to horses, two to sheep, and two to goats. In another 137 outbreaks sausage meat was the vehicle. A large percentage of these epidemics followed the use of " emergency-slaughtered " meat, and the common organism found in cases of bovine origin was "*S. enteritidis* of Gaertner". Lentz (1924) noticed a marked increase in the number of epidemics of meat poisoning in Prussia during the years immediately following the First World War. He attributed this increase to the frequent use of " emergency-slaughtered " meat at the time. He regarded the " enteritidis " group of bacteria as the most common cause of the disease and believed that these organisms were identical to those usually associated with " Kälberruhr " and septic infection of cows during parturition.

Several other workers have reported outbreaks of Salmonella meat-infection and an extensive literature has accumulated on the subject, but it is not our intention to review this literature. However, attention is directed to the important rôle played by cattle in causing epidemics of food infection, particularly through the medium of milk.

A great deal of evidence has been obtained to show that the milk of cows shedding " Gaertner bacilli " in the faeces may be contaminated with these bacteria and that such milk may serve as a vehicle for Salmonella food-infection.

Although it is conceded that these organisms may be discharged in the milk when the cow suffers from septicaemia or when the udder is infected, most of the available information indicates that the contamination occurs during or after the withdrawal of the milk and that the faeces of a carrier-animal is an important contaminant.

Although Bugge and Dierks (1922) succeeded in isolating " Gaertner bacilli " from the udder of a cow that was affected with septicaemia and gastro-enteritis, they believed that the milk was contaminated with faecal material.

In a study of " Gaertner " infections in adult cattle Lehr (1927) isolated these bacilli from the milk of cows that were excreting the organisms in the faeces, and Rudolf (1928) recovered paratyphoid bacilli from the udders of five cows that were suffering from mastitis. Poppe (1931) reported outbreaks of Salmonella infection in man traced to a focus of *S. dublin* infection in the udder of a cow and to contamination of the milk with dung. John (1946) also reported that the milk of cows, which were infected with *S. dublin,* was frequently contaminated with infected faeces. Moreover, Standfuss, Wilken and Sorrenson (1932) showed that calves might become infected when nursed by cows which were discharging " Gaertner bacilli " in the faeces; but they believed that the presence of " Gaertner bacilli " in the milk should be attributed to faecal contamination. Wundram and Schönberg (1931) found that the faeces of a clinically healthy carrier-cow could contaminate the milk with *S. typhi-murium.* Clarenburg (1931), however, believed that, although the milk was often contaminated by the faeces, infection might also occur in the udder.

Miessner and Köbe (1929) by regularly examining the milk of a carrier-cow, which was discharging " *S. enteritidis* Gaertner " in the faeces, could not find these organisms more than twice during a period of 30 days, and they attributed the presence of these bacteria to faecal contamination of the milk. Standfuss and Wilken (1933), after carefully examining the milk of two cows that were excreting " Gaertner bacilli " in large numbers with the faeces, could not find a single milk sample that was infected. They concluded, therefore, that Gaertner bacilli occurred in the milk only as a result of contamination with excreta. For a period of 18 months Rievel (1934) regularly examined the milk of four cows which were excreting *S. dublin* intermittently with the dung and found that, provided it was carefully drawn and not contaminated with faeces during the handling, the milk remained free from infection .

One of the first records of a milk-borne epidemic of Salmonella food-infection is that of Fischer (1906), involving 50 persons. The milk that was implicated was bulked with the milk derived from two cows that had been suffering from enteritis. Paratyphoid organisms were later recovered from the internal organs of these cows. Bainbridge (1912) reported an outbreak in which 100 persons became affected with gastro-enteritis after they had partaken of some milk obtained from a recently-calved cow. This cow was obviously sick at the time of milking. " Gaertner's bacillus " was isolated from the milk as well as from the evacuations of some of the patients. Another epidemic, involving 523 persons, was studied by Kerr and Hutchins (1914), who traced the source of infection to a sick cow. "*S. enteritidis*" was isolated from the dejecta of the patients and from the organs of the cows. Bugge and Dierks (1922) described an outbreak of gastro-enteritis affecting a group of persons, and traced the source of infection to a cow that was suffering from acute diarrhoea and septicaemia, " Gaertner bacilli " having been isolated from the udder of the cow. Grimsted (1923) attributed an outbreak of enteritis in the Aalberg Hospital in Denmark to "*B. paracoli*" (*S. dublin*) which he isolated from the stools of the patients and from the internal organs of the cow that had supplied the mlik. In an epidemic of gastro-enteritis involving 497 persons Kinlock, Smith and Taylor (1926) traced the source of infection to the milk. They recovered "*S. enteritidis*" from the milk, from the dejecta of the patients, and from the udder and flesh of one of the cows in the herd which supplied the milk.

Lutje (1926) reported "Gaertner" infection in two children who became infected after receiving milk from a cow that had to be "emergency-slaughtered".

In view of the work of Bruce White (1929) and of Smith and Scott (1930) the labelling assigned to the organisms described in these outbreaks can no longer be accepted, and there seems to be very little doubt that *S. dublin* is the cause of the great majority of the milk-borne epidemics reported. Moreover, Smith and Scott (1930) suggested that *S. dublin* should be regarded as the organism responsible for the milk-borne epidemics of food infection reported at Newcastle (1910), Nottingham (1919), Cork (1921), and London (1928). Finally these authors described three cases of continued fever in which *S. dublin* was incriminated.

Souper, Smith and Stephen (1930), McAllan and Howie (1931), and Smith (1933, 1934) reported various outbreaks of *S. dublin* infection in man, some of which involved infants and children. In one epidemic, involving three members of a family, there appeared to be a spread of the infection from case to case. These authors showed that the disease in children was usually associated with septicaemia and meningitis. Moreover, Bruce White (1929) found that the cases of meningitis in children described by Pesch (1926) were due to *S. dublin*. But other strains of Salmonella have also been incriminated as the cause of meningitis in infants and children. Thus, Guthrie and Montgomerie (1939) recorded eleven cases of meningitis in infants, only one of which was due to *S. dublin*, the other ten having been caused by *S. enteritidis;* and Henning, Rhodes and Gordon-Johnstone (1941) reported a case of meningitis in a child due to *Salmonella kaapstad*.

An epidemic of "Gaertner" food-infection, in which cheese was the vehicle, was described by Bourmer and Doetsch (1928). The milk used for the cheese-making was derived from a herd harbouring a cow that was discharging "Gaertner bacilli" in the faeces, but no organisms could be found in any of the milk samples studied. Another outbreak in which milk contaminated with *S. dublin* was the vehicle, was reported by Poppe (1931). The milk was derived from a carrier-cow that was discharging *S. dublin* in the dung, and later it was found that she had in addition a small focus of *S. dublin* infection in the udder.

Conybeare and Thornton (1938) traced the cause of an outbreak of gastro-enteritis, involving 100 persons, to bottled milk contaminated with *S. dublin*. By means of serological tests an attempt was made to detect carriers in the herd supplying the milk. Three cows were found giving an H-agglutination reaction of at least 1:400, and from the faeces of one of them *S. dublin* was isolated. In each of two extensive outbreaks of a milk-borne Salmonella infection, involving 373 and 280 persons respectively, Tulloch (1939) traced the source of infection to a cow affected with septicaemia. *S. dublin* was isolated from the milk, from the stools of some of the patients, and from the udder and internal organs of the cows.

Sutherland and Berger (1944) and also Rankin and Slavin (1947) traced an epidemic of Salmonella infection, affecting 162 persons, to milk contaminated with *S. dublin*. By means of an agglutination test of the whey they detected an apparently healthy carrier-cow which was excreting large numbers of *S. dublin* in the faeces. They regarded a whey titre of merely 1:64 as being an indication of the carrier state. But the milk of the carrier-cow taken directly from the udder was not infected, and these authors presumed that *S. dublin* was either discharged intermittently or that the milk became contaminated during the handling.

More recently two epidemics of *S. dublin* food infection were reported in the Lancet (1947a and 1947b). Milk was believed to be the vehicle in both outbreaks. In one epidemic 97 persons were affected, and *S. dublin* was recovered from the milk, from the stools of some of the patients, and from the dung of one cow and two calves in the herd supplying the milk. The organism was later also recovered from the organs of the cow. The other outbreak involved 133 persons, and *S. dublin* was isolated from the stools of three of the patients; but, although there was very strong evidence implicating the milk as the vehicle, no definite proof could be obtained.

In spite of the extremely wide distribution of *Salmonella dublin* infection in cattle, particularly calves, in South Africa, there are not many records of food infection implicating this organism. The first apparent case which came to the notice of the author occurred on a farm that was badly infected with calf paratyphoid. Although a proper diagnosis has never been made, there is very strong circumstantial evidence that the infection was of bovine origin and that the cause was *S. dublin*. The victim was a child of about twelve months old. This child had been regularly fed on boiled milk until a few weeks before it fell ill, when it received raw milk from the infected herd. No facilities existed for refrigeration on the farm. The child developed a continued fever and, later, meningitis. Faeces and cerebrospinal fluid were examined bacteriologically and a diagnosis of "paratyphoid", based entirely on biochemical tests, was returned. The author was unable to obtain a culture of the organism and therefore could not examine it serologically. But on several different occasions he had succeeded in isolating strains of *S. dublin* from the organs and faeces of calves on the farm, which was known to be notoriously infected.

The first outbreak of food infection proved to have been caused by *S. dublin* was reported by Henning (1938). It involved ten natives who had partaken of the meat of a calf that had died from calf paratyphoid. *S. dublin* was isolated from the blood of one of the patients who had died. Although material from the calf was not available for study, there is no doubt that the calf's meat was responsible for the epidemic, as the herd, of which the calf was a member, was badly infected with *S. dublin* at the time.

More recently Pullinger and Scott-Millar (1945) reported two epidemics of food infection on the Witwatersrand. In both of these, milk contaminated with *S. dublin* was the vehicle. In one of these outbreaks over a hundred persons were involved, and *S. dublin* was recovered from the milk, from the dejecta of some of the patients, and from the faeces and internal organs of a cow which was a member of the herd supplying the milk. Agglutination tests of all cows in the herd, including the carrier, were performed, but not a single animal gave a reaction which could be regarded as being above the range of normal agglutination. In the second outbreak over 150 persons were affected; and milk was also the vehicle of infection. But, although *S. dublin* was isolated from the milk and from the evacuations of some of the patients, the source of the contamination could not be traced.

Summary and Conclusions.

1. The incidence of calf paratyphoid in various countries and its relationship to disease in adult cattle, in other species of animals, and in human beings are reviewed.

2. The isolation of 507 strains of Salmonella from outbreaks of paratyphoid in calves is reported. Of these 491 were identified as *S. dublin*, eleven as

S. typhi-murium, four as *S. enteritidis*, and one as *S. bovis-morbificans*. In addition the isolation of twelve further strains of *S. dublin* from adult cattle is recorded.

3. The significance of *Salmonella dublin* as an etiological agent in paratyphoid of cattle, particularly calves, is discussed.

4. For the detection of *S. dublin* or other forms of Salmonella infection either faeces, bile, blood or liver, and spleen cultures were made, and the suspected sera tested for "O" and "H" agglutinins. In some cases only one of these tests was possible, but at other times two or more were performed. In addition a pathological study of the liver and spleen was made whenever possible. With very few exceptions the results obtained from a pathological examination of suspected organs corresponded to the results of the bacteriological study. But in many positive cases of calf paratyphoid faeces and blood cultures were negative and a negative agglutination test was obtained. Sometimes faeces cultures were positive when the serological test was negative or vice versa. In other cases the presence of a carrier was spotted first by a positive agglutination test. For the detection of carriers, therefore, both faeces cultures and serological tests should be performed.

5. The agglutinogenic response of infected, carrier, or immunized animals generally resulted in the production of practically only "H" agglutinins. "O" agglutinins were seldom present in significant amounts, and when they were present the titre was generally extremely low in comparison with that of the "H". These results do not agree with the previous observations of Henning and his co-workers (1939, 1946, 1942). These workers found that the sera of birds acting as carriers of *S. typhi-murium* or of horses affected with a latent or chronic *S. abortus-equi* infection responded chiefly to "O" agglutination, and that the "H" agglutination was either poor or entirely absent.

6. The rôle played by rodents and the so-called Rat "Viruses" in the dissemination of Salmonella infection is discussed.

7. The significance of *S. dublin* as an etiological agent of food infection in human beings is discussed.

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