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STUDIES ON A COMMON SOURCE EPIZOOTIC OF BOVINE SALMONELLOSIS IN MANITOBA

by

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ABSTRACT

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A. A. VAN DREUMEL

During a period from January to June, 1966, a severe outbreak of bovine salmonellosis occurred in Manitoba. The disease was diagnosed clinically in approximately 50 herds. Salmonella organisms were isolated from carcasses or specimens on 24 different farms involving approximately 2,379 cattle The most frequently isolated serotype was S. newport (92 per cent).

The source of infection was traced to bonemeal and mineral supplements. The bonemeal and one of the mineral mixtures originated at one local feed mill. Out of 20 bonemeal samples collected at the affected farms during or shortly after outbreaks occurred, 19 yielded Salmonella organisms, most of which contained more than one serotype. Cultures of three mineral samples were also positive for Salmonellae.

The serotypes isolated from cattle corresponded with those isolated from unused portions of bonemeal or mineral on 16 premises. The total morbidity on the 24 farms amounted to 525 animals (18 per cent) with a mortality rate of 127 animals (24 per cent of clinically affected animals).

Salmonella organisms (including S. newport) were isolated from bonemeal samples at retail outlets, bags stored at the feed mill and the environment of the mill. Bones used for the production of the bonemeal and other

animal by-products used as feed ingredients were collected from two rendering plants which supplied the feedmill and these were positive for Salmonellae.

There was a 100 per cent increase in 1966 in the incidence of human infections with S. newport in Manitoba compared to the previous year. None of the human cases could be traced to the bovine outbreak. However contaminated beef products may have been an important source of infection.

Stricter sanitary conditions in rendering plants and feed mills could prevent similar recurrences of contamination of animal feeds with Salmonellae. This in turn would reduce the risk of infection in animals and man.

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INTRODUCTION

Salmonellosis is one of the most important zoonosis in many countries. During the last twenty years there has been a significant decrease in the incidence of *S. typhi* infection in humans in countries of the Western hemisphere. In contrast, the incidence of paratyphoid infections in man has greatly increased during this period. A similar increase in the incidence of salmonellosis has been reported in the animal population during this period. *S. typhimurium* is the organism most frequently isolated in both groups, but all serotypes are considered to be potential pathogens for man and animals. It is generally agreed that animals and food products of animal origin are the most important reservoirs of Salmonellae for humans. There are numerous sources of infection for the animal or bird population. It may be introduced into these by infected animals or birds of the same or different species, contaminated feed or water, fertilizers or by man himself. The literature contains many reports where these various sources are incriminated. The evidence in some of these papers is well substantiated while in others the evidence is only circumstantial.

The salmonella outbreak in Manitoba cattle presented an ideal opportunity to study the epizootiology and epidemiology of infection with *S. newport* for several reasons. There were no previous isolations of this serotype in cattle in the province. The history in the herds where the disease was first diagnosed soon suggested bonemeal as the source of infection.

There are numerous reports in the literature which describe the prescence of Salmonellae in animal

feeds and animal by-products used as feed ingredients. However many workers have questioned the importance of these organisms in these products with regard to actually being able to produce outbreaks of salmonellosis. Examples of such outbreaks have been reported mainly in poultry.

Since there appeared to be a definite need for more accurate information on the incidence and source of salmonella infections in animals, it was proposed to further study the epizootiology and epidemiology of this paticular outbreak.

The study included collection of information on morbidity and mortality, clinical symptoms, serological reactions, pathological lesions, effectiveness of therapy and with emphasis on the epizootiology and the epidemiology. Investigations extended into the feed mill where the bonemeal was produced and the rendering plants which supplied the bones and other animal by-products. The incidence of S. newport infection in humans in Manitoba was also investigated.

REVIEW OF LITERATURE

Definitions

Before attempting to describe a disease caused by a certain type of organism, it would appear to be proper to define the organism itself. The following definition of Salmonellae was adopted by the International Association of Microbiologists (38):

"The genus Salmonella consists of serologically related Gram-negative, non-sporing rods, corresponding to Salmonella typhi in staining properties and morphology, showing with certain exceptions, a motile peritrichous phase in which they normally occur. They do not ferment adonitol lactose, and sucrose, nor liquefy gelatin, nor produce indole, nor decompose urea, nor form acetyl-methyl-carbinol. They regularly attack glucose with, but occasionally without gas production. They do not ferment salicin promptly but in some cases delayed fermentation occurs.

All members of the genus have an antigenic structure by which they can be recognized. All the known types are pathogenic for man, animals or both. By international agreement, serologically related types are considered to belong to the genus Salmonella even if their behaviour differs from the above properties. (fermentation of lactose or sucrose, liquefaction of gelatin, or production of indole.) No organism possessing aberrant cultural or biochemical properties is to be included in the genus Salmonella unless it contains O and H antigens typical of the Salmonella genus".

In this thesis the term "serotype" will be used to refer to members of the genus Salmonella. The term "salmonellosis" will refer to salmonella infection

with clinical symptoms, "salmonella infection" means infection without symptoms. The term "carrier" refers to animals which excrete Salmonella organisms in their feces. The excretions may be constant, intermittent or latent when a stress factor is needed to induce excretion of the organisms. (26). According to Buxton (10), Salmonella organisms have been isolated from various sources but their natural habitation is the intestinal tract of animals and under favourable circumstances most species may become symptomless carriers.

It is not within the scope of this work to completely review the incidence of bovine salmonellosis with all the serotypes which were involved. The review will be limited to the most common serotypes associated with salmonella outbreaks in various species of animals where feed or feedstuffs were incriminated as a source of infection. The incidence and serotypes involved in bovine salmonellosis on the North American continent will be discussed in greater detail.

Incidence and Serotypes in Cattle in Countries Outside the North American Continent

Henning (33) reviewed calf paratyphoid and he pointed out that the pathogenicity of any strain of Salmonella is not strictly specific. He mentioned that S. typhimurium was the most cosmopolitan serotype which may infect any mammal or bird. Other serotypes appeared to more or less host specific: S. typhi and S. paratyphi, A, B and C for man, S. pullorum and S. gallinarum for poultry, S. choleraesuis for swine, S. dublin for cattle, S. abortivoequina for pregnant mares and S. abortusovis for pregnant ewes. This worker found S. dublin as the most common source of calf paratyphoid, 491 (97 per cent) were caused by

S. dublin.

Buxton (10) listed 28 serotypes which were isolated from calf material received at Veterinary Investigation Centres in England and Wales. During the three year period from 1958 to 1960 inclusive, a total of 918 Salmonellae were isolated,, 61 per cent of these were S. dublin, 35 per cent S. typhimurium and the remaining four per cent were "exotic" serotypes.

Field (22) suggests that any serotype other than S. dublin, infecting cattle will likely be cosmopolitan in nature, i.e. they are not restricted in their habitat to any one species of host. This author noted that bovine salmonellosis is enzootic in many European countries, notably Holland, Germany and Denmark and is responsible for rather heavy losses in young animals. S. dublin was the serotype most commonly isolated in these outbreaks. This worker felt that because salmonellosis occurred mainly as sporadic cases and not as herd outbreaks, the disease never received the attention it deserved in Great Britain. A survey carried out by this writer during 1946-1947 in South Wales showed that on 50 out of 68 farms on which cases of salmonellosis occurred only one animal in each herd developed clinical symptoms of the disease. Severe outbreaks in adult cattle were regarded as being exceptional. S. dublin was the causative organisms at 102 out of 106 farms with bovine salmonellosis. S. Typhimurium was isolated on the four remaining farms. Eighty-eight per cent of the outbreaks occurred during the months of May to October inclusive thus suggesting a seasonal incidence.

Gibson (25) stated that S. dublin was the

predominant serotype in calves in those areas where salmonella infection is endemic in adult and yearling cattle. Previous studies had also shown that S.dublin was the predominant serotype in these older cattle. Calfhood infection was invariably the result of direct or indirect contact with carrier animals. This author also emphasized that the proportion of cases due to S. typhimurium infection was higher in purchased calves than those reared at home. The increased demand for calves for rearing as beef with the large scale movements of these calves through sales etc. was suggested to be a predisposing factor for the apparent increase of S. typhimurium infection. It was felt that adult cattle constitute a likely source of S. typhimurium infection in calves. However, due to the lack of host-specificity other sources should be investigated including poultry, other species of animals, wild birds, feed and water. Similar recommendations were made for bovine infections with other less common or "exotic" serotypes.

Dennis (13) reviewed the incidence of salmonellosis in domestic animals in Western Australia. He concluded that salmonellosis is uncommon in Australia as a whole. In Western Australia a total of only 19 Salmonellae were isolated from cattle for the period of 1939 to 1964. Twelve of the 13 typed outbreaks of infection in cattle were due to S. typhimurium. Next to S. pullorum, S. typhimurium was the most frequently encountered serotype in all species of domestic animals. Since 1950 S. typhimurium became the most common serotype in all species of domestic animals. This serotype also caused serious outbreaks in post-parturient sheep. S. dublin was not isolated from any species in this survey.

The most extensive review on Salmonella infect-

ion in cattle was probably written by Gibson (26). He concluded that the serotype most commonly associated with bovine salmonellosis in areas where the disease is endemic is S. dublin. The following countries were mentioned as examples where the disease was endemic: Germany, Holland, Denmark, the British Isles and Ireland. A high incidence of S. dublin was also reported for South Africa, Venezuela, and Brazil. The serotype was further reported from various countries such as India, Kenya, Israel, Nigeria, Australia and the United States. Thus, S. dublin is widely distributed and presents a major problem in a number of countries. This worker also concluded that S. typhimurium is usually present but with a lower incidence, in any area which S. dublin occurs. In those areas where S. dublin is absent, then S. typhimurium usually constitutes the chief salmonella infection of cattle. He also suggested that the prescence of serotypes other than S. dublin or S. typhimurium, is usually due to contamination of feedstuffs, or to cross-infection from other animals or birds. In his experience such infections are commonly subclinical and seldom establish themselves in a herd. Infection of adult cattle, i.e. over six months of age, was considered to be sporadic.

Stevens (64) compared the incidence of S. dublin and S. typhimurium infection in calves in the United Kingdom. Three striking changes became apparent in the calf salmonellosis in 1965:

(a) There was a significant increase in the total number of incidents. In 1964 there were 954 isolations compared to 1,728 in 1965.

(b) There was also an increase in the proportion of outbreaks due to S. typhimurium. The

ratio of S. typhimurium to S. dublin infection changed from 1:3 in 1964 to 3:4 in 1965.

(c) Sixty-seven per cent of S. typhimurium isolated in 1965 belonged to the same phage type, namely type 29.

It was felt that the increase in bovine salmonellosis was real and not the result of improved laboratory techniques. The increases were thought to be due to crowding of calves on breeding farms, transportation, passage through sales barns, and further distribution of infected animals over widely scattered areas. S. typhimurium phage type 29 was also resistant against several drugs commonly used for prophylactic treatment of various conditions in feeder calves.

Guinee, et. al.(31) investigated the source of S. typhimurium infection in veal calves on three farms. On two of the farms the calves were kept with pigs in the same barn. The third farm was free of pigs. The calves were cultured for the presence of Salmonella throughout the feeding period and after slaughter. The incidence of S. typhimurium was significantly higher where calves were kept with pigs, compared to the group where calves only were present. These workers suggested that the environment, (including pigs) is the most important factor in the pathogenesis of Salmonella infections in veal calves.

In summary, it appears that bovine salmonellosis is a serious problem in many countries. S. dublin appears to be the predominant serotype in those areas where the disease is endemic. The source of infection for this serotype is almost invariably a carrier animal. S. typhimurium is the next most common serotype isolated from cattle with salmonello-

sis. The source of infection is most likely a carrier animal from the same or different species, however alternative sources of infection should be investigated. These potential sources include poultry or wild birds, feed, water and fertilizers. It is apparent that in very few cases of bovine salmonellosis caused by serotypes other than S. dublin, epizootiologic studies were able to establish the source of infection.

Incidence and Serotypes in Cattle in the United States

There have been relatively few reports on bovine salmonellosis in the North American literature until the last decade, when there has been an apparent increase in the frequency of these reports. This apparent increase is probably real in part but improved methods of isolation undoubtedly account for a certain per centage of these cases. In a review on Salmonellosis, Edwards (18) stated: " In the case of Salmonellosis it is impossible to determine accurately the incidence of the condition in either animal or man. No systematic method of reporting salmonellosis in animals has been established and one must rely only on summarries of isolations and identification of causative agents that are dispersed throughout the medical and veterinary literature to estimate the frequency with which the bacteria occur among animals." In other words the number of reported cases is not necessarily a true reflection of the incidence of bovine salmonellosis as it occurs in the field.

Edwards et al (16,17) and Bruner et al (8) reviewed the Salmonella serotypes cultured in the United States during a period of 1934 to 1947. In cattle, S. typhimurium was responsible for 16 outbreaks

of salmonellosis, S. choleraesuis for 10, S. newport for two and S. dublin for 19. The authors suggested that the low incidence of salmonellosis in the United States could be due to the apparent absence of S. dublin throughout the greater part of the country. All cultures of S. dublin from various species came from areas west of the Rocky Mountain range.

One of the bovine outbreaks caused by S. newport occurred in adult steers suffering from enteritis. Most of the cultures of S. newport, were from sporadic cases or small outbreaks of enteritis in which only a few individual animals in the herd were affected. A few of the cultures were from sporadic cases of abortion.

These workers also mentioned that mixed infections with different serotypes in one particular animal or group of animals are not uncommon. This phenomena was especially common in poultry outbreaks. Only one case of a multiple infection occurred in a cow, where S. derby, S. Bredeny and S. worthington were cultured from the feces.

Moran (43) reported on 850 Salmonella cultures which were isolated in the United States from animal sources in 1957 and typed serologically at the Communicable Disease Centre in Georgia. There were only two cultures of Salmonella reported in cattle. This number is very low compared to 86 isolations from swine and 622 from poultry.

Schroeder, et al (59) described an outbreak of food poisoning in 47 humans. There was highly suggestive circumstancial evidence that certified raw milk was the source of infection. Subsequent culturing of fecal samples in the herd where the milk originated revealed S. dublin infection in three cows. Two of

these proved to be active carriers. None of the animals were clinically ill nor was there any history of salmonellosis in the herd.

Rokey (53) reported on an outbreak of S. dublin in dairy-calves in Arizona. The serotype was isolated from a three week old calf from a large dairy herd in which a mortality of 33 per cent and a morbidity of 25 per cent was reported in the young calves.

The same author reported on the isolation of S. dublin from other calves. (54). In the same paper, the S. dublin was also described in horses, chickens, dogs, mice, rabbits and doves, thus indicating that species other than bovines can be infected with S. dublin. Average calf mortality in the herds infected with S. dublin was 48 per cent with a range of 30 to 85 per cent. Morbidity was almost 100 per cent in one herd. The average morbidity in all herds was 65 per cent.

Mann (41) is one of the North American workers who investigated the incidences of Salmonellae in animals in abattoirs. Four groups of animals, representing 10 animals in each group were positive for Salmonellae. The samples were taken from mesenteric lymphnodes and pooled for each group. The most commonly isolated serotype was S. muenchen.

Ellis (19) felt that salmonellosis among dairy and beef animals was more prevalent than reports would indicate. Fourty outbreaks were diagnosed in cattle in Florida over a two year period, 18 of these being in calves. S. newport was one of the serotypes isolated. Only in one outbreak was the same serotype found in the cattle and feed. An estimated total of 200 calves died as the result of Salmonella infection. The author pointed out that the findings probably represented a small sampling of the entire state.

Moran (44) reviewed the occurrence and distribution of Salmonellae in animals in the United States during the period of 1957 to 1961. Some 6,216 cultures of Salmonellae from animals were serotyped in the Enteric Laboratory at the Communicable Disease Centre, Georgia. These cultures came from more than 35 different animal species and included 86 different serotypes. There were 439 isolations from cattle. S. typhimurium was the most common serotype. It accounted for 42 per cent of all serotypes isolated from cattle. The next most common serotypes were S. newport with 13 per cent and S. dublin and S. enteritidis, each with 11 percent. Thus there was a definite increase in total numbers of isolations and proportion of S. newport infections in cattle, compared to previous reports. This serotype was recovered from 17 other species and it was also common in turkeys. S. dublin was still confined to the western states.

Other isolations of S. newport from cattle with Salmonellosis have been reported recently. Moore, et al (42) isolated S. newport in 80 per cent of fatal cases of enteritis in cattle. The authors suggested that this serotype may cause serious losses in cattle under certain stress conditions. S. newport was also isolated during an outbreak of severe diarrhea in a large dairy herd in Florida (63).

Rude (57) reported 46 cases of S. typhimurium infection in cattle, nine involved mature cattle and 37 occurred in calves. The possible sources of infection were investigated but none were found. S. typhimurium was also responsible for a severe outbreak of salmonellosis in a feedlot with 1,000 head of cattle, (48).

Rothenbacher (56) described salmonellosis in calves on 39 Michigan farms. On 26 of these farms a mortality rate as high as 23.6 per cent was recorded. A total of 48 isolates were serotyped, 44 of these being S. typhimurium and the remaining four S. newport. The average age of the calves that died from acute or sub-acute salmonellosis was 13.7 days. Carrier animals were thought to be the source of infection.

In summary, it appears that the incidence of bovine salmonellosis is increasing in the United States. Prior to the last decade, S. typhimurium and S. dublin were reported as the predominant serotypes in cattle. More recent reports indicate a significant increase in the occurrence of S. newport in cattle, placing it second to S. typhimurium. The geographical distribution of S. dublin remains confined to the western states and this probably explains the relatively low incidence of bovine salmonellosis in the United States as compared to other countries. The sources of most infections in cattle were not established.

Incidence and Serotypes in Cattle in Canada

Gibson (26) felt that salmonella infection in cattle was uncommon in Canada. If the number of reported cases are any indication of the incidence of a disease, then Gibson would appear to be correct in his supposition. Undoubtedly many sporadic cases of salmonellosis are never published.

Schofield (58) published the first report of a fatal outbreak of enteritis in adult cattle due to S. typhimurium in Canada. In the same paper he also referred to an isolation of S. enteritidis variety dublin made by Bain, from one fatal case of enteritis in a cow. In a ten year survey, 26 salmonella isol-

ations were made from cattle in Alberta (3). S. typhimurium was the most commonly isolated serotype, accounting for 17 isolations, followed by S. newport with four isolations.

Avery and Niilo (2) reported on an outbreak of salmonellosis in a beef herd in Alberta.

S. typhimurium was responsible for an outbreak of salmonellosis in a dairy herd in Eastern Ontario. (65). The authors observed that the incidence of salmonellosis in that area had been extremely low. During the previous 10 years 850 bovine carcasses were necropsied and Salmonellae were never isolated. The source of the organism was not determined.

In summary, it appears that the incidence of bovine salmonellosis in Canada is low. Where the condition was diagnosed, it appeared as sporadic cases only. The predominant serotype appears to be S. typhimurium.

Salmonellae in Feeds and Feed Ingredients

There is a vast number of reports which deal with the problem of Salmonella contaminated animal and poultry feeds. Most of these reports are on surveys conducted by government and commercial investigators. The results are generally expressed as per centages of Salmonella positive samples with lists of the different serotypes isolated. The degree of contamination i.e. number of Salmonellae per unit of sample was seldom investigated.

Erwin (20) was one of the first workers to carry out a survey on Salmonella contamination of commercially prepared poultry feeds in North America. Two hundred and six samples were tested, 77 of these producing Salmonella-like colonies on differential media. Seventy-

three of these cultures were finally identified as Paracolon species, one as Proteus mirabilis and only three as S. oranienburg.

Watkins et al (67) cultured 200 samples of animal by-products for Salmonella organisms. Thirty-seven samples (18.5 per cent) were found to be contaminated. Most of the samples were contaminated with several serotypes. One sample yielded eight different serotypes. The study indicated that re-contamination following cooking is primarily responsible for the presence of salmonella organisms in animal by-products. The authors suggested that this recontamination occurred because the same workers were used in both contaminated and "sterile" areas of the plants. There was no direct evidence that these feeds were sources of infection in poultry and livestock.

In England a study was carried out to determine the incidence of Salmonellae in imported feeds and animal-byproducts used as feed ingredients (52). Sixteen per cent of the meat products, 11 per cent of the whale products, and 15 per cent of the fishmeals were positive for Salmonellae. The authors observed that S. senftenberg was the most frequently isolated serotype from the feeds. However, this serotype was rarely cultured from animals or poultry. S. thompson was the most common serotype in poultry and this serotype was recovered from the feed only once. S. choleraesuis was never isolated from the feed but it is the most common serotype in swine.

In view of the isolations of Salmonellae from imported animal by-products in England, a similar survey was carried out in Holland (66). The contamination of fish and whalemeal was less compared to the products imported into England. A significant propor-

tion of the imported meatmeal, bloodmeal and bonemeal were contaminated with Salmonellae. S. typhimurium was rarely present in these samples. The authors therefore concluded that infection with S. typhimurium in swine was mainly due to sources other than animal-meal and fishmeal.

Shotts et al (60) took samples from feed, swine carcasses, slaughter and rendering facilities and pork sausages in a particular area of Florida. Fifty-six finished feed samples were examined and 16 per cent were found to be positive for Salmonellae. A total of 38 serotypes were recovered. Nearly one half of these serotypes were recovered in more than one phase of these studies. Two serotypes (S. derby and S. anatum) were isolated at least once from all phases. In seven instances, the same serotype was found to be present in the feed, swine and packing plant environment. Nine serotypes were isolated from swine and from sausages but not from feeds, while 21 serotypes were isolated from feed but not from animals or sausages.

Grumbles and Flowers (29) demonstrated that protein supplements of vegetable origin may contain Salmonella organisms. Six different serotypes were isolated from seven of 136 samples of cottonseed and soybean oil meal. None of these isolations were associated with clinical disease in animals or poultry.

In Ontario, Wright et al (70) cultured 179 samples of animal byproducts intended for feed supplements for Salmonella species. Out of 78 samples of dry rendered tankage 14.7 per cent yielded 18 Salmonella isolates. Five samples yielded more than one serotype. Of 101 samples of wet rendered tankage examined from 26 different suppliers, only eight of these were infected with Salmonellae. A total of five

different serotypes were isolated.

Salmonella were isolated from four samples of meal after 12 months storage at 8° C. There were no Salmonellae in any samples stored at room temperature for a similar period.

Harvey et al (32) collected crushed bone samples imported from India and Pakistan when they arrived at the docks in Cardiff, England. These bones were intended for the preparation of animal feed-stuffs. Fifty-six of the 57 samples were positive for Salmonellae (98.2 per cent) and 56 different Salmonella serotypes were isolated. In many samples more than one serotype was present. In one sample as many as 14 serotypes were present.

In Manitoba, Isa et al (35) cultured 281 feed samples. Of these approximately 15 per cent contained Salmonella organisms. Five samples contained three different serotypes and six other samples contained two serotypes. A total of 15 different serotypes were cultured. Meat and bonemeal were most heavily contaminated with the greatest variety of serotypes. Finished cattle feeds were free of Salmonella organisms.

During a survey of 18 Wisconsin rendering plants Moyle (46) found 10.8 per cent of the samples collected were contaminated by Salmonellae. There was some correlation between storage time and methods and incidence of Salmonellae in a plant. Eighty-one per cent of the samples that were stored for long periods contained Salmonellae as compared to seven per cent of the samples stored for shorter periods. The study failed to show much correlation between sanitation and incidence of Salmonellae in the plant products. This worker suggested that substances antagonistic to bacterial

growth, such as free fatty acids, may be responsible for the lack of such correlation.

The problem of Salmonella in feedstuffs was the topic of an article in a recent issue of "Feedstuffs" (11). It was pointed out that the United States Food and Drug Administration considers all animal feeds or feed ingredients to be adulterated and subject to seizure if they are contaminated with Salmonella organisms. A commercial laboratory carried out a three year survey on a variety of feedstuffs, including some of plant origin, and found three per cent were contaminated by Salmonellae. The highest percentage of contamination was consistently found in meatmeal. Thirteen per cent of 319 samples tested contained Salmonellae. In another survey the United States Department of Agriculture tested 12,618 samples taken from 26 different states. This survey showed that over one third of the animal products were contaminated with Salmonellae.

Further studies were carried out in 1963 and 1964 on 269 carloads of meatmeal, 27.5 per cent of these loads being found contaminated with 27 different serotypes of Salmonellae. A number of these serotypes were isolated from turkeys which were fed on feed containing the meatmeal. However, there were no outbreaks of salmonellosis in turkeys which could be specifically traced to feed as the source of infection. Another 213 carloads were tested in 1965 and 30.99 per cent were positive for Salmonellae. Out of 171 carloads of meatmeal tested in 1966, 32.75 per cent were contaminated. Samples from certain suppliers were often more heavily contaminated than samples from others. This often was the result of heavy contamin-

ation by one particular serotype. There was no correlation between serotypes isolated from meatmeal and turkey breeder hens in 1966. Pelleting and granulation completely eliminated Salmonellae from several feeds. These procedures were recommended for the control of Salmonellae in feedstuffs. The importance of producing Salmonellae-free animal by-products was also emphasized.

Kampelmacher et al (37) attempted to determine the temperature and heating time which would be necessary to decontaminate fishmeals from Salmonellae. Experiments were carried out in the laboratory and under field conditions. The results indicated that a product free from Enterobacteriaceae including Salmonellae is obtained on heating at 80 to 85° C for 30 minutes. The effect of this treatment on feeding value was not determined.

Edel et al (15) fed 120 pigs with pelleted meal and another 40 pigs with unpelleted meal. The feed for both groups came from the same lot. In the group fattended on unpelleted feed, 18 per cent of the animals were positive for Salmonellae. The group fed on pelleted feed was free of Salmonellae.

In a review on the importance of animal feeds and fertilizers in the spread of Salmonellae, the W.H. O. expressed concern over the increasing number of reports dealing with Salmonella contamination of these products (51). It was emphasized that uncommon serotypes are frequently present. There was a need for further investigations to determine the sources and degree of this contamination and the possibilities of sterilizing the products. It was strongly recommended that in the plants where these feeds and fertilizers are made, there should be a strict separation between the "unclean" section where the raw materials are kept

and the "clean" department where the sterilized meal is milled, sacked and stored. Contamination by rodents and birds should also be prevented. The possibility of contaminating feed mills and products manufactured in feed mills, with contaminated animal by-products was also discussed.

Salmonellae in Feeds and Feed Ingredients as Sources of Infection in Animals

The papers reviewed so far, reported the prescence of Salmonellae in feeds without relating the organisms to disease in animals.

References where Salmonellae in feeds were incriminated as sources of infection for animals are less numerous.

Morehouse and Wedman (45) realized that there were many reports of Salmonellae in animal by-products or rations containing animal by-products. They noted that these reports often failed to explain the significance of the prescence of these bacteria for a particular species of animal. Therefore, a special committee composed of members of the Animal Disease Eradication Division of the United States Department of Agriculture was named to study the spread of Salmonellae and other disease causing organisms in poultry and livestock by means of animal by-products. During their survey, the committee found that 59 Salmonella serotypes were isolated from 14 different animal by-products. Reports tabulated from 31 states, indicated that isolation attempts were made from 5,712 samples. Of these samples, 718 contained Salmonellae.

Among the five products containing the highest percentage of Salmonella contaminated samples, the

known different serotypes recovered ranged from 17 in dog food to 34 in meat scraps. Serotypes most frequently recovered included S. montevideo, S. senftenberg, S. typhimurium, S. cubana, S. infantis and S. oranienburg. The authors felt that recontamination of animal by-products after their processing is the main cause of Salmonella in them. Rodents, wild birds, dogs and humans handling these products were suggested as possible sources of this contamination. The authors stated "Definite evidence that animal by-products in rations are sources of causative organisms responsible for specific field occurrences of salmonellosis is lacking. The potential disease threat posed by these organisms in animal by-products is worthy of further analysis." One of their recommendations was that attention should be given to the numbers of organisms found in the by-product samplings to evaluate their significance. The committee agreed that there was at least a potential disease problem concerning the possible "seeding" of poultry and livestock with a variety of Salmonella serotypes by-products.

It was pointed out that even small numbers of Salmonella organisms may be capable of producing carriers regardless of the source of exposure.

Griffin (28) described an explosive outbreak of infection with S. newport in two guinea pig colonies and a mouse colony. All colonies received a commercially prepared dog food. Investigations revealed the presence of Salmonella organisms in three different dog feeds. One of these contained S. newport. A different serotype (S. senftenberg) was isolated from meat scraps and a sample of dog food as delivered from the pelleting machine in one mill.

Galton et al (24) cultured a total of 159 samples of dehydrated dog food in Florida. Salmonellae were isolated from 26 (26.5 per cent) of 98 samples of dog meal. "Pressed" dog foods (dog "bones", biscuits, flakes, kibbled products and "candy") were all negative on culture. Those products containing the largest amounts of meatmeal commonly yielded Salmonellae. Seventeen different serotypes were isolated from the dog meal samples. All except one serotype were previously cultured from dogs in Florida and 15 of these were also isolated from humans. The authors stressed that commercially prepared animal foods may be an important source of infection of Salmonellae.

Chicken livers were thought to be the source of infection during an outbreak of food poisoning due to S. hadar and S. infantis in Israel. Hirsch et al (34) therefore attempted to trace the source of infection for the poultry. Chicken feed, including bone meal was found to be contaminated by 11 serotypes. Most of the organisms were of the same serotypes as those commonly isolated from humans suffering of gastro-enteritis in Israel. The bones were sterilized by steam at high pressure, ground, and the resulting meal was spread on the ground to dry. There was ample opportunity for these piles to become contaminated by mice, rats, cats, pigeons and wild birds. The authors recommended improved storage facilities to prevent contamination after cooking.

Newel et al (47) did a slaughter house survey for salmonella infection in swine and attempted to relate these findings to contaminated feeds on the farm. Cecal swabs from 489 pigs killed, showed a salmonella isolation rate of two per cent. Three per

cent of meat samples and 70 per cent of sausage samples from these pigs contained Salmonellae. Rectal swabs were taken from 192 pigs on five farms where the infected pigs originated. Nine per cent of those examined were positive for Salmonellae. There was no evidence of clinical salmonellosis in any of these pigs. Twenty-four of the feed samples taken on these farms contained Salmonellae. The same serotypes were found in the meal at the mill and in the fish-and bonemeal before mixing.

The following chain of events was suggested: contaminated feed results in carrier pigs which are used for human food causing clinical salmonellosis in humans.

Williams Smith (69) studied the effects of feeding pigs on fish-and bonemeal naturally contaminated with Salmonellae. The fishmeal made up ten per cent of the ration. It contained two different serotypes. The probable number of Salmonella organisms was 50 per 100 grams of fishmeal. Sixteen different serotypes were cultured from the bonemeal and it contained approximately 700 Salmonella organisms per 100 grams. Bonemeal formed two per cent of the final ration. This feed mixture was fed for 50 days and it failed to produce any clinical symptoms or macroscopic lesions. Salmonellae were isolated from cecal swabs, rectal contents and mesenteric nodes. The sub-maxillary, retropharyngeal, and hepatic lymphnodes and liver, spleen, bile, kidney and lungs were all negative on culture. The longer the contaminated ration was fed, the higher the incidence of infected mesenteric lymphnodes. Nineteen out of 139 (14 per cent) feces cultured were positive for Salmonellae during the 50 day experimental period. The first isolation

was made on the 14th day after the contaminated feed was first given. None of the animals became permanent excretors of Salmonellae. Serum samples were tested for somatic antibodies and all were negative. Eighteen different serotypes were found in the fish-and bonemeal, 12 in the feces, and only five in the mesenteric lymphnodes. Four of the latter were S. typhimurium. The author suggested that S. typhimurium may have a selective action in swine, since S. typhimurium was probably present in the meals in much smaller numbers than most of the other serotypes.

In a separate experiment, two four month old calves were fed bonemeal containing S. dublin and S. typhimurium for a period of one month. The calves remained healthy and neither serotype was cultured from their feces. When slaughtered, all internal organs, including the mesenteric nodes, were negative for Salmonellae. The serum samples did not contain antibodies against either serotype.

Pomeroy and Grady (50) examined 980 samples of animal by-products from 22 states. Salmonella organisms were present in 175 samples (17.8per cent) which consisted of 43 different serotypes and six unidentified serotypes. Out of 283 meat scrap samples, 83 were contaminated. Many of the serotypes recovered from the animal by-products had also been isolated from poultry and livestock submitted to the diagnostic laboratories. Only in a few instances were the serotypes encountered in the feed also isolated from animals or poultry receiving the feed. The authors recommended that every effort be made to eliminate the contamination of feed ingredients with Salmonellae.

Boyer et al (7) found that of 22 serotypes isolated from poults, 11 were also isolated from feed and/or feed ingredients during the same period. Four serotypes isolated from feeds were not isolated from poults consuming this feed, and 11 serotypes were isolated from poults but not from the feed. The authors suggested that uneven distribution of Salmonella organisms in the feed samples and their methods of sampling and culturing were probably fators accounting for failure to always find the same serotypes in the poults and feed. The breeder flocks and hatcheries were free of Salmonellae and since these birds were raised in isolation, infection from the environment was unlikely. Often, more than one serotype was recovered from the feed.

References which incriminate the feed as a source of Salmonella infection in cattle are extremely rare.

Gray et al (75) reported an outbreak of bovine salmonellosis on nine dairy farms in Australia. The incriminated sources of infection were a cattle concentrate and a bonemeal saltlick, the contaminated ingredient being the bonemeal. Six different serotypes were isolated from eight cattle on six different farms. The most common serotypes isolated from the cattle were S. typhimurium and S. newport, neither of which were recovered from the bonemeal. The authors suggested that the failure to isolate these serotypes from the cow- and bonemeal may have been due to either sampling errors or to the possible prescence of separate reservoirs of these organisms in the outbreaks under consideration. However, migratory birds present in the district failed to yield Salmonella organisms and no other reservoirs could be found. Examination

of six samples of concentrate collected from affected farms over a five week period resulted in the regular isolation of Salmonellae. Eleven serotypes of Salmonellae were isolated, two of which corresponded to strains isolated from cattle. Further studies on bonemeal revealed that all samples collected from four different producers were contaminated with Salmonella organisms. One of these suppliers produced 40-50 tons of bonemeal per week for the production of cowmeal. There were seven different serotypes present in these bonemeal samples. Two serotypes were recovered from a heavily contaminated sample which had been stored on a farm for 13 months. Multiple infections in individual animals were recorded and the authors suggested that this may be a more frequent occurrence than is commonly supposed.

The enormous potential of bonemeal as a possible source of infection for livestock was stressed.

Limited studies revealed a very probable connection between the bovine cases and human gastroenteritis with milk as a suspected vehicle. Thirteen of the serotypes present in the bonemeal were also isolated from humans.

Results of their experiments indicated that pelleting of feedstuffs containing up to 17 per cent of contaminated bonemeal, eliminated the Salmonella organisms.

In Alberta, Avery and Niilo (2) described an outbreak of salmonellosis in a range herd of 350 head of cattle. A total of seven serotypes were isolated, four of which were in both bonemeal and animals. One of the serotypes present in tissues and bonemeal was S. newport. Two of the serotypes were also cultured from various specimens taken at the

rendering plant where the bonemeal originated. The authors felt that the most likely source of contamination was dust and the use of contaminated equipment after the cooking process. Thorough cleaning and disinfection of the plant eliminated the organisms from subsequent samples. Bonemeal was fed to one cow on an experimental basis at a level of 2.2 lb per day for 45 days. The animal failed to develop clinical salmonellosis but excreted the organisms in the feces. Salmonella organisms could not be recovered from the various organs on post-mortem. The bonemeal was estimated to contain 18,000-80,000 Salmonella-like organisms per gram.

Knox et al (39) reported on a milkborn outbreak of S. heidelberg infection in England. There were 77 clinical cases and 46 carriers. The infection was traced to a cow which suffered from mastitis. S. heidelberg was isolated from the udder on post-mortem. The same serotype was isolated from meat-and bonemeal at the feedmill which supplied cattle cake to the farm where the cow originated. The cake did not contain any animal proteins. Two of 23 samples of cattle cake collected at the plant were positive for Salmonellae but none of the samples contained S. heidelberg. The authors noted that the same machines were used for the mixing of the bone- and meatmeal and the cattle cake. Contamination of the cattle cake may have occurred in this machine.

These workers observed that S. dublin was the most common organism responsible for milkborn outbreaks of human salmonellosis in the United Kingdom until 1950. Thereafter, S. typhimurium became the most common serotype. More recently, other less common serotypes have appeared. It was suggested that the

change in the serotypes may be related to the increased use of Salmonellae contaminated animal feedstuffs imported into the United Kingdom.

Gibson, in his review, (26) concluded that there is only circumstancial evidence that contaminated feedstuffs may act as the source of some Salmonellae infections in cattle. He felt that S. dublin infection seldom if ever arises in this way because of the rarity of this serotype in feed. He pointed out that S. typhimurium is less rare in feedstuffs and it could be significant, particularly if it has a selective action on its host. He also admitted that the evidence is more convincing when the less common serotypes are considered, especially those previously rare or unrecorded in a given country. In his experience, these "exotic" serotypes rarely caused clinical disease in cattle. He suggested that the chief significance of their prescence in feedstuffs is their ability to produce carrier animals. These carriers could then act as a source of human infection.

Wilder (68) stated that feeds or feed ingredients are often blamed for the transmission of salmonellosis although the specific serotype responsible for the outbreak may not be found in the feed. He felt that a specific serotype could be present in low numbers in the feed and it might not be detected because of overgrowth of other serotypes that may be present in greater numbers. On the other hand, he also suggested, that when a serotype is present in sufficient numbers to cause an outbreak of salmonellosis, it should be possible to detect it. The author recommended that perhaps some other sources of contamination should be investigated more thoroughly before assuming that the feed, or any single feed ingredient is the culprit. Many

questions regarding the level and incidence of contamination remain unanswered. He emphasized that most feed ingredients become contaminated after they have been sterilized. The importance of adequate sanitation in the rendering plant, during transportation and in the feedmill was stressed.

MATERIALS AND METHODS

Sources of Material

All isolations were made from specimens or carcasses received at the Manitoba Provincial Veterinary Laboratory. In general, these specimens or carcasses were referred to the laboratory by veterinary practitioners. A standard case history form accompanied each carcass or specimen. An identical form was completed at the laboratory when the submission was made by the owner. (See Appendix A).

In most instances, carcasses or specimens were submitted at the onset of a herd outbreak. Data on morbidity and mortality rates were therefore incomplete at that time. The owners were approached eight to ten months later and final adjustments on these data were made.

Bonemeal and mineral samples were usually submitted with carcasses or specimens. In some cases these samples were collected at the farms during field investigations. Where it was possible, samples were taken from unopened bags on the farm and were transported in plastic bags.

During the investigations, it became apparent that all bonemeal had been sold in plain, unlabelled, paper bags which originated at a local feed mill. Dust and spillage samples were taken from the conveyor system in this feedmill on March 23rd, 1966. At this time, samples were also taken from bags of bonemeal stored at the mill. These bags represented three different lots of bonemeal which were produced in January, April and May.

On March 30th, 1966 a representative of the feedmill submitted bones and "wet underground tankage" from an abattoir, and meatmeal from a rendering company.

The bones were to be used for the production of bonemeal. The tankage and meatmeal were to be used as ingredients in prepared feed.

Bonemeal samples were also received from 15 retail outlets or clients of the feed mill on April 18th, 1966.

More samples were taken in the feedmill on May 27th, 1966. This time, samples were collected from the conveyor system and also from various areas in the environment of the mill e.g. dust on shelves, walls, floors, stairs, machines, mixing rooms and from finished premixes.

Autopsy Procedures

An autopsy was carried out on all carcasses received at the laboratory. The macroscopic lesions were recorded on the reverse side of the history form. (Appendix B). The following tissues were routinely submitted for bacteriological examination: spleen, liver, gallbladder, ileum, and mesenteric lymphnodes.

Tissues which were suitable for histopathological examination, were fixed in 10 per cent, buffered formalin and prepared routinely for sectioning. They were sectioned at four to five microns in thickness and stained with Harris hematoxylin and eosin.

Certain sections of gut, lymphnodes, liver and spleen were also stained for bacteria with a modified Gram stain (12).

Isolation Procedures

Standard cultural and biochemical procedures (38) were employed for the primary isolation of Salmonellae from animal specimens. The following special media

were routinely used: Maconkey agar, Selenite F broth* and Tetrathionate broth.**

Bonemeal was inoculated in 10-15 gram amounts into 50 mls of Selenite F and Tetrathionate broth and incubated at 37 C. The broths were subcultured onto MaConkey agar before incubation and for three successive days after incubation. Any non-lactose fermenting colonies were treated in the same manner as those isolated from autopsy material. If the serotype isolated from the bonemeal did not correspond with that isolated from animals in the herd, two more isolations were attempted. When, after three attempts, the serotypes still did not correspond, it was assumed that that particular bonemeal sample did not contain the serotype which was isolated from the animals in the herd of origin.

Mineral mixtures containing visible bone chips were sieved and the chips were cultured as above. The residual mineral mixtures, or those lacking visible bone chips, were cultured after certain preliminary steps were taken. Direct inoculations of this material into broths was consistently negative for Salmonellae. Because such mixtures contain approximately 25 per cent salt, further cultures were attempted after dilution of the mixture to the strength of normal saline i.e. 1 gram of the mixture per 30 mls sterile distilled water. After being mixed thoroughly, the mixture stood at room temperature for one hour. The supernatant fluid and the sediment were then cultured in the usual manner. Samples which failed to

*Baltimore Biological Laboratories
**Difco Laboratories

yield any non-lactose fermenting colonies after three consecutive attempts, were regarded as being free of Salmonellae.

Pure cultures which reacted positively with Salmonella Polyvalent O Diagnostic Antiserum* were forwarded to the Manitoba Provincial Medical Laboratory for serological identification.

Bacterial counts were done on five random samples of bonemeal to determine the degree of contamination. This procedure was carried out after the samples had been stored at room temperature for a period of approximately one year. Samples of bonemeal in two gram amounts were thoroughly mixed with 100 mls of peptone water. This mixture was allowed to stand for five minutes. Duplicate volumes of 0.05 mls were pipetted from the middle zone of the mixture and streaked over separate MaConkey agar plates. After 24 hours incubation, the number of lactose and non-lactose fermenting colonies were counted. The non-lactose fermenting colonies were then identified by polyvalent O Salmonella Antiserum

Serological Procedures

Serum samples were obtained from animals in affected herds to relate antibody levels to the Salmonella serotypes isolated from clinical cases and/or bonemeal on the same premises.

Since S. newport was the most common organism encountered, all serum samples were tested with this antigen. In those herds where other serotypes were isolated from the animals or where titres against S. newport were below 1:20, the serum samples were

* Difco Laboratories

also tested with S. oranienburg and S. worthington antigen. These were the next most common serotypes isolated from animals and bonemeal.

A total of 157 single serum samples were tested. These were taken at various intervals after infection. Paired serum samples were taken from 11 animals two to four weeks apart.

Fecal samples and serum samples were collected from a number of animals in three different herds at several intervals after the outbreaks occurred. These samples were taken to determine the incidence and persistence of carrier animals. One herd with 83 animals was tested 3, 8, 8.5 and 9 months after the initial infection.

Samples from clinically healthy animals on infected premises, and from 100 cattle elsewhere in the province, were included in the test for comparison.

Somatic antigens were prepared from 18 hour subcultures of the primary isolates. These were harvested in normal saline and boiled for one hour. The antigen was then further diluted with sterile saline to conform to MacFarlands standard number two. (6×10^8 organisms per ml). Antigens were prepared from cultures of S. newport, S. worthington, and S. oranienburg.

The flagellar antigens were prepared according to Gard's method(38).

The tube agglutination test was carried out according to Kaufman's method (38) with a few minor modifications. Instead of 0.2 mls antigen and 0.2 mls diluted serum, 0.5 mls of each were used. The antigen and serum were incubated at 37 C for 18 hours.

RESULTS

Isolations from Animals, Bonemeal and Mineral Samples on Affected Farms

Salmonella organisms were isolated from animal tissues or feces which originated at 24 different farms. These farms had a total cattle population of approximately 2,379 head. Seventeen of the farms with salmonellosis were located in the "Interlake Area" which is situated between Lake Winnipeg and Lake Manitoba. Three other farms were located between Lake Manitoba and Dauphin Lake. The four remaining farms were situated in the southern and western parts of the province. (Fig. 1)

The most commonly isolated serotype was S. newport. It was recovered from 42 out of 46 animals. The 42 animals came from 20 different herds. In 13 of these herds S. newport was cultured from 19 carcasses, and in seven other herds , from 21 fecal samples. S. oranienburg was cultured from fecal samples in two herds. Multiple infections were encountered in two herds. In one of these herds, S. newport and S. worthington were isolated from tissues of the same carcass. In the other herd, S. newport and S. oranienburg were recovered from fecal samples.

Except in one instance, all outbreaks occurred during the period from January to June, 1966.

In 22 herds, clinical salmonellosis appeared a few days after bonemeal was first fed, or after a new bag was started. A similar history was given in two other herds where mineral was added to the ration. The serotypes isolated from the cattle corresponded with those isolated from unused portions of bonemeal or mineral on 16 premises. On 15 of these premises S. newport was isolated from these products and anim-

als. S. oranienburg was recovered from the feces and a mineral supplement on one farm. In another herd, S. newport was isolated from the feces, but no Salmonellae were cultured from the available bonemeal or mineral supplement. Different serotypes were recovered from animals and bonemeal in six herds. The bonemeal had been completely consumed on one farm and could therefore not be cultured. In 17 herds, more than one serotype was recovered from the bonemeal after repeated culturing. The relative frequency with which different serotypes were isolated from animal tissues, feces, bonemeal and mineral supplements is indicated in Table I.

Next to S. newport, S. oranienburg was the most frequently isolated from bonemeal. This serotype was cultured from animals on only three occasions. The data suggest that S. newport is more pathogenic for cattle than S. oranienburg.

The last outbreak occurred in September, 1966. The owner of this herd had bought the farm in the summer. Bonemeal was found in a shed and fed to the animals when they returned from pasture in the fall. Salmonellosis occurred within one week after the bonemeal was fed.

Morbidity and Mortality Rates

The total morbidity was 525 animals which was 18 per cent of the population at risk. The mortality rate was 24 per cent of the clinically affected animals . The highest morbidity and mortality occurred in calves less than one week of age and cows which were either pregnant or post-parturient. Fourty-four cows either aborted or produced stillborn calves. Most of the abortions occurred during the last month of pregnancy. The total morbidity and mortality

rates are given in Table II.

In six herds, the number of animals present in each age group was known. Therefore, the morbidity and mortality rates can be expressed as percentages as shown in Table III.

The data in Table III support more accurately what was generally indicated in the overall findings in Table II. The morbidity and mortality rates also varied widely from herd to herd.

Prophylactic and therapeutic treatment was initiated in all herds as soon as a positive diagnosis was made. Without these treatments, the figures for morbidity and mortality would undoubtedly have been higher.

Clinical Symptoms

The symptoms in the various age-groups were different in severity and duration only, the calves succumbing more rapidly than the adult animals.

The most outstanding symptom was a severe watery diarrhea, soon followed by the presence of mucus, blood and fibrinous casts in the feces. The feces often had a foul odor. There were also reports of severe tenesmus and evidence of abdominal pain. Dehydration, depression, anorexia and polydipsia were constant findings. Temperatures as high as 106 F were recorded. Many calves became comatosed as early as 12 hours after the onset of symptoms.

Many of the calves which died during the first week of life were weak at birth. A number of cows retained their afterbirths and developed a purulent metritis.

Macroscopic Lesions

In general the carcasses were dehydrated and

chronic cases were emaciated. The hindquarters were stained by dark fecal material. The enteric lesions varied from congestion of the mucosa in acute cases to a hemorrhagic necrotic enteritis with green-yellow casts in the more chronic cases. (Fig. 2). The intestinal contents varied from watery yellow fluid to bloodstained mucus. In the early forms the casts consisted of thin strands of sloughed mucosal lining changing to thick, firm, green-yellow casts in the more advanced form.

The mesenteric lymphnodes were invariably two to four times their normal size and extremely edematous and hemorrhagic. Similar but less severe lesions were seen in other lymphnodes.

A fibrinous pericarditis, pleuritis and peritonitis was observed in three calves. The joint cavities of several calves contained an excessive amount of synovial fluid, but purulent arthritis was not observed. Different degrees of pulmonary congestion and edema were constantly present. In a few chronic cases, the apical and cardiac lobes were consolidated. The livers were swollen with slightly rounded edges, and in more advanced cases minute foci of necrosis were visible under Glisson's capsule. The gall bladder always contained a copious amount of thick, dark green, mucoid bile. Congestion of the spleen with subcapsular ecchymotic hemorrhages was seen in most carcasses. Ecchymotic and petechial hemorrhages were present on the serosal surfaces of acute cases. Three post-parturient animals which were autopsied had a necrotic, purulent metritis. The uterus contained a large amount of foul-smelling, dark brown fluid. Attempts to culture Salmonella organisms from the uterine fluid failed.

Microscopic Lesions

Tissues from nine carcasses were examined histologically. The intestinal mucosa was necrotic with sloughing of epithelial cells into the lumen. The sloughed epithelial cells and neutrophiles formed a pseudodiphtheritic membrane. (Fig. 3.). The lamina propria was heavily infiltrated by both mono- and polymorphonuclear leucocytes. In some sections the inflammatory reaction extended into the serosa, resulting in in perivascular necrosis with lymphocytic cuffing(Fig.4). An acute lymphadenitis of the mesenteric lymphnodes was present in all sections examined. The lesion was characterized by areas of coagulative necrosis with almost complete obliteration of germinal centres in the cortices. (Fig. 5). These areas varied greatly in size and were surrounded by a reticulo-endothelial cell reaction at the periphery. The smaller areas resembled paratyphoid nodules in the liver. (Fig. 6). The sinuses were packed with lymphocytes.

The lesions in the spleen were characterized by congestion, hyperplasia of the reticulo-endothelial cells and degeneration of lymphoid follicles. Foci of necrosis similar to those seen in the mesenteric lymphnodes were often present in the red pulp. Gram negative bacilli were demonstrated in some of these necrotic foci.

The sinusoids in the liver were engorged with blood and the Kupfer cells were hyperplastic. So-called "paratyphoid" nodules were seen in five carcasses. Some nodules consisted of early foci of acidophilic necrosis without a significant cellular reaction.(Fig 7) Others had a prominent reticulo-endothelial cell reaction.(Fig. 8). Occasionally, similar nodules were attached to the necrotic endothelium of the hepatic

veins (Fig. 9).

In the kidneys, the glomerular tufts were hypercellular and some of the capillaries contained hyaline thrombi. Toxic nephrosis with the presence of proteinaceous casts in the convuluted tubules was present in most sections. Paratyphoid nodules were not observed in the kidneys.

The lesions in the lungs were those of interstitial pneumonia.

A fibrinous meningitis was seen over the cerebellum in one calf (Fig. 10). The endothelium of the meningeal arterioles was necrotic resulting in fibrinoid thrombosis. The meningeal exudate consisted of a moderate amount of fibrin containing mostly lymphocytes, histiocytes and a few neutrophiles. (Fig. 11). The brain peranchyma was edematous.

Interstitial hemorrhages were evident in the adrenal cortices.

Treatment

The majority of the affected animals were treated by veterinarians or by the owners under veterinary supervision. The effectiveness of treatments is based on reports from veterinary practitioners. A sensitivity test was done on seven isolates of S. newport. All seven isolates were sensitive to chloramphenicol and neomycin, six to nitrofurazones, and tetracyclines, five to oxytetracyclines, and chlortetracyclines. Reports indicated that losses in calves were reduced with prophylactic and therapeutic use of chloramphenical intramuscularly and nitrofurazones with astringents orally. Oral administration of nitrofurazones or sulfadimidine and sulfathiazole in combination with astringents were reported to be effective in control-

ling further losses in adult animals. Complete recovery generally occurred in calves, but many cows did poorly afterwards. In herd # 23 (Table III), 14 out of 36 cows were culled for this reason.

Isolations Made From Rendered Products

Two different rendering methods were employed in the two plants from which animal by-products were received.

One of the plants used the "dry rendering" method. Here, whole carcasses were crushed, then they were passed through steamjackets at 100 C for four to six hours. The surplus fat was removed as tallow. The endproducts were meat- and bonemeal, which were stored in a separate storage area. Although efforts were made to prevent recontamination of these products, employees moved from the clean area into the contaminated area and vice versa. Rodents, flies and birds could also move into these areas. S. oranienburg, and S. tennessee* were isolated from meatmeal stored in this plant. (Fig. 12).

The second rendering plant used the "wet rendering" method. It consisted of boiling the heads and legs in open vats at 100 C for 16 hours. The bones were stored until they were collected by trucks for delivery to the feed mills. The construction in this plant was such that the prevention of recontamination was almost impossible. Personnel moved back and forth from the contaminated area into the storage area, and hygiene was generally very poor (61). S. oranienburg and S. worthington* were isolated from bones, and S. newport and S. oranienburg* were isolated from wet tankage

* Isolations made at the Ontario Veterinary College.

produced at this plant. (Fig. 13).

It is interesting to note that two different laboratories isolated different serotypes from the same samples. This is probably due to the selection of different colonies for serological typing.

Isolations Made From the Environment and From Stored Products in the Feed Mill

Four out of five samples taken from the conveyor system in March were positive for Salmonellae. S. newport was one of the serotypes isolated. Two samples out of three different lots of bonemeal stored in the feedmill were also positive on culture. The bag of bonemeal produced in January contained two serotypes, S. worthington and S. oranienburg. A bag from the lot produced in April contained S. worthington. Salmonellae were not isolated from a bag produced in May. (Fig. 13).

Only two samples out of 17 taken from the feedmill in May yielded Salmonellae. The areas and products which were sampled and the serotypes isolated are shown in Table IV. A general cleanup had been carried out prior to this sampling.

All 15 bonemeal samples received from 15 retail outlets in April were positive for Salmonellae. Seven samples contained S. oranienburg, four S. worthington, three S. newport and one S. bareilly.

Results of Bacterial Counts on Bonemeal

The results of bacterial counts on five bonemeal samples are given in Table V. The average total bacterial count for the five samples was 73×10^3 bacteria per gram. Of these, 23.3 per cent were Salmonella-like organisms. The term "Salmonella-like organisms" is used since not all non-lactose "poly-O

positive" colonies are Salmonellae. Other enteric organisms such as certain Paracolon species may give similar reactions.

Serological Results

One hundred control samples which were taken at random from cattle throughout Manitoba had somatic titres of 1:20 or lower. Any titres of 1:40 or over were therefore considered to be significant. Gibson (26) also considered a somatic titre of 1:40 and a flagellar titre of 1:60 significant.

The results of 157 single serum titres against the somatic antigen of S. newport are presented in Table VI. Most samples taken from animals which consumed bonemeal had significant somatic titres against S. newport but not against other serotypes (S. oranienburg, S. worthington) present in the bonemeal. A number of animals which never consumed the bonemeal but had contact with infected animals also developed significant titres.

The results of 11 acute and convalescent somatic titres are given in Table VII.

Flagellar agglutinin levels were determined for five of these samples for comparison. The flagellar titres appeared to rise less rapidly than the somatic titres. A significant drop in the somatic titres was accompanied by a similar decline in the flagellar titres in animal number two. In contrast, the flagellar titres remained constant when the somatic titres declined in animal number three. Thus, in the latter animal the flagellar titres were more persistent. Antigenic response to the two phases of the flagellar antigen differed in all animals except number three.

The relatively high acute titres in animals 2,

3, 4, 5, 6 and 7 tend to suggest that these animals were exposed to infection earlier than is indicated in the table. There were significant changes in most titres over relatively short periods of time. Thus, these titres are additional evidence that the animals had been infected by S. newport.

These serum samples were also tested against S. oranienburg and S. worthington and all somatic titres were 1:20 or less.

Incidence of Carrier Animals

The results of periodic fecal and serological examinations in three herds are given in Table VIII. Two out of a total of 467 fecal samples taken at different intervals were positive for S. newport. These two samples were taken in herds numbered one and two, six months after the initial outbreaks. The fecal samples taken in herd number three at 8, 8.5, and 9 months post-infection were all free of Salmonellae. Thus, this herd was free of carrier animals at this time. Although, the feces were negative on culture, several animals retained significant somatic serum titres. In the first test, 10 animals had significant titres. This number rose to 17 in the second test. The third test revealed only one significant titre in 88 animals. Cattle which had a significant titre in the first test were also positive in the second test, however the titres were lower. The data indicate that carrier animals are no serious problem with S. newport infection in the bovine species.

TABLE I

FREQUENCY WITH WHICH DIFFERENT SALMONELLA SEROTYPES WERE ISOLATED FROM ANIMALS, BONEMEAL AND MINERAL SUPPLEMENTS

Serotype	Animals	Bonemeal	Mineral
S. newport	42	24	3
S. oranienburg	3	18	4
S. worthington	1	9	2
S. bareilly	-	3	-
Total	46*	54	9

*24 premises

TABLE II

TOTAL MORBIDITY, MORTALITY, ABORTIONS AND STILLBIRTHS IN 24 HERDS AFFECTED BY SALMONELLOSIS

Age Group	Morbidity	Mortality*	Aborted/ Stillborn
Less than 1 week	187	47	44
1 week-6 months	77	11	
Over 6 months	261	69	
Total	525(18%)	127(24%)	

*Number of clinically affected animals which died.

TABLE III

MORBIDITY AND MORTALITY RATES IN DIFFERENT AGE GROUPS IN SIX BEEF HERDS AFFECTED BY SALMONELLOSIS

Herd No.	Age* Group	Number of animals in each group	Morbidity %	Mortality %**	Aborted or Stillborn
6	A	50	100	26	3
	B	4	100	0	
	C	70	60	14	
9	A	70	3	0	0
	B	50	0	0	
	C	120	4	20	
11	A	136	50	2	1
	B	114	4	0	
	C	150	13	15	
16	A	25	60	64	0
	B	0	0	0	
	C	47	4	50	
20	A	15	100	7	0
	B	25	88	0	
	C	70	11	9	
23	A	81	16	70	5
	B	0	0	0	
	C	120	30	30	

*A = less than 1 week old.
B = one week to 6 months old.
C = over 6 months old.

**Percentage of clinically affected animals that died.

TABLE IV

AREAS AND PRODUCTS SAMPLED IN THE FEED MILL IN MAY WITH SEROTYPES ISOLATED

Sample	Isolate
Turkey starter	Negative
Rendering meatmeal	"
Mineral mixture	"
Cattle concentrate	"
Soybean meal	"
Oil cake meal	"
Cattle trace mineral	"
Phosphate	"
Beet molasses	"
Salt	"
Floor dust - bagging room	"
Shelf dust - " "	"
Floor dust - premix "	"
Surface dust from bags - premix room	"
Surface dust from walls- " "	"
Surface dust from legs of conveyor, motor pipes and auger	S. worthington
Surface dust from concrete piles, auger and walls	S. oranienburg
Dust from stairs	Negative
Contents from meat auger	"
Contents and residue in the grinder	"
Premixer	"
Dust in grain grinder room	"
Mixing room	"

TABLE V

RESULTS OF BACTERIAL COUNTS ON FIVE BONEMEAL SAMPLES*

Sample number	Lactose fermenting colonies	Non-lactose fermenting "poly O positive" colonies
1	50	13
2	68	12
3	70	20
4	80	20
5	96	20
Average	73	17

*Number of organisms in thousands per gram of bonemeal.

TABLE VI

RESULTS OF 157 SINGLE SERUM TITRES AGAINST THE SOMATIC ANTIGEN OF S. NEWPORT

Percentage of tested animals	Somatic Titres
50	1/20 - 1/80
20	1/80 - 1/320
30	1/320 - 1/2560

TABLE VII

ACUTE AND CONVALESCENT SOMATIC AND FLAGELLAR SERUM TITRES AGAINST S. NEWPORT ANTIGEN

Animal	Days Post-infection*	Somatic Titre	Flagellar Titre Phase 1	Phase 2
1	1	1:10	1:10	1:10
	28	1:2560	1:160	1:80
2	14	1:2560	1:320	1:640
	30	1:320	1:20	1:80
3	7	1:2560	1:640	1:640
	24	1:160	1:640	1:640
4	7	1:160	1:10	1:40
	30	1:2560	1:40	1:320
5	7	1:160	1:10	1;10
	30	1:2560	1:80	1:320
6	0	1:320	N.D.	N.D.**
	21	1:640	"	"
7	14	1:640	"	"
	30	1:320	"	"
8	7	1:80	"	"
	21	1:640	"	"
9	7	1:20	"	"
	30	1:80	"	"
10	7	1:40	"	"
	30	1:80	"	"
11	0	1:10	"	"
	30	1:320	"	"

*Number of days after onset of clinical symptoms.

**N.D. = Not done

TABLE VIII

INCIDENCE AND PERSISTENCE OF CARRIER ANIMALS IN THREE HERDS AFFECTED BY SALMONELLOSIS

Herd	Number of Animals Tested	Months Post-infection	Number of Positive Fecal Samples	Somatic Titres against S. newport						
				0	10	20	40	80	160	320
1	30	3.0	0	2	12	12	2	2	0	0
1	83	6.0	1	83	0	0	0	0	0	0
2	50	6.0	1	37	3	5	8	0	1	0
2	52	15.0	0	52	0	0	0	0	0	0
3	82	8.0	0	49	5	16	6	2	2	0
3	82	8.5	0	18	20	25	11	6	0	0
3	88	9.0	0	75	9	3	1	0	0	0

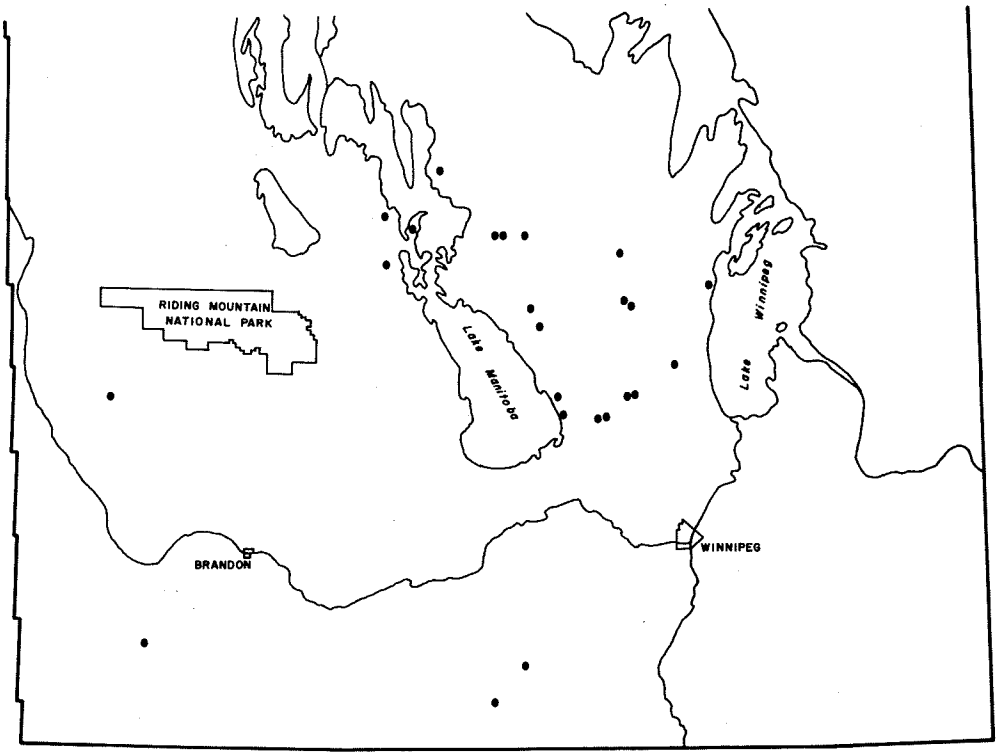

Fig. 1. Distribution of herds with bovine salmonellosis in Manitoba.

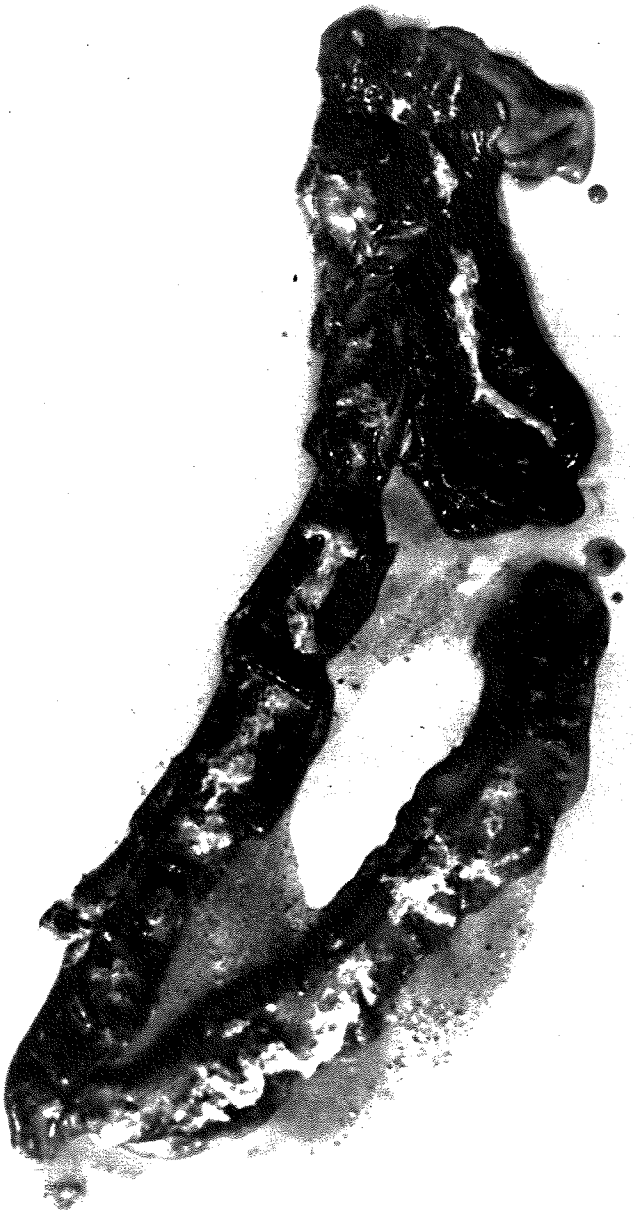

Fig. 2. Early cast-formation in ileum and cecum due to S. newport infection in a calf.

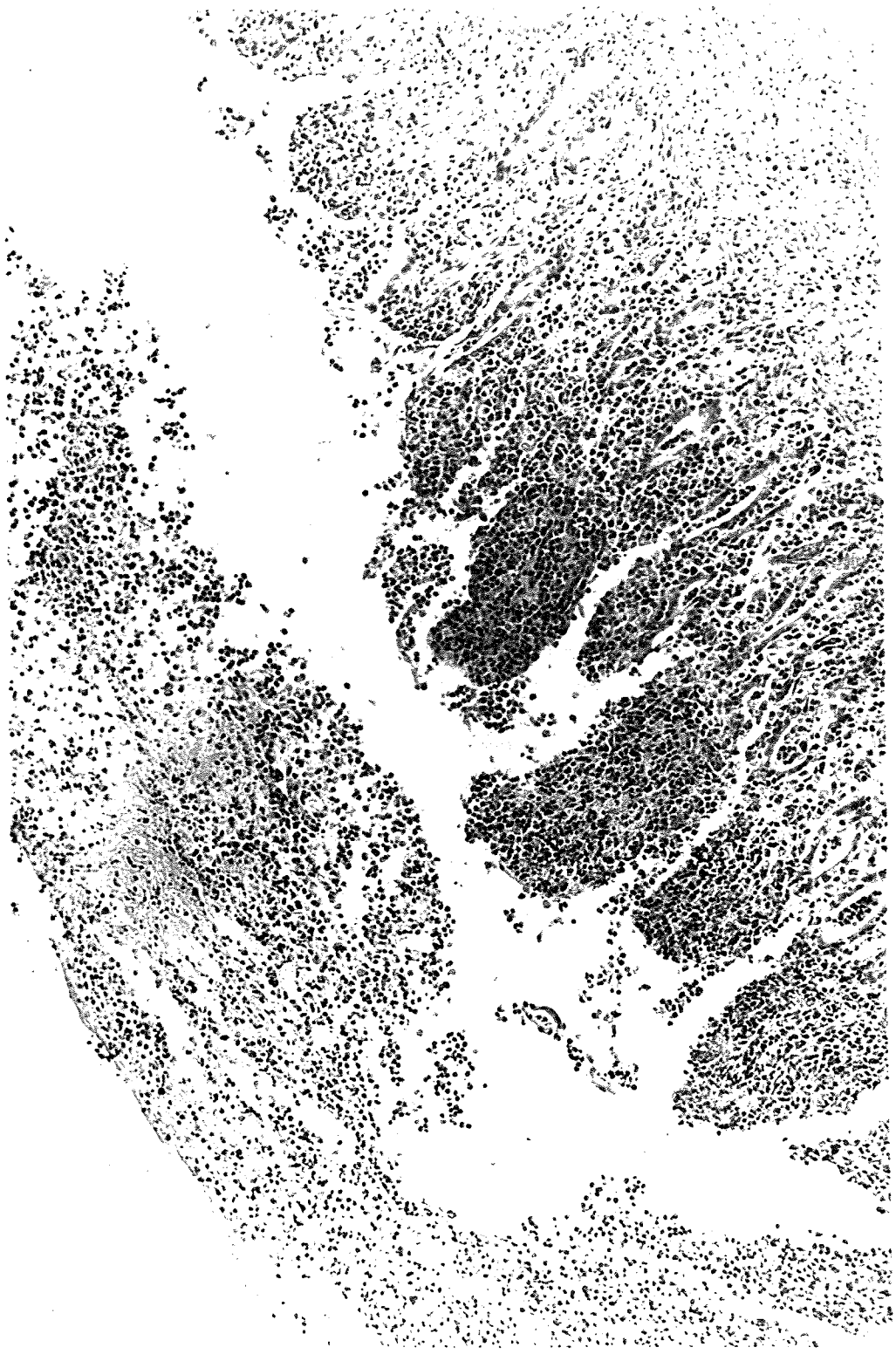

Fig. 3. Pseudo-diphtheritic membrane (left) in the small intestine of a calf caused by S. newport infection. Note heavy leucocytic infiltration of the lamina propria (right)(x 100)

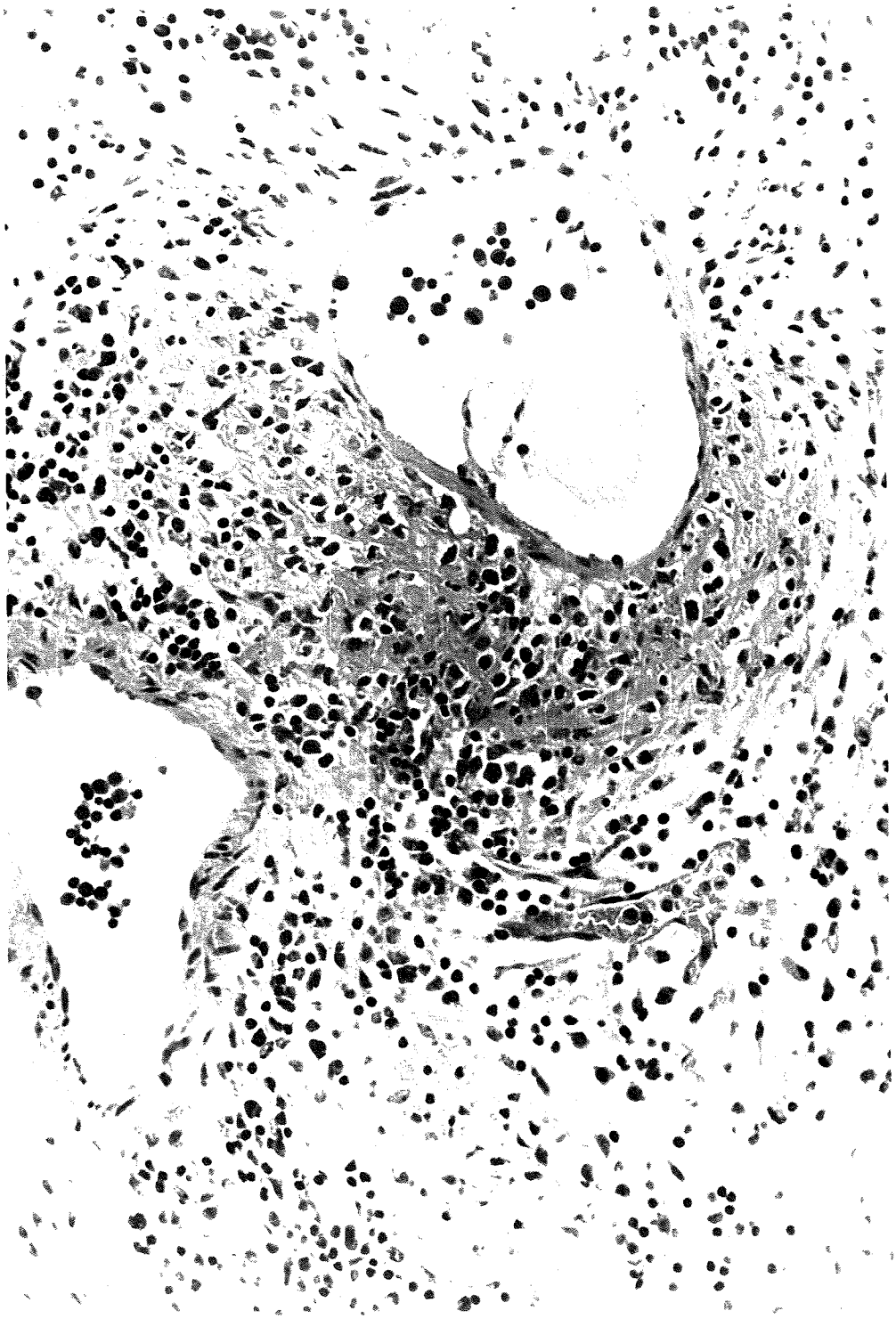

Fig. 4. Section of intestinal serosa from a calf which died of salmonellosis. Note the perivascular necrosis with a predominantly lymphocytic reaction (x 400).

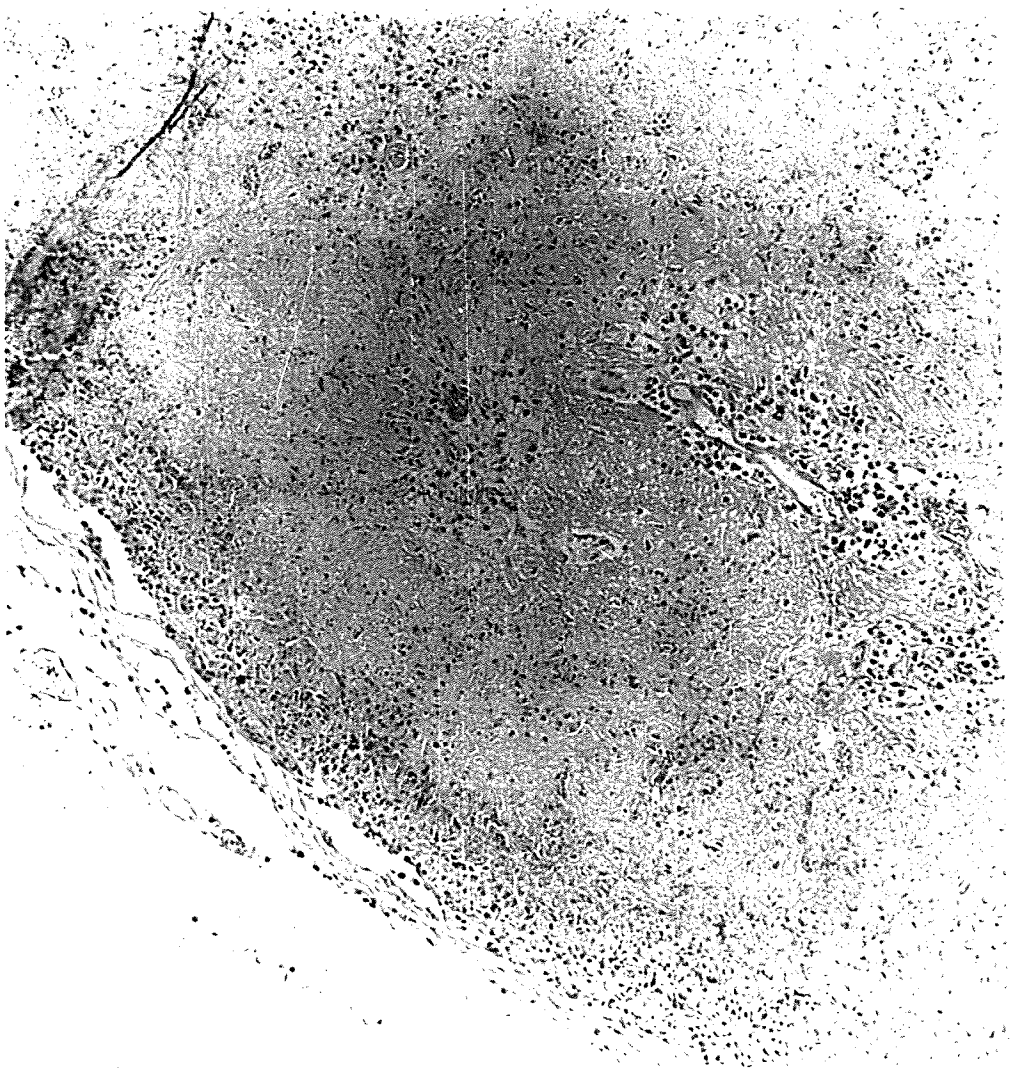

Fig. 5. Extensive necrosis in a mesenteric lymphnode from a calf which died of S. newport infection. The germinal centres are completely destroyed (x 100).

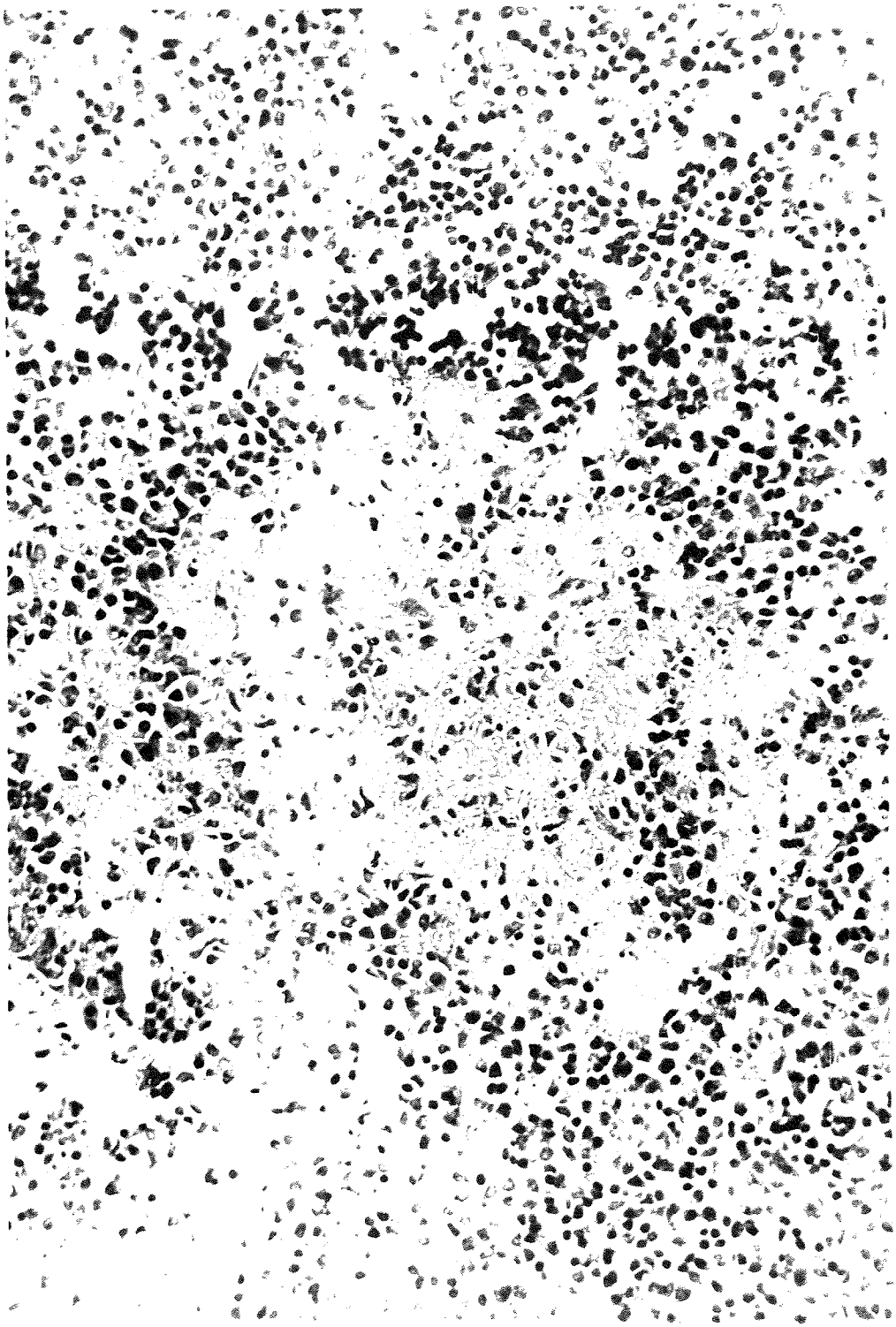

Fig. 6. Paratyphoid nodule in a mesenteric lymphnode from a calf which died of S. newport infection.

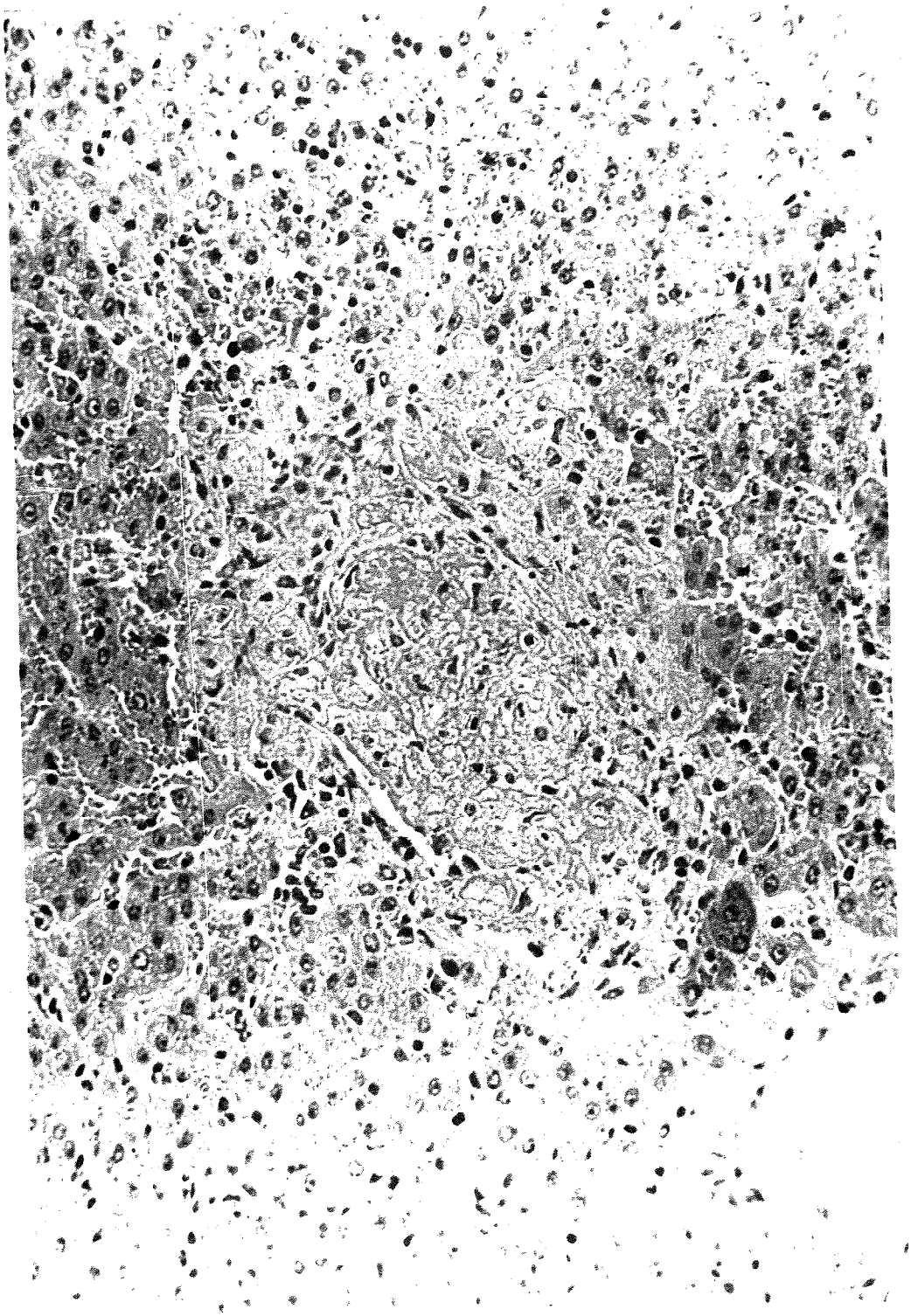

Fig. 7. Early paratyphoid nodule formation in the liver parenchyma due to S. newport infection in a calf (x 400).

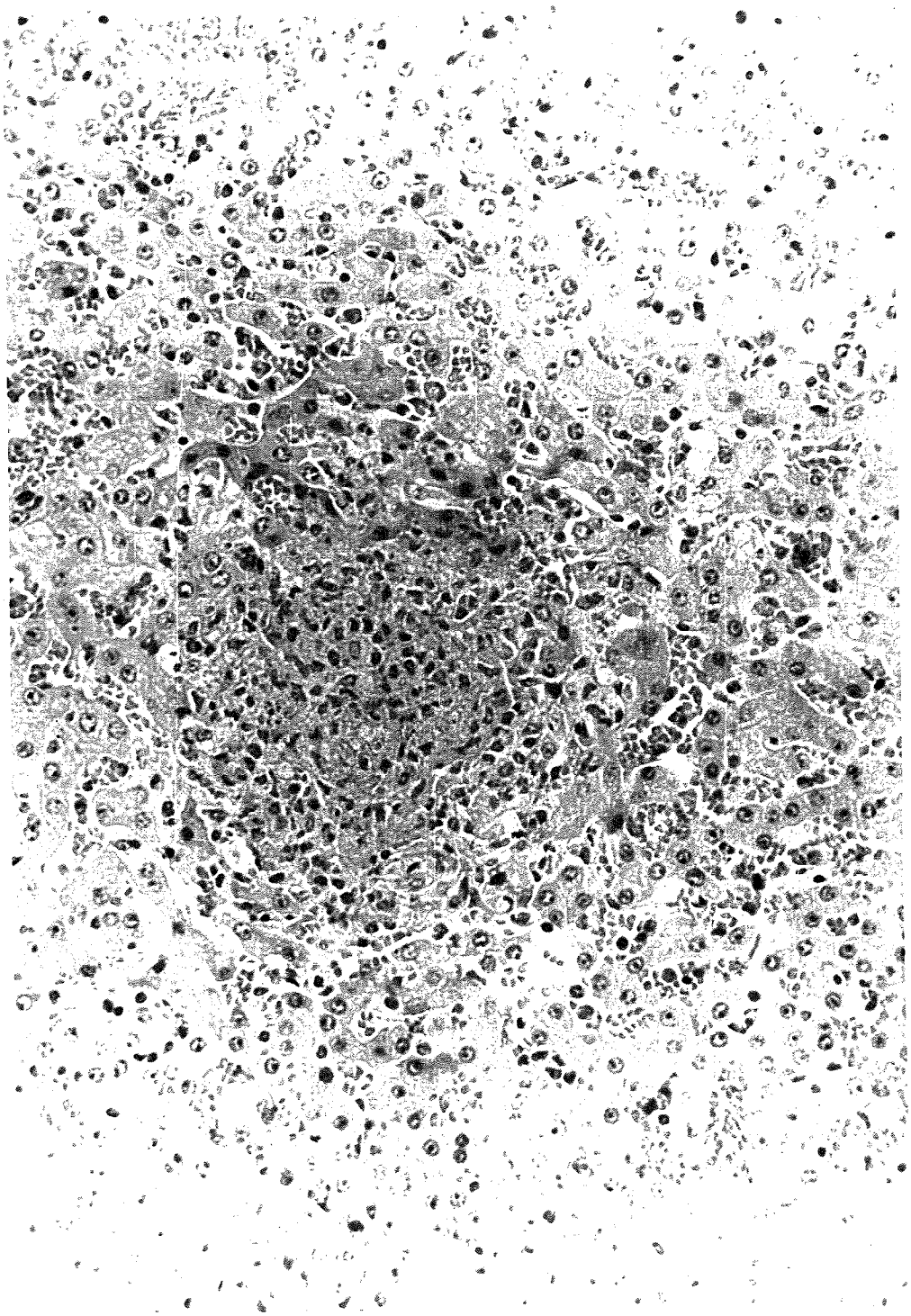

Fig. 8. Late paratyphoid nodule in the liver parenchyma due to S. newport infection in a cow. Note increased numbers of reticulo-endothelial cells compared to fig. 7. (x 400)

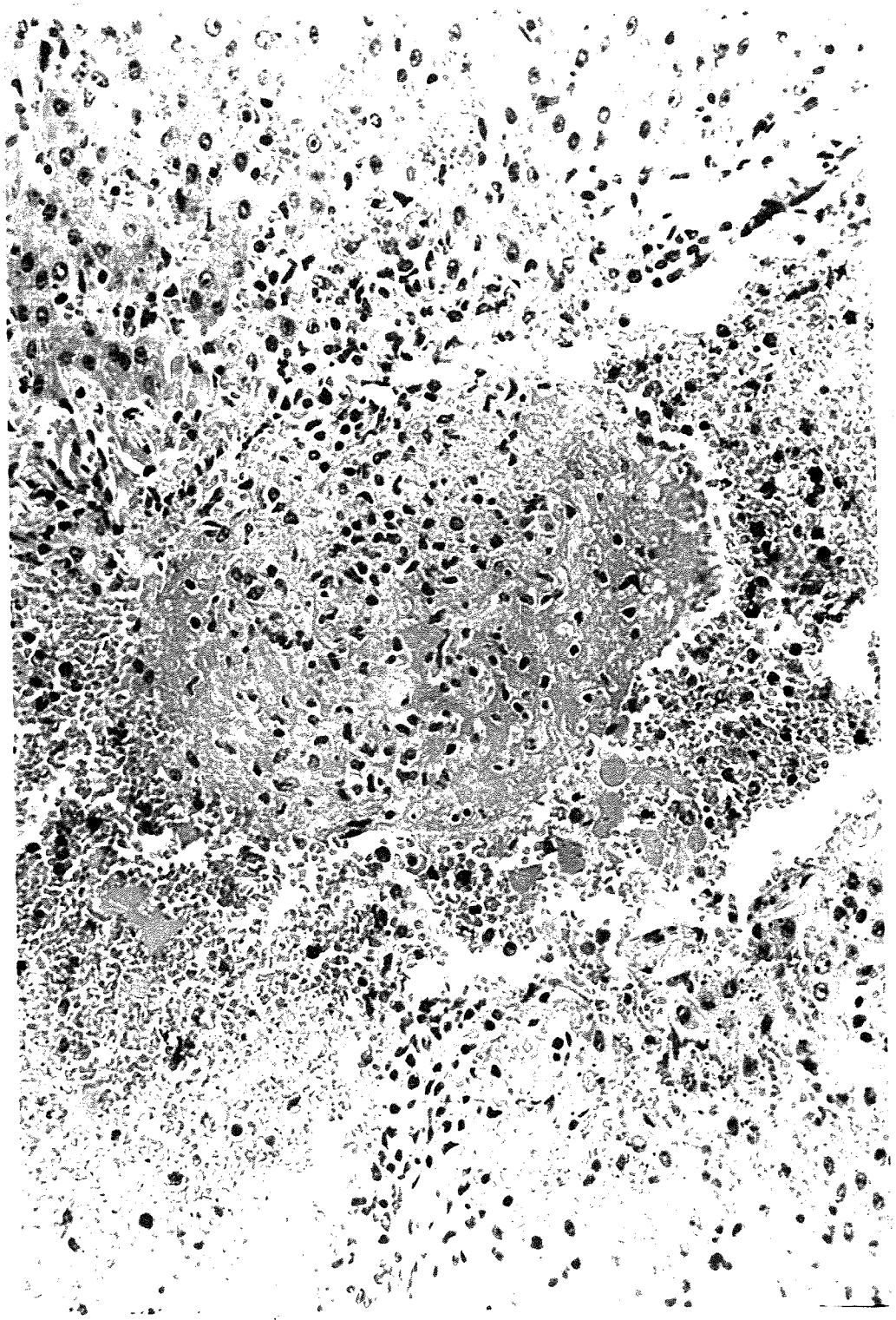

Fig. 9. Paratyphoid nodule attached to the wall of a hepatic vein. Calf with S. newport infection (x 400).

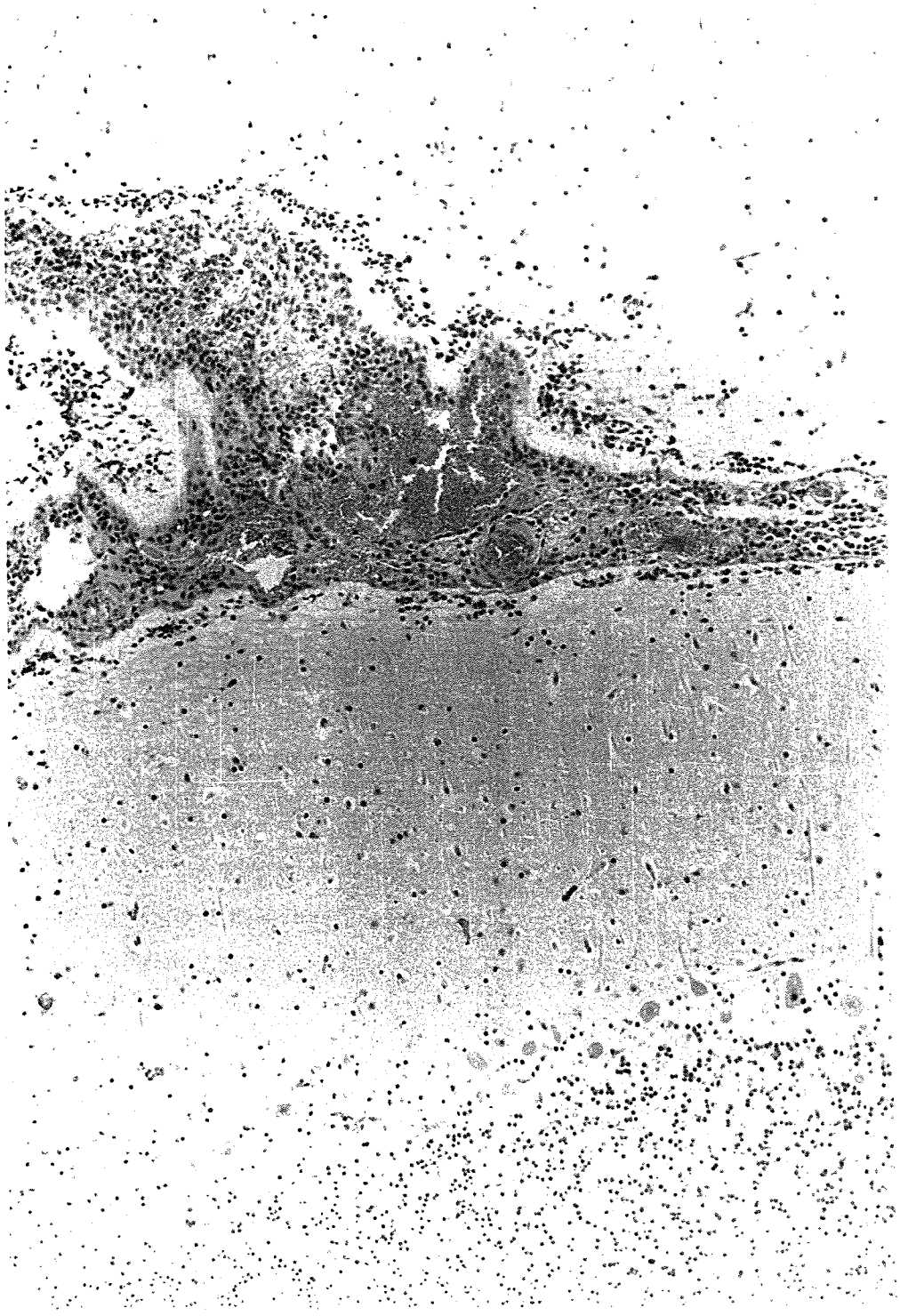

Fig. 10. Fibrino-purulent meningitis of the cerebellar meninges in a calf with S. newport infection. (x 100)

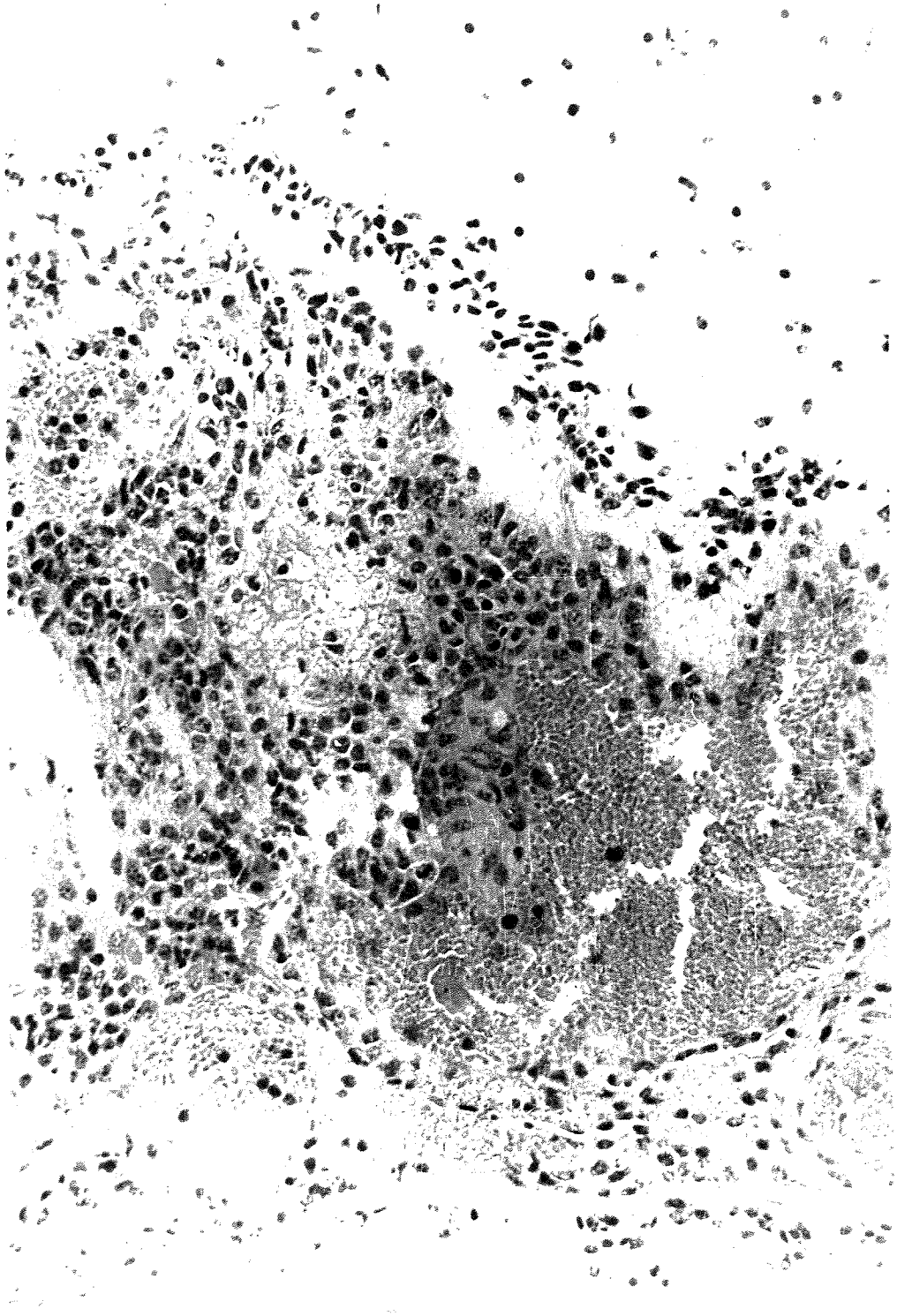

Fig. 11. Fibrino-purulent meningitis in a calf with S. newport infection. Note predominance of lymphocytes, histiocytes with a few neutrophiles in the exudate. (x 400)

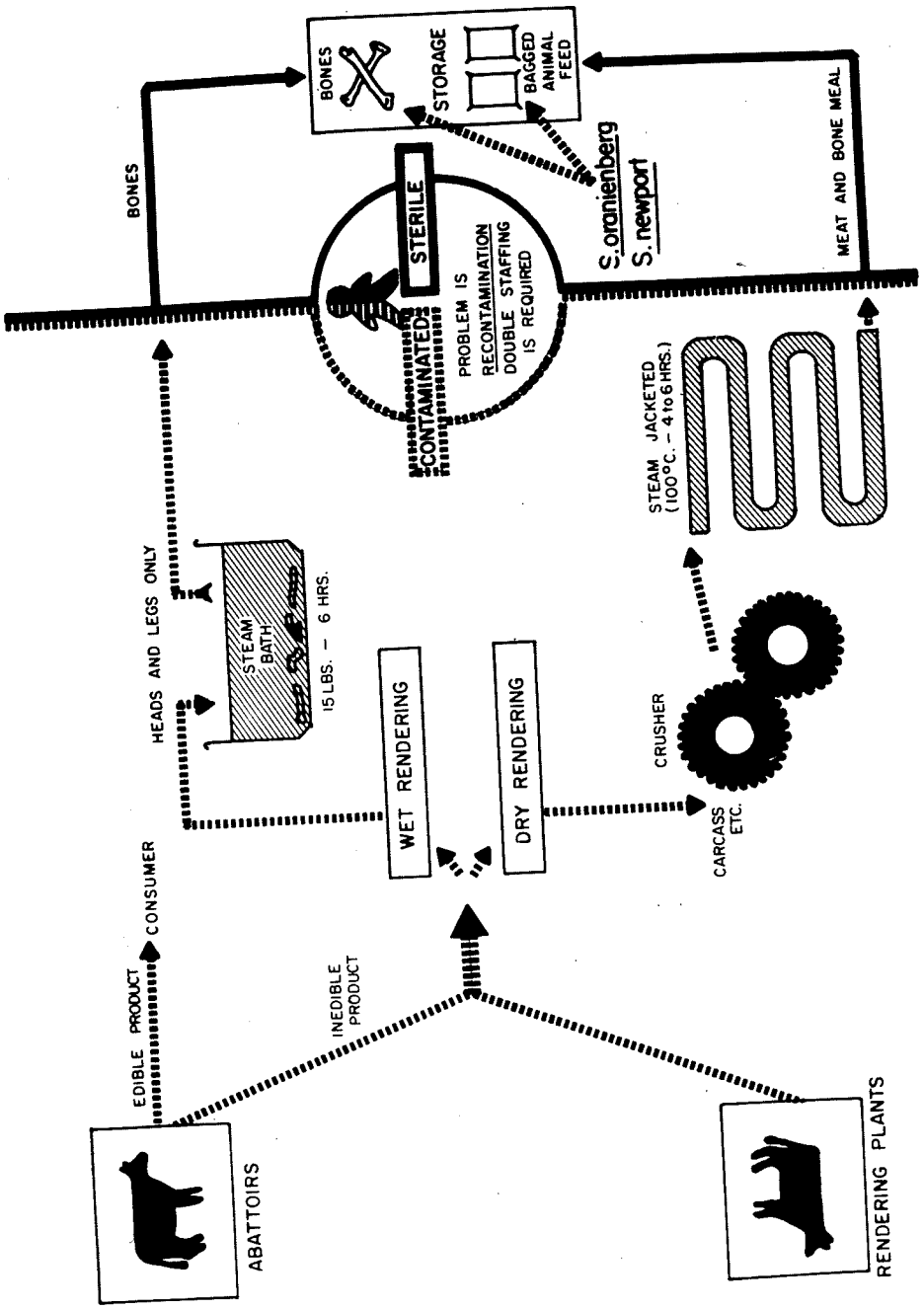

Fig. 12. Illustration of the two rendering methods which were used for the production of animal by-products and the Salmonella serotypes isolated from these products.

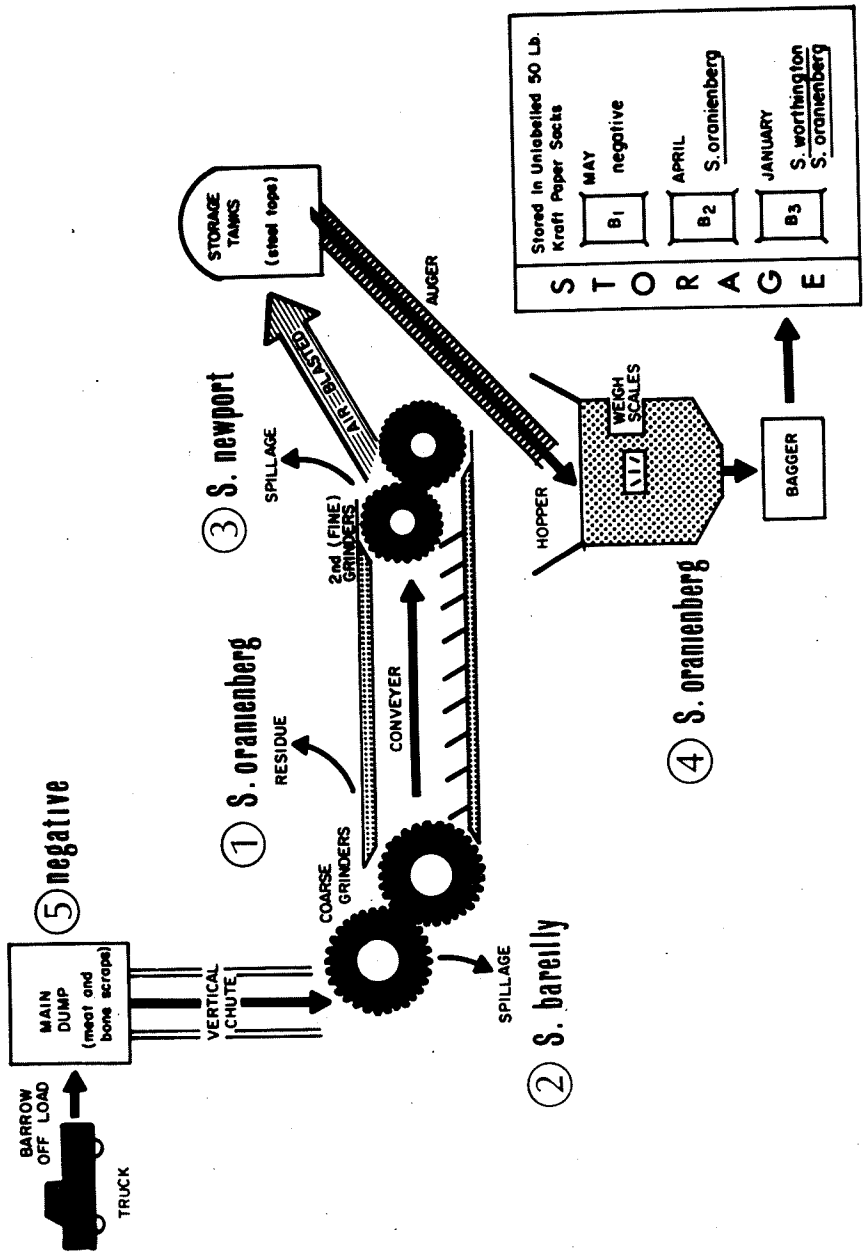

Fig. 13. Areas and products in the feed mill from which various salmonella serotypes were isolated.

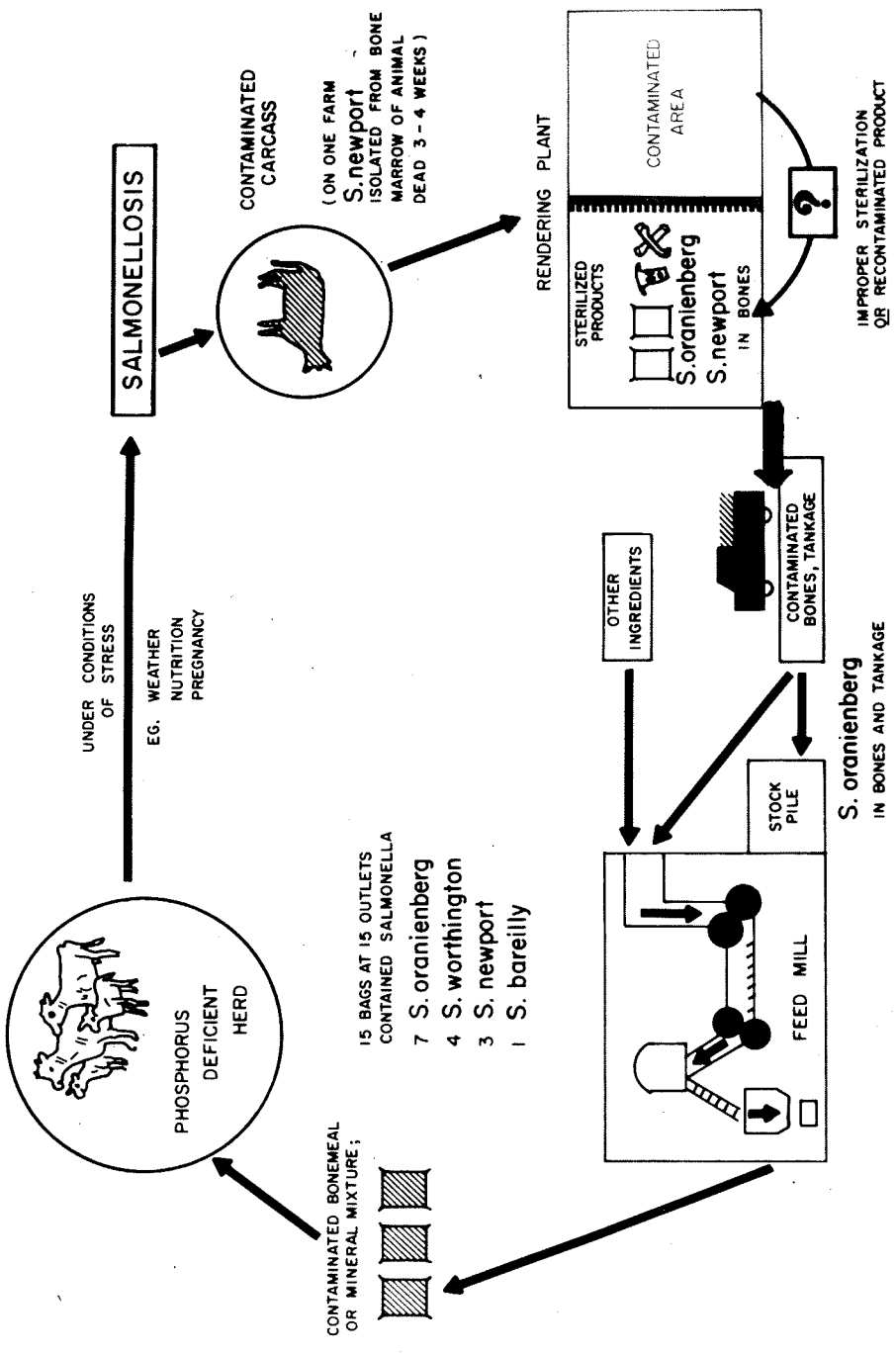

Fig. 14. The sequence of salmonella contamination and infection of products and animals, as suggested by the results of the investigation.

DISCUSSION

Predisposing Factors

There are very few reports of common source epizootics of salmonellosis in animals. It was previously indicated that consumption of salmonella-contaminated feeds rarely result in clinical outbreaks of salmonellosis in animals. (26). Experimental evidence indicates that ingestion of Salmonella organism does not necessarily produce salmonellosis in animals or man.

The question arises: "Why did the outbreak in Manitoba reach epizootic proportions?"

The evidence that bonemeal was the source of infection is overwhelming. In nearly every herd the history indicated that salmonellosis occurred shortly after bonemeal was added to the ration. The bacterial counts of the bonemeal revealed an average of 17×10^3 Salmonella-like organisms per gram.

Feeds containing much smaller numbers produced carrier animals but no clinical symptoms or lesions on post-mortem (69). Bonemeal containing 18,000-80,000 Salmonella-like organisms per gram caused salmonellosis in beef-cattle in Alberta. (2.). The relative frequency with which these serotypes were isolated suggest that S. newport and S. oranienburg were probably present in greatest numbers. The most conclusive evidence that bonemeal was the source of infection was provided by the isolation of the same serotypes from bonemeal and animals on 16 farms. S. newport accounted for 15 of these isolations.

Serum titres supported bacteriological and pathological evidence that S. newport was the primary pathogen. S. oranienburg was also isolated from fecal

samples, however, this was probably the result of passive passage through the gastro-intestinal tract. This serotype was not isolated from tissues. All somatic serum titres against S. oranienburg were below significant levels. i.e. less than 1:20. S. worthington was cultured from tissues of of only one carcass. This carcass also yielded S. newport. The possibility of S. worthington being a primary pathogen was also ruled out on serological grounds.

S. newport was the primary pathogen in those herds where the serotypes in the animals and bonemeal were different. Failure to isolate S. newport from the bonemeal in all herds, may have been due to errors in sampling or selection of the wrong colonies for serological identification. It is highly unlikely that S. newport originated in different reservoirs on these farms. Most of the herds were relatively isolated and rarely was there a history of introduction of livestock. Other feed-ingredients were considered as unlikely sources, since native hay formed the bulk of the ration occasionally supplemented with a small amount of grain. All herds were beef-herds and other domestic animals were rarely present. Wild animals or birds were also considered to be improbable sources of infection since there was little contact with these species.

Although bonemeal was the primary source of infection, vertical and horizontal transmission undoubtedly occurred once the infection became established in a herd.

The ingestion of large numbers of infective organisms over relatively short periods of time by a highly susceptible host was probably the primary factor responsible for this epizootic. Several authors

have stressed the importance of predisposing factors in the etiology of the salmonellosis. These factors reduce the host's resistance making the animals more susceptible to infection, or activating an infection which is already present. Factors which have been mentioned are; age, intercurrent disease, malnutrition, transportation, weather, management, pregnancy and calving. (2, 10, 22, 25, 26, 30, 42, 56, 64).

Figures in Tables II and III indicate a definite age susceptibility. It was previously mentioned that many calves were born weak. These calves and those which were aborted or stillborn were probably infected intra-uterine. S. newport was recovered from one aborted fetus. Buxton (10) felt that young animals were more susceptible because; a) Immunity was only passive. b) Actively growing tissues are more susceptible to infection. c) Poor sanitation in environmental stress.

Poor sanitation and environmental stress were probably the most significant predisposing factors for calves in this outbreak. The calves were either born outside or in open sheds. The average temperature during the period of most outbreaks was 17.4 F. Sudden changes in weather were also common at that time of year. Calves which were not infected intra-uterine would have been exposed to large numbers of infective organisms present in the feces of clinically affected adult animals. These animals were generally kept in corrals where feces and urine accumulated in puddles. A high morbidity and mortality with salmonellosis in calves less than one week old is not considered to be typical (21). Rothenbacher (56) reported 13.7 days as the average age of death in his survey. It appears

that when the dams are infected by the disease and calves are debilitated when born, losses may occur at an earlier age.

Marginal nutrition, pregnancy, calving and stress associated with weather and housing were considered to lower the resistance of cows.

Figure one indicates that the majority of the herds with salmonellosis were located in the Interlake Area. A large proportion of cattle in this area is raised almost entirely on native roughages. A study of the nutritional value of forages in the Interlake Area revealed that the native hays were deficient in phosphorous and crude protein, and were poorly digestable. Cultivated hays were adequate in their protein and calcium contents but the phosphorus levels were marginal. The use of a phosphorus supplement, such as bonemeal or dicalcium phosphate was recommended for this area. (27). Pica and a craving for minerals is commonly seen in farm animals in these areas when the soil or feed is not supplemented with phosphorus. In one herd, salmonellosis occurred three days after 14 head of cattle consumed 50 lbs. of bonemeal within two days. Consumption of large amounts of bonemeal over short periods of time was also reported in other herds. It is therefore reasonable to assume that the animals received a high dosage of infective organisms because of consumption of large amounts of heavily contaminated bonemeal. The high incidence of salmonellosis in the Interlake Area can probably be explained on the basis of the feeding of more contaminated bonemeal than in other areas of the province, with marginal nutrition as a predisposing factor.

All herds involved were cow-calf operations. Problems usually occurred first in pre- and post-

parturient animals. The inclement weather was probably another stress factor for adult animals.

It is very difficult to pin-point any precipitating factor which would lower the resistance of the host most. Certainly, pregnancy and calving were stress factors present in all herds and these may well have been the most important predisposing factors. Herd number six in Table III was well managed and nutrition was considered adequate. However, the morbidity and mortality rates in the cows and their calves was higher than in any other herd. Thus, under range conditions, losses of both cows and their calves due to salmonellosis can be considerable.

Observations on Carrier Animals

The results of surveys in three herds indicate that the incidence and persistence of carrier animals in this outbreak were not significant. Further evidence to support this suggestion is supplied by the absence of any new cases in the spring of 1967.

According to Gibson (26) persistence of infection in a herd depends on: a) The extent to which other cattle in the herd become infected. b) The persistence of infection in individual animals of the same or different species present in the herd. c) The longevity and possible multiplication of the organism after excretion. In this outbreak, many animals in a herd became infected. There are no data available on the longevity of S. newport in feces and water. There is no reason to believe that these data would differ greatly from those obtained for S. dublin. Field (21) found that S. dublin survived for 73-119 days in feces in pasture. S. dublin survived for 150 days in feces on soil, 163 days in feces on grass, 307 days in feces

on stone and 115 days in feces in pond water. (25,26) The conditions for survival of S. newport in this outbreak were probably quite favorable. This was particularly true during the latter part of the spring. The limiting factor appears to have been persistence of infection in animals in the herd. It appears that cattle are able to cast off an infection with S. newport when the environmental stress factors are removed i.e. when adequate pasture becomes available, the weather improves and the calving season subsides.

Animals with significant serum titres do not necessarily excrete Salmonellae in their feces. Table (VIII). Other workers have reported similar findings with S. dublin infection in cattle. (14, 22, 33, 49). They also noted that animals which excrete Salmonellae in their feces do not necessarily have high titres. These workers recommended that both fecal and serological examinations should be carried out to detect carrier animals in a herd.

Cattle seem to differ in their antigenic response to somatic antigens of S. newport and S. dublin. Henning (33) found that in cattle infected with S. dublin, the antigenic response appears to be confined to the production of flaggellar agglutinins, as the somatic agglutinin titre of the serum rarely exceeds 1:5. The results of this investigation indicate that cattle are able to produce significant titres against somatic antigens of S. newport, and these titres can be used as additional evidence of recent infection with this organism.

Sequence of Salmonella Contamination and Infection

The probable sequence of salmonella contamination and infection in this epizootic is shown in figure 14. It is not clear how the Salmonella organisms were initially introduced into this cycle. Circumstancial evidence indicates that a contaminated carcass or carcasses entered the rendering plants. S. newport was isolated from the bonemarrow of a frozen bovine carcass which had been dead for three to four weeks. This carcass was destined to be rendered.

Recontamination of the cooked products was the most likely mode of transmission in the rendering plants. The temperatures at which the rendering products were heated should have been sufficient to destroy all Salmonellae present in the carcasses.

The results of the investigation in the feed mill indicate that the machines, conveyors, and augers etc. became heavily contaminated. It is conceivable that other feedproducts were also contaminated by this equipment. The degree of contamination in prepared feeds was probably significantly lower than in the bonemeal because of the dilution factor. There was no evidence that prepared feeds caused clinical outbreaks of salmonellosis. There was some evidence that carriers were produced by these products. For example, S. newport was isolated from turkey poults which were fed a starter ration produced in the same feed mills. This serotype was never before cultured from turkeys in Manitoba. Unfortunately, the starter feed was never submitted for culture. One of the mineral mixtures which was found to be a source of infection for cattle was also produced in this feed mill.

The importance of salmonella contaminated feeds

in the spread of Salmonella organisms becomes particularly evident, when one considers that these feed mills produce large quantities of feed which are distributed over widely scattered areas.

Public Health Aspects

S. newport was the fourth most common serotype- six per cent of all isolations- cultured from man in Canada in 1964. (71). In 1965 there were 418 isolations of S. newport which made it the second -14.4 per cent- most common serotype in man. This represented an increase of 147 per cent over the 169 cases reported in 1964. (72). In 1966, S. newport remained the second most common serotype (12.5 per cent),next to S. typhimurium which accounted for 27.1 per cent of 2,551 Salmonellae isolated from humans in Canada (73).

Of the 1,037 non-human salmonella isolations reported in Canada during 1965, S. newport accounted for 24 (2.5 per cent). (72) In 1966, there were 1,048 non-human salmonella isolations in Canada, 122 (11.6 per cent) of which were S. newport (73). The increase in non-human isolations in 1966 was mainly due to isolations made in Manitoba from cattle and bonemeal.

The incidence of S. newport in humans in Manitoba increased from nine cases in 1965 to 22 cases in 1966. Most of these cases occurred in the metropolitan area of Winnipeg. A thorough investigation of all human isolations failed to establish a direct relationship with the bovine cases. (62). There was no other obvious reason for the increase in the incidence of human isolations in Manitoba.

At this time there is no known reason for the rapid increase of human isolations of S. newport on a national basis. (74). Yurack feels that a widely

distributed food could be the most likely source of S. newport for humans.

There are numerous examples where animals or animal products have been incriminated as sources of infection for man (5, 21, 23, 39, 60).
A few of these references deal specifically with beef or veal as reservoirs of infection for man (1, 40).
Milkborn outbreaks of salmonellosis in humans were common prior to pasteurization of milk.(5).

There is no evidence that beef products were a source of infection for humans during the bovine epizootic in Manitoba. However, one would be inclined to feel that the sudden increase in S. newport isolations from humans during and after the bovine epizootic was more than just a coincidence. Investigations revealed that animals from infected herds were shipped for slaughter during and after outbreaks. Carcasses from clinically affected animals would show obvious lesions and these would certainly be condemned by meat inspectors. However, carrier animals do not show any gross lesions. These carcasses cannot be detected without bacteriological cultures of certain organs. Surveys have shown that Salmonella organisms in carrier animals are located mainly in the intestine, mesenteric lymphnodes and particularly in the gall-bladder. When these structures are cut or ruptured during the slaughtering process the meat of these carcasses may become contaminated. Routine meat inspection procedures as they are practiced in Canada to-day do not include bacteriological cultures of carcasses and carrier animals therefore pass undetected. It is obvious that contaminated products from these carrier animals may further spread Salmonella organisms into other food products via processing equipment.

Kung, (40) described an outbreak of salmonellosis in humans due to S. newport which was traced to contaminated beef from a cow. Bowmer (5) stated that cattle - next to swine - are the most common reservoir of salmonellosis among the large meat-producing animals.

Recommendations for Prevention of Similar Outbreaks.

Much has been written about the control of salmonellosis in both animals and man. Bowmer (5), made fifty recommendations for the control and ultimate eradication of salmonella infection. Some of these were particularly applicable to the bovine epizootic and these only will be enlarged upon here.

The most obvious place to start is the control of salmonellosis on the farm. In spite of the widespread state of S. newport infection witnessed during the Manitoba outbreak, it was not possible to either quarantine herds or to control the movement of infected cattle. In fact, infected animals were shipped for slaughter through stockyards. Carcasses of animals which died of salmonellosis were also picked up for rendering. If salmonellosis had been made a reportable disease as was recommended by Bowmer, the infected herds could then have been quarantined. Any animals shipped from these herds could have been tagged in such a way as to allow easy identification in packing plants and rendering plants. The carcasses of the tagged animals could then have been examined bacteriologically.

Salmonellosis in animals could be made a reportable disease under either a Provincial or a Federal Contagious Diseases Act.

Recommendations made to the feed industries to develop techniques that would prevent contamination of feed products, were, in the light of this investigation, well founded. Periodic inspections of rendering plants, including culturing of samples of animal by-products produced in these plants, should be a routine procedure. Similar procedures could be adapted to feed mills using animal by-products as feed ingredients.

As noted in the literature review, recontamination of cooked animal by-products is a common problem in rendering plants. This appeared to be the primary pathway of transmission of Salmonellae from the unfinished to the finished product. Every effort should be made to prevent recontamination of cooked end-products, through high standards of plant hygiene.

Bowmer also recommended that in each province, state, or other suitable area, a salmonella-working group should be formed. These groups would consist of representatives of all government agencies involved in human and animal health, agriculture and food and feed processing. The need for such a group was clearly evident during the bovine epizootic in Manitoba. Many questions regarding the degree of contamination of rendering plants, packing plants and foods remain unanswered. Some of these questions might have been answered through cooperative action by different members of such a working group on a provincial level.

These regional groups could also assist in developing a more sensitive national surveillance program on incidence, epidemiology and epizootiology of salmonellosis.

SUMMARY

The investigation revealed the following important facts:

1. Feeding of salmonella-contaminated bonemeal to cattle, under certain stress conditions, can result in an epizootic of salmonellosis.

2. Abortions, stillbirths and high morbidity and mortality rates in newborn calves and their dams caused serious economic losses on affected farms.

3. The clinical symptoms, macroscopic and microscopic lesions in cattle infected by S. newport are similar to those reported for infections with other Salmonella serotypes in this species.

4. Recontamination of cooked animal by-products in the rendering plants was the most probable source of Salmonellae in the bonemeal.

5. The environment and equipment in feedmills may become contaminated by animal by-products containing Salmonella organisms. "Seeding" of other feeds with Salmonellae could occur in such contaminated feed mills.

6. S. newport appears to be more pathogenic for cattle than S. oranienburg.

7. Cattle infected by S. newport develop significant somatic and flagellar agglutinins against this serotype.

8. The carrier state in cattle infected by S. newport is of shorter duration than the carrier state with S. dublin infection.

9. There was a 100 per cent increase in the number of isolations of S. newport from humans in Manitoba in 1966 compared to 1965. S. newport was the primary pathogen in the bovine epizootic, however, none of the human cases could be traced directly to infected cattle or beef products.

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APPENDIX A

History Sheet - Enclose with specimen. Complete each pertinent section.

Your Clinic No.___ Date Sent ____ Date Rec'd ____ Lab No. _____

Owner _____ Address _____ Telephone _____

Veterinarian _____ Address _____ Telephone _____

Submitted by _____ No.Alive _____ No.Dead _____

Species/Breed ____ Age____Sex____ Weight___ Duration of illness__

Portions/Specimens _____

Herd Size _____Morbidity _____Mortality _____

Elapsed time between death & autopsy___ Time of death: Day__Hour__

Ration in detail _____

Recent changes in feed _____

Possible source of poison _____ Source of water _____

Management & Breeding _____

Vaccinations and/or treatment _____

_____

Clinical signs/autopsy findings _____

_____

_____

_____

_____

_____

_____

_____

APPENDIX B

Laboratory Necropsy & Histopathology:

Laboratory Findings:

Diagnosis:

_____

Pathologist