SC

A PRELIMINARY SURVEY OF SALMONELLAE IN CATTLE SLAUGHTERED AT THE ADDIS ABABA AND DIRE DAWA ABATTOIRS

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ABSTRACT

In an attempt to assess the incidence of Salmonellae in apparently healthy cattle slaughtered at the Addis Ababa and Dire Dawa abattoirs, a total of 1571 organ samples were collected, out of which 971 were obtained from the former and 600 from the latter.

The 971 samples from Addis Ababa abattoirs included 315 spleen, 303 mesenteric lymph node and 353 small intestine samples and these yielded 16 (5.1%), 10 (3.3%) and 8 (2.3%) strains of Salmonellae, respectively. The remaining 600 samples collected from cattle slaughtered at the Dire Dawa abattoir were 200 spleen, 200 mesenteric lymph node and 200 small intestine samples and from these 14 (7%), 3(1.5%) and 8 (4%) strains of Salmonellae were isolated, respectively. From this finding the incidence of Salmonellae in the cattle slaughtered at the Addis Ababa and Dire Dawa abattoirs was found to be 5.1% and 7% respectively. No significant difference was observed ($P \leq 0.05$).

Serogrouping of the 59 Salmonella isolates recovered from the 1571 samples showed that, with 24 (40.7%) strains, group D was the most frequent serogroup, to be followed by C_1 with 13 (22%) strains, B with 11 (18.6%), C_2 with 6 (10.2%) and E with 5 (8.5%). A comparision of the relative frequency of the serogroups in the samples from the two abattoirs

also revealed that the most frequent serogroup was D. The relative incidence of the rest of the serogroups was, however, found to be variable.

Of the two culture media used viz., Desoxycholate Citrate Agar and Brilliant Green MacConkey Agar, the former was found to be slightly superior in supporting the growth of more strains, both in number and type, of Salmonellae encountered in this work.

INTRODUCTION

The causative agents of the diseases known as salmonellosis are found in the Division Protophyta, Class Schizomycetes, Order Eubactariales, Family Enterobacteriaceae, Tribe Salmonellae and the Genus *Salmonella* (Breed *et al.*, 1967).

The genus *Salmonella* is composed of motile bacteria that confirm to the definitions of the family Enterobacteriaeae and the tribe Salmonellae. Urease is not produced, sodium malonate is not utilized, gelatin is not liquefied and growth does not occur in medium containing potassium cyanide. Lysine, arginine and ornithine are decarboxylated. Acid is produced in Jordan's tartrate medium. Dulcitol is fermented and inositol is utilized by numerous strains. Sucrose, salicin, raffinose, and lactose are not fermented (Edwards and Ewing, 1972).

The first member of this group to be studied was the typhoid bacillus, originally observed in human tissues by Eberth in 1880 and cultured by Gaffkey in 1884. Soon afterwards, Salmon and Smith reported on an organism (*Bacillus cholerae-suis*) which they isolated from diseased pigs in 1885, and similar isolations were made in the following years from a wide variety of human and animal sources (Hagan and Bruner, 1961 and Buxton and Fraser, 1977). The name *Salmonella* is in memory of Salmon and his early work on these organisms (Edwards & Ewing, 1972 and Buxton and Fraser, 1977).

Salmonellae readily grow on a variety of artificial media. Isolation of the organisms from suspected specimens is done on differential and selective media which are specifically designed to favor the growth of these and closely related organisms, and to supress unwanted contaminants. The preliminary identification of the isolates is made by studying their biochemical properties. This method, however, is of limited value. Therefore, the identity of isolates is confirmed by serology (Hagan & Bruner, 1961 and Prost & Riemann, 1967).

The serological identification and classification of Salmonella organisms is based on the analysis of the antigens present on the cell surface (somatic antigens) and the flagella (flagellar antigens). The surface of the bacterial cell contains lipopolysaccharide-protein complexes which constitute the somatic (O) antigens. Most Salmonella serotypes possess flagella and are thus motile. The flagella are composed of monomeric protein (flagellin) and constitute the flagellar (H) antigens. A detailed study of these antigens has shown that there is a very large number of O and H antigens and their identification is used as the basis for the classification of these organisms into serotypes (Buxton and Fraser, 1977). Therefore, identification of a given serotype of Salmonella is done after complete elucidation of the O and H antigens, by using monovalent antisera produced against specific antigens.

In addition to serological identification, phage-typing has also been used in identifying members of the genus Salmonella. Different strains of a particular serotype can at present be distinguished only by this method (Prost and Riemann, 1967). By means of this technique it is often possible to determine whether the likely origin of S.typhimurium infection in a group of animals was derived from a particular source of domesticated or wild animals. In addition, phage-typing is often of value in tracing the possible origins of human infections with S.typhimurium, when these are derived from direct contact with diseased animals, from human foods of animal origin of from another human source (Buxton and Fraser, 1977). Phage-typing, therefore, helps in distinguishing strains of the same serotype which are otherwise indistinguishable by serologic identification, because there can be more than one strain in the same serotype. In relation to this the work of Anderson (1964), as quoted by Prost and Riemann (1967), indicates that 48 phage-types of S.paratyphi B can be distinguished, as well as more than 35 types of S. typhimurium.

In most publications nomenclature of Salmonellae follow the traditional binomial way of naming viz., the genus name Salmonella followed by the species name which in most cases takes the name of the place from where it was first isolated. Therefore it is common to see names like S.havana, S.london, etc.

A different system of nomenclature for the genus Salmonella has been proposed by Edwards and Ewing (1972), because according to them the traditional way of naming is illegitimate in that it appears to accord species status to all serotypes of Salmonellae. According to these authors, only three species are recognized in the genus Salmonella. The three species are S. choleraesuis, S.typhi, and S.enteritidis. All Salmonellae other than S.choleraesuis and S. typhi are serotypes and biosero-types of S.enteritidis. Therefore serotypes (ser) and bioserotypes (bioser) of S. enteritidis are recorded in the manner indicated in the examples that follow:

Old designation	Present designation
S. typhimurium	S. enteritidis ser Typhimurium
S. paratyphi A	S. enteritidis bioser Paratyphi A

The term bioserotype is used in connection with certain serotypes of S. enteritidis that possess unique biochemical characteristics. Unnamed serotypes are recorded as in the following example: S. enteritidis ser 58: a:- (Edwards and Ewing 1972).

However, the new system of naming Salmonellae has not been used in most of the articles published since then. Up to the present, well over 2000 serotypes of Salmonellae have been isolated from several sources and they have been named with the traditional nomenclature.

Salmonellosis is a unique disease which has a world-wide distribution and the causative microorganism is commonly found in the intestinal tract of animal species, so that we would probably look upon it in much the same way as we look upon coliforms, if it were not for the fact that it is a pathogenic organism (Prost and Riemann, 1967).

A striking feature about the natural distribution of Salmonellae is the relative host-specificity of a few serotypes, and the ubiquitous habits of the majority (Buxton and Fraser, 1977). It is the lack of host-specificity in the majority of the serotypes and the resulting free mobility of the organisms amongst different animal species that have been responsible for its cosmopolitan distribution. Its common occurrence in a wide range of hosts and its pathogenic activities in both man and other animal species have made Salmonella a typical zoonotic microorganism. Therefore, with regard to their pathogenic feature, most of the types show no specific association with a given host species or pathological process. Only a few are able to produce conditions that are regarded as pathological entities.

As a consequence, Salmonellae can be classified into three groups, according to their pathogenic properties:

1. Salmonellae specifically pathogenic for man, producing typhoid and paratyphoid febrile diseases and which tend to become endemic in the population. These include S.typhi,

S. paratyphi A, B and C and S. sendai. More than 90% of the cultures come from man. However, domestic animals and other lower animals that live around man act as reservoirs of these organisms. Several studies support this last statement. For example, out of the strains of Salmonella tested at the National Salmonella and Escherichia Center of India, 12 strains of S. typhi, 5 strains of S. paratyphi A, 22 strains of S. paratyphi B and one strain of S.paratyphi C were isolated from laboratory animals, cattle, goats, sheep, pigs and poultry (Basu et al., 1975). Le Minor et al (1971) also reported that several strains of S. paratyphi B have been isolated from cattle. After mentioning that S. typhi is essentially a strictly human pathogen, Morse et al (1976) reported a single isolation of the organism from animals. In another report of Salmonella strains examined at the National Center of France Le Minor et al (1978) indicated that a few strains of S. typhi were isolated from other animals. A report of the Veterinary Services of the Ministry of Agriculture of France, indicates that of Salmonella isolates examined, S. paratyphi B was isolated from cattle, horse-flesh and milk, chicken and eggs (Ministere de L'Agriculture, 1978 and 1980). Le Minor et al.,(in press) also stated that several strains of S. paratyphi B that were isolated from horses, chicken and cattle were amongst other isolates examined at the National Salmonella Center between 1977-1979. From the above it can be seen that those Salmonellae specific to man can also be isolated from other animals.

2. Salmonellae specifically pathogenic for animals include S. abortusequi, which causes abortion in horses, S. abortusovis, a caustive agent of abortion in sheep, S. choleraesuis and S. typhisuis, swine pathogens, S.pullorum and S. gallinarum, pathogens of birds of different kinds, and several others. These organisms may be isolated from other animal species other than the ones mentioned above. Le Minor et al (in press) and Basu et al., (1975), in the works cited earlier, indicated the isolation of S. abortusovis from cattle, S. choleraesuis from man, S. pullorum and S. gallinarum from cattle and man and S. abortusequi from cattle, thus showing the presence of other reservoir hosts, though they may not show the typical symptoms during infection.

3. Nonhost-specific Salmonella include the vast majority of the serotypes which occur mainly in animals and often produce non-specific pathological processes (Hagan and Bruner, 1961; Prost and Riemann, 1967). This group includes some 1, 300 distinct serotypes of S. enteritidis that seemingly attack man and other animals with equal facility and no evident difference (Report, 1970). After mentioning that members of this group are ubiquitous and potentially pathogenic for animals and man alike, Buxton and Fraser (1977) indicated that the most outstanding example is S.typhimurium

which is frequently the cause of the disease throughout the world in a variety of animal species, as well as in man. The latter is best supported by the works that are cited eariler which indicate the recovery of S. typhimurium and other nonhost-specific strains both from animals and man (Le Minor et al., 1978 and in press; Ministere de L'Agriculture, 1978 and 1980, and Basu et al., 1975).

In the Report (1970) cited earlier, it was also mentioned that the nonhost-specific group accounted for 96.7% of 23, 414 cultures from all sources, and 94.8% of 12,267 from man during 1948-58 in the U.S.A. In the same report it was indicated that of 19,723 cultures isolated from man in 1967 in the same country, 17,977 (91.3%) isolates belonged to this group.

From the foregoing brief discussion, it can be seen that the majority of Salmonella show no host-specificity and only a few are host-specific even though the host-specific strains can also be found in abnormal hosts. The greatest danger of infections, both to man and other animals, is posed by the nonhost-specific serotypes which are quite abundant and have a wide distribution.

Salmonellae are not only confined to animals and man. Even though their native habitat is the intestinal tract of animals, including man, they can easily spread to other environments where they may surivive and even multiply (Report, 1970).

These organisms are commonly isolated from human foods such as eggs and egg products, meats and meat products, milk and milk products, etc. (Weiseman and Carpenter, 1969; Patterson, 1969; Report, 1970 and Baker et al., 1980).

Several reports also indicate the isolation of Salmonella from various animal feed stuffs (Report, 1970; Klindova and Havelka, 1973 and Stott et al., 1975). In the review of Williams (1975) on environmental considerations in salmonellosis, it was mentioned that bone meal and fish meal were found to contain a variety of Salmonellae.

The work of Smith and Grau (1974) and that of Harbourne et al. (1978) show that members of this genus have been isolated from abattoir effluents, effluent waters and river sites. Mueller (1979) reported isolating the organisms from drinking water. Salmonellae were also recovered from water flowing off feedlot surface (Miner et al., 1967), from animal manure disposal (Will et al., 1973) and from feedlot manure (Clinton et al., 1979). Bishop et al, (1980) in a report on the persistence of some groups of organisms in dairy-waste solids, have indicated that Salmonella organisms could persist in the waste matter for a fairly long period of time. A similar report on the persistence of S. abortusovis in soil shows that the organisms have been isolated from soil 162 days after artificial contamination (Tadjebakhche and Nazari, 1974). Following application of effluent from apparently healthy pigs to land, S. havana was isolated from kilogram samples, irregularly and at a low frequency for eight months from soil, and for nearly two months from pasture (Chandler and Craven, 1981). The organisms can survive in dust and animal manure oxidation ditches for a reasonably long period of time (Morse and Duncan, 1974). The above and several other works show the existence of Salmonellae in the environment outside their natural hosts.

A significant portion of the animal population, both wild and domestic, is exposed to the infective organisms from different sources and, therefore, are infected, though the degree of infection varies amongst different animal species and from place to place. Infection of animals by these bacteria, in most cases, results in illness which may culminate either in death or in ultimate recovery.

The mortality and/or morbidity of animals due to salmonellosis, specially in domestic animals, will have a direct and an indirect effect on the well-being of mankind.

Domestic animals which suffer from the disease usually have a reduced performance, which is reflected in term of decreased milk production in dairy cattle, decreased rate of weight-gain for beef cattle, swine, lambs, broilers and turkeys, together with decreased egg production, etc., thus reducing the benefit which man would otherwise get from his domestic animals. Verterinary payments, costs of preventive measures, loss of consumer confidence in dairy and poultry products (especially eggs), reflected in decreased price at the producer level and cost to the processor for recalling, reprocessing, and/or destroying livestock, dairy and poultry products found to be contaminated with Salmonella after processing and/or distribution in channels of trade, are but a few of the other costs incurred by the disease. Not only might infection with Salmonellae cause morbidity and mortality among the animals and the resultant economic losses, but considering that many of the chains of infection include both animals will have an effect on man (Report, 1970). Therefore, salmonellosis in animals, especially in domestic animals, is not only the problem of the animals but also that of man.

Several factors are involved in determining the degree of incidence of salmonellosis in domestic animals. For the sake of convenience these factors are grouped into two, viz., factors of human origin and those of animal origin.

In the effort of trying to make maximum use of his domestic animals, man has unknowingly been the cause for spreading and/or increasing the incidence of many animal diseases. One such activity of man is modern-day animal husbandry. In relation to this last point the Report of the Committee on Salmonella states the following: "some current methods of animal production are associated with an increased possibility of salmonellosis. For example, mass-production involving calves, beef cattle, swine, and poultry in relatively small areas, increases the potential spread of salmonellosis among these animals. Due to the increasing demand for poultry, there are larger breeding flocks and greater concentration of birds during transportation, slaughter, and processing, all of which aid the transmission of Salmonella among the birds" (Report, 1970). In the same report it was also indicated that the incidence of non-human salmonellosis, especially that of domestic animals, increased three-fold between 1963 and 1967 in U.S.A. Even though this increase might reflect increased surveillance, the major factor is the development of modern methods of animal production. Considering animal housing as a factor, Williams (1975) stated that the continuous rearing of large number of young animals in buildings will lead to a considerable build-up of pathogenic organisms, particularly Salmonellae. The situation could, however, be checked by periodic emptying, cleansing and disinfection of animal accomodation. To illustrate the latter point, Williams (1975) cited a case of young calves infected within two to four days after being introduced into a house. The calves were infected by S. dublin which was shed by a carrier cow and the bacteria had survived for months on the walls of a loose-box.

Management systems have a significant influence on the incidence of salmonellosis in groups of animals. While the infection rate in cattle tied in individual stalls is usually low, there is a positive correlation between the incidence of salmonellosis and the use of loose housing. Transmission of Salmonellae amongst animals housed in the same housing is possible via droplets, because the infective organisms are expelled within droplets in the expired breath (Williams, 1975).

Easy transmission of Salmonellae amongst newly-hatched chicks has been enhanced by the introduction of large egg incubators. Industrially-produced animal feeds serve as good vehicles for the dissemination of the disease-causing organisms both nationally and internationally (Williams, 1975). The incorporation of antibiotics in animal feeds has been responsible for the ellimination of drug-sensitive organisms and the proliferation of drug-resistant strains (Report, 1970 and Morse and Duncan, 1974).

Animals to be slaughtered have to be transported from farms to saleyards and finally to abattoirs or directly from farm to abattoirs. Transmission of Salmonellae can be effected by fecal contamination, especially when animals are crowded in railway wagons, saleyards, etc. In one investigation, the mean infection rate in calves on the farm was estimated at 0.5%, it increased to nearly 36% after the animals were kept in holding-pens for two to five days (Report, 1970). The work of Grau et al. (1968) shows that Salmonella spp. were detected commonly in railway wagons, saleyards and abattoir holding-pens through which cattle pass from farm to slaughter. In the same paper it was also pointed out that the percentages of animals with Salmonellae in the rumen increased with the period between leaving the farm and slaughter.

During transportation, animals are fed intermittently. This is also true in saleyards and abattoir holding-pens. The alternation of feeding and starvation plays a role in the establishment of infection of animals by Salmonellae. This is best shown by the work of Brownlie and Grau (1967) who found that growth of Salmonellae and E. coli type I occurred during starvation, and resumption of feeding after starvation caused further multiplication. They have also shown that stravation for 2 or 3 days was generally followed by infection of the small intestine, with Salmonellae persisting in the feces for at least a week. On the other hand, regular feeding facilitated the rapid elimination of infective organisms, and viable organisms in the feces were rarely detected (Brownlie and Grau, 1967).

The role of the human carrier as a reservoir for animal infection has to be mentioned also. The problem of the human carrier is emphasized when the problem is seen in view of the nonhost-specificity of the majority of the Salmonella serotypes, which can freely pass from one host to the other. Infectious diseases such as salmonellosis are often occupational and carriers of the organisms are more common in such individuals than other people. This has been confirmed by several workers (Beceril *et al*., 1979; Cedida *et al*., 1979 and Gracey *et al*., 1980). In addition to the human carrier that poses a more or less permanent threat of infection to other humans and animals, the role of the mechanical carrier, who spreads the infective organisms from pen to pen in piggeries and other animal-rearing places, is significant (Williams, 1975).

Some animal behaviour aids the transmission of *Salmonella* organisms in the herd or flock. Animals like chickens tend to stay together most of the time. This gregarious nature facilitates cross-infection. The habit of licking each other brings about transmission of the bacteria. The works of several investigators as quoted by Williams (1975) confirm the presence of Salmonellae in the saliva of calves infected with the organisms. This leads to the contamination of nursettes, buckets and drinking-bowls and infection could then spread to other members of the group. Cannibalism, which is a common phenomenon amongst large flocks of birds, can also help the transmission of the infective organisms. The habit of eating fecal matter, coprophagy, is another aspect of the behavior of several animals which may enhance the transmission of Salmonellae within a species, or in different animal species. The above are examples of the different behavior of animals which may contribute to the incidence of salmonellosis in different groups of animals.

Several studies have been made to determine the incidence of Salmonellae in apparently healthy cattle in different countries round the world. The carriage-rate ranges between zero and well over 70%. These variations in the carrier-rate in different places could be accounted for by the factors mentioned earlier in this paper, and possibly others.

In the preceeding introduction attempts have been made to point out the importance of the Salmonella problem. It has also been briefly mentioned that Salmonellae are unique organisms in that the majority of them are not host-specific and therefore are of both public health and veterinary importance. The latter condition is emphasized in under-developed countries like Ethiopia, where the standard of living of the majority of the population is very low; man and his domestic animals living together in close proximity, (most of the time in the same hut), which facilitates the easy transmission of the disease. In addition to this, the livelihood of the majority of the population is closely tied to cattle, because the latter are used in subsistence agriculture. The problem can be aggravated by certain cultural practices like eating raw meat, drinking raw milk, etc.

Annual Reports of the Ministry of Public Health of this Country show that the problem exists in the human population. So far no published work has appeared on salmonellosis in cattle in Ethiopia, with the exception of a few isolations from domestic animals with clinical disease, made by the Central Veterinary Laboratory at Debre Zeit. As a result, the incidence of Salmonellae in cattle, and the potential health hazards to cattle and man, is obscure. It is therefore the objective of this work to at least partly answer this question.

MATERIALS AND METHODS

Sample collection

A total of 1571 organ samples was collected from cattle slaughtered at the Addis Ababa and Dire Dawa abattoirs between January 1, 1982 and March 11, 1983.

Samples were collected between 5 p.m. and 7 p.m. in Addis Ababa on the days the samples were collected. Nine Hundred and Seventy One organ samples viz., 315 spleen, 303 mesenteric lymph nodes and 353 small intestine (with contents) were collected from the Addis Ababa abattoirs. The samples from the three organs were taken from different animals because the slaughtering method did not allow the taking of samples of the different organs from the same animal. Between 5 and 10 gram samples of the organs were collected in plastic bags (previously kept in boiling water for 30 min.) and were sealed over a candle flame. The samples thus collected were immediately taken to the laboratory for bacteriological examination.

From cattle slaughtered in the Dire Dawa abattoirs, 600 organ samples viz., 200 spleen, 200 mesenteric lymph nodes and 200 small intestine (with contents) were collected. On each day of sampling, the samples were collected between 10:00 p.m. in the evening and 5:00 a.m. in the morning. In this case, samples from each of the three organs were taken from a single animal. To avoid the loss of organisms as a result of the time lapsed during transportation to the laboratory, which is located in the College of Agriculture at Alemaya (approximately 45 km from Dire Dawa), the samples collected were inoculated on the spot into 50 ml conical flasks containing about 30 ml of Selenite broth (Oxoid, CM 395). Before inoculation each organ sample was surface-sterilized by burning over a spirit lamp and minced into smaller pieces with sterile scissors. These were then taken to the laboratory for incubation and further bacteriological examination.

Cultural Methods

The samples collected from the Addis Ababa abattoirs were also surface-sterilized over a bunsen flame, minced into samller pieces with sterile scissors and inoculated into 125 ml conical flasks containing about 100 ml of selenite broth (Oxoid, CM 395). In both cases, incubation was at 43°C for 48 h (Harvey and Philips, 1961; Report, 1964; Harvey and Price, 1968 Harvey et al., 1966; Merahan and Hawksworth 1969).

A loopful of each of the growth from each specimen was subcultured on Desoxycholate Citrate Agar (Oxoid, CM 227) and MacConkey Agar (Oxoid, CM 115) containing 0.004% Brilliant Green (BDH) (Iveson & Mackay - Scollay, 1969, Cruickshank et al., 1975 and Chau & Leung, 1978). The plates were incubated at 37°C for 48 h. Non-lactose fermenting colonies resembling those of Salmonella were transferred into freshly-prepared slants of Triple Sugar Iron Agar (TSIA) (Oxoid, CM 277) and Lysine Iron Agar (LIA) (Oxoid, CM 381) by touching the center of the suspect colony once with a straight needle. The TSIA and LIA slants were incubated at 37°C for 24 h. Those tubes with typical Salmonella reactions were selected and further identification carried out using standard biochemical and serological methods (Edwards and Ewing, 1972).

Biochemical Identification

The purity of the cultures was tested regularly on Brilliant Green Agar (Oxoid, CM 263) before biochemical tests were performed. Pure cultures from the Brilliant Green Agar were transferred into urea slants (Oxoid, CM 33), motility test medium, tryptone water medium, citrate slants (Oxoid, CM 155), glucose and sucrose fermentation tubes with inverted Durham tubes. Andrade's indicator was used in the fermentation tubes. The inoculated and uninoculated control tubes were incubated at 37°C for 24 h. Cultures that were urease, indole and sucrose negative, motile and glucose positive were transferred onto Blood Agar Base (Oxoid, CM 55) with 5% defibrinated sheep blood. The blood agar plates were incubated at 37°C for 24h and the growth on these plates was used for serological studies.

Serological identification

Confirmation of the Salmonellae was done by polyvalent O antisera obtained from the Pasteur Institute in Paris. The confirmed cultures were counter-checked and serogrouped by BBL Poly O (A-E) and group-specific O antisera (A, B, C_1, C_2, D, E and Vi) obtained from the Central Laboratory and Research Institute (CLRI) in Addis Ababa. The serologic identification was done by the standard slide agglutination test and agglutination was observed by holding the slides against a dark background (Edwards and Ewing, 1972).

RESULTS

Salmonellae were isolated from 59 of 1571 organ samples collected from cattle slaughtered at the Addis Ababa and Dire Dawa abattoirs. Out of these 34 isolates were recovered from 971 samples collected from Addis Ababa and the 600 samples examined from those slaughtered at Dire Dawa abattoirs yielded 25 strains.

From cattle slaughtered at the Addis Ababa abattoirs 315 spleen, 303 mesenteric lymph node and 353 small intestine samples were examined and these yielded 16 (5.1%), 10 (3.3%) and 8 (2.3%) Salmonellae, respectively. The 25 strains of Salmonellae that were isolated from samples obtained from Dire Dawa abattoirs were recovered from 14 (7%) of 200 spleen, 8 (4%) of 200 small intestine and 3 (1.5%) of 200 mesenteric lymph node samples (Table I).

The 59 isolates obtained from the two abattoirs were serogrouped into five known serogroups, as shown in Table II. The relative incidence of the different serogroups in relation to the total strains isolated from the two places is as follows: with 24 (40.7%) strains, group D ranked first; followed by C_1 with 13 (22%) strains, B with 11(18.6%), C_2 with 6 (10.2%) and E with 5 (8.5%) strains. No organism belonging to group A was recovered from samples collected from cattle slaughtered at the two abattoirs.

Table III provides a comparison of serogroups of Salmonellae obtained from the two sites. In both places the most prevalent serogroup is D. From the Addis Ababa abattoirs, 14 (41.2%) strains belonging to this group were recovered and similarly 10 (40%) strains from the Dire Dawa abattoir. The relative incidence of the other serogroups is variable in the two places. Group C_1 with 10 (29.4%) strains ranks 2nd in Addis Ababa while this group with only 3 (12%) strains ranks 3rd in Dire Dawa. The 2nd most prevalent serogroup in Dire Dawa is B, with 7 (28%) strains while this same serogroup with 4 (11.8%) strains takes the 3rd place in Addis Ababa. Even though the difference is not great, C_2 with 4 (11.8%), strains from Addis Ababa shows an incidence a little higher than that recovered from Dire Dawa samples. In the latter, there were only 2 (8%) strains from this group. A comparison of strains that belonged to group E shows that with 3 (12%) strains, Dire Dawa abattoirs yielded slightly more than those from cattle slaughtered at the Addis Ababa abattoirs, which yielded only 2 (5.9%) strains.

The relative distribution of the Salmonella serogroups in the different organs collected from cattle slaughtered at the Dire Dawa and Addis Ababa abattoirs is shown in Table IV. The maximum number of strains recovered was from spleen samples in both places. Sixteen (47.1%) strains from 4 serogroups and 14 (56%) strains belonging to 5 serogroups were isolated from spleen samples collected from cattle slaughtered at the Addis Ababa and Dire Dawa abattoirs, respectively. Out of the 16 strains obtained from Addis Ababa spleen samples, 1 belonged to serogroup B, 6 to C_1, 3 to C_2, 6 to D and none to groups A and E. The distribution of the 14 isolates from Dire Dawa was: 3 of B, 1 each of C_1 and C_2, 6 of D, 3 of E and none of A. The organ that ranks 2nd in the number of strains from Addis Ababa samples is the mesenteric lymph node. A total of 10 (29.4%) strains, of which 4 were in serogroup C_1, 1 in C_2, 3 in D, and 2 in E, were recovered from this organ. The mesenteric lymph node samples from Dire Dawa, on the other hand, yielded the least number of strains. Only 3 (12%) strains were isolated from samples of this organ in the latter case. Out of the 3 strains, 1 belonged to C_1, 1 to C_2 and to B. Eight (23.5%) strains, of which 3 were B and 5 were D, were recovered from small intestine samples collected from the Addis Ababa abattoirs. The 8 (32%) strains that were isolated from small intestine samples of Dire Dawa belonged to groups B (4), C_1 (1) and D (3).

The relative efficiency of the two culture media viz., Brilliant Green MacConkey (BGM) and Desoxycholate Citrate Agar (DCA) was compared (Tables V and VI). A comparison of the number of serogroups recovered on both media shows that of the 17 strains that belonged to B, 8(10.1%)

were obtained from BGM and 9 (11.4%) from DCA; of the 16 C_1 strains, 8 (10.1%) strains each from both media; of the 9 C_2 strains, 4 (5.1%) from BGM and 5 (6.3%) from DCA; of the 31 D strains, 14 (17.7%) from BGM and 17 (21.5%) from DCA and out of the 6E strains, 1 (1.3%) was obtained from BGM and 5 (6.3%) from DCA.

Some of the strains were isolated simultaneously media, while others were recovered on one of them. Out of the 79 strains, 40 (50.6%) strains were isolated on both media, while 15 (18.9%) strains were recovered from BGM alone and 24 (30.4%) from DCA (Table VI). The breakdown of this general statement is as follows: of the total of 17 B strains, 12 (70.6%) were recovered on both, 2 (11.8%) on BGM and 3 (17.6%) on DCA; of the 16 C_1, strains, 6 (37.5%) on both, 5 (31.3%) on BGM and 5 (31.3%) on DCA; of the 9 C_2 strains, 6 (66.7%) on both, 1 (11.1%) on BGM and 2 (22%) on DCA; of the 31 D strains, 14 (45.2%) on both, 7 (22.6%) on BGM and 10 (32.3%) on DCA, and of the 6 E strains 2 (33.3%) were recovered on both, none on BGM alone and 4 (66.7%) on DCA.

Table I: Number and types of organs examined and found *Salmonella* positive from Addis Ababa and Dire Dawa abattoirs.

Organ	Addis Ababa			Dire Dawa		
	Number examined	Number positive	Percent positive	Number examined	Number positive	Percent positive
Spleen	315	16	5.1	200	14	7
Small Intestine	353	8	2.3	200	8	4
MLN*	303	10	3.3	200	3	1.5

* MLN = Mesenteric tymph Node.

Table II: Relative incidence of the serogroups isolated at Addis Ababa and Dire Dawa abattoirs.

Serogroup	No isolated	% isolated
A	-	-
B	11	18.6
C_1	13	22.0
C_2	6	10.2
D	24	40.7
E	5	8.5
All serogroups	59	100

Table III: Relative incidence of *Salmonella* serogroups in Addis Ababa and Dire Dawa abattoirs.

	Addis Ababa		Dire Dawa	
Serogroup	Number isolated	Percents isolated	Number isolated	Percent isolated
A	-	-	-	-
B	4	11.8	7	28
C_1	10	29.4	3	12
C_2	4	11.8	2	8
D	14	41.2	10	40
E	2	5.9	3	12
All serogroups	34	100	25	100

Table IV: The relative distribution of Salmonella serogroups in the different organs examined at the two places

	Addis Ababa			Dire Dawa		
Serogroup	Spleen	MLN*	Intestine	Spleen	MLN	Intestine
A	–	–	–	–	–	–
B	1	–	3	3	–	4
C_1	6	4	–	1	1	1
C_2	3	1	–	1	1	–
D	6	3	5	6	1	3
E	–	2	–	3	–	–
Total	16(47.1%)	10(29.4%)	8(23.5%)	14(58%)	3(12%)	8(32%)

* MLN = Mesenteric trymph node

Table V: Relative efficiency of Brilliant Green MacConky Agar (BGM) and Desoxycholate Citrate Agar (DCA) for the recovery of Salmonella

Serogroup	No. recovered on BGM	No. recovered on DCA
A	-	-
B	8(10.1%)	9(11.4%)
C_1	8(10.1%)	8(10.1%)
C_2	4(5.1%)	5(6.3%)
D	14(17.7%)	17(21.5%)
E	1(1.3%)	5(6.3%)
All serogroups	35(44.3%)	44(55.6%)

Table VI: Rate of recovery of the different *Salmonella* serogroups on each or both of the two different media

Serogroup	BGM & DCA	BGM	DCA	Total
A	-	-	-	-
B	12(70.6)	2(11.8)	3(17.6)	17
C_1	6(37.5)	5(31.3)	5(31.3)	16
C_2	6(66.7)	1(11.1)	2(22.2)	9
D	14(45.2)	7(22.6)	10(32.3)	31
E	2(33.3)	0	4(66.7)	6
All serogroups	40(50.6)	15(18.9)	24(30.4)	79

DISCUSSION

Earlier it has been indicated that due to problems posed by the slaughtering methods in the Addis Ababa abattoirs, samples of the three organs examined were not from the same animal. Instead, organ samples were from different animals. However, by taking a reasonable number of samples of each organ, and using the organ that yielded the maximum number of the organisms as an index, the carrier rate can be approximated. Therefore, the discussion of the findings in Addis Ababa should be seen with this in mind.

From the findings of this work, the carrier rate of cattle slaughtered in the Addis Ababa abattoir can be approximated as 5% on the basis of the spleen-positivity rate since spleen yielded the highest number of strains. A closer look at the course of infection of this disease justifies the use of such organs as the liver, kidney and spleen as indices in determining the carrier rate in apparently healthy cattle. Recovery of Salmonellae from intestine and contents, mesenteric lymph nodes and rumen are indicative of relatively recent infections, possibly acquired in abattoir holding-pens, transportation vehicles or saleyards. Therefore, the latter may not reflect the actual carrier rate in the farms where the animals are bred. Hence the carrier rate in apparently healthy cattle slaughtered at the Addis Ababa abattoirs is around 5.0%.

The work in Dire Dawa shows that the carrier rate of cattle slaughtered in the abattoirs of this town can be approximated as 7%.

A comparison of the incidence at the two places shows that there is no significant difference ($P \nless 0.05$). The slight difference could be due to the differences in the conditions of the abattoirs in Addis Ababa and Dire Dawa. In the Dire Dawa abattoirs, the animals to be slaughtered are brought in to a single room where they are stunned, slaughtered, skinned, gutted and dismembered. The whole process is done in the same room on the floor. It is not uncommon to see a bull waiting to be slaughtered, others lying on the floor being skinned and gutted and heaps of beef waiting to be transported, in the same room. Though water is splashed on the floor every now and then, the floor is permanently covered by blood and gut contents. The chances of cross-contamination is greater here. The conditions in the Addis Ababa abattoirs, on the other hand, are better. Though the actual stunning, slaughtering, and skinning is done in the same room, the chances of cross-contamination are much lower because the animals are found on the floor only during slaughtering and the removal of the guts. All of the other processes are done while the animals are suspended from hooks on their hind legs. The floor is cleaner because it is continuously washed by water from hoses. The carcases are also continuously washed by jets of water as they are moved from one area to another on rails. Therefore, the chances of cross-contamination are greatly reduced. Earlier, it has been mentioned that all possible attempts have been made to minimize or eliminate contaminants from the surfaces of the samples by keeping them over a bunsen flame. This does greatly reduce, but may not totally eliminate the contaminants and therefore the role of slaughtering methods in the two abattoirs cannot be totally ruled out. It can be one of the reasons that may account for the slight difference between the two abattoirs. It may also be due to actual differences in the carriage-rates of cattle slaughtered at the two places.

The carrier rates for the two places are not far from those obtained elsewhere, and is within the range (0-20%) given in the Introduction. However, it differs from values obtained by some workers. For example, Smith and Buxton (1951), in a report of a survey made of healthy adult farm animals in England and Wales, showed that 0.4% of the cows examined were found to beinfected. One factor that may partly account for this difference could be the material they used as an index, viz, the feces. The feces may not serve as a good site to look for Salmonellae because fecal excretion of the organisms is intermittent and sampling could be done at the wrong time. The differences is most probably due to the difference in health and nutritional status. Salmonella infections tend to establish in cattle that are starved rather than in those that are fed well. In well-fed cattle the organisms are eliminated quickly and infections do not establish easily (Brownlie and Grau, 1967).

In many cases carriage-rates which are much higher than obtained in this work have been reported. Grau and Brownlie (1965) reported a 45% carriage rate in Australia. Kheifets et al., (1973) found a value as high as 57% in cattle examined in USSR. A carriage rate of 76% was reported by Samuel et al., (1980). Samuel et al., (1981) have also

have also reported finding 77% of carriers. Several factors could account for these differences. They could be due to differences in time spent in transportation wagons, saleyards and abattoir holding-pens of cattle slaughtered here at the Addis Ababa and Dire Dawa abattoirs, from those for which the above carriage-rates relate. In practically all of the cases above, the cattle have spent a minimum of 4 days in abattoir holding-pens. If the time spent in transportation and saleyards is considered, the time spent between the farm and slaughter will definitely increase. Earlier it has been mentioned that the longer the time between the farm and slaughter, the higher the chances of cross-infection due to crowding and the establishment of infections due to intermittent feeding and therefore the higher the incidence will be (Prost and Riemann, 1967 and Report, 1970). Consideration of the cattle slaughtered at the Addis Ababa and Dire Dawa abattoirs shows that a relatively short time is spent between the farm and slaughter. In the first place, animals do not spend more than one day in saleyards because farmers bring their animals to local markets on market days and take them back home if they are not sold. Secondly, the contribution of transport wagons is minimal or nil be because all of the cattle slaughtered at the Dire Dawa abattoirs and some of those slaughtered at the Addis Ababa abattoirs, are brought to these places on foot. Some of those brought to the latter place are transported by trucks and train, a trip that usually takes not more than a day. Thirdly, animals to be slaughtered in the Addis Ababa and Dire Dawa abattoirs are brought to the abattoirs on the day they are to be slaughtered. Therefore, the chances of cross-infection are minimal or very low.

The differences could also be attributed to differences in animal husbandry practices. In places where intensive modern-day animal husbandry is practiced the incidence could also be higher because under such circumstances cross-infection could increase as the result of crowding in relatively small areas. The use of factory-produced feeds could also serve as a means by which the infective organisms are disseminated. Most, if not all, of the cattle slaughtered at the two abattoirs in Ethiopia are obtained from peasants, who in most cases possess only a few head of cattle and never use such things as factory-produced animal feeds, thus almost eliminating the chance of infection from such sources. The above may account for the differences in carriage rates of cattle examined here and in the places mentioned earlier.

A comparison of the carriage rate obtained here to work done elsewhere in Africa shows that it is higher than some and lower than others. For example, Zwart (1962), in Ghana found an incidence rate of 21.3%, a rate significantly higher than the one obtained here. Khan (1970), on the other hand, has reported finding that only 1.6% of cattle examined in the Sudan were positive for Salmonellae. Of the cattle slaughtered at Dar es Salaam abattoir, about 6.16% were found to be carriers (Hummel, 1974). A carrier rate as high as 9.3% has also been reported for Egypt (Abdel Galii et al., 1972). Even though there are significant differences in the two cases i.e., between the highest (21.3%) and the lowest (1.6%), the difference from the other two is not significant. The differences could be due to differences in animal production practices, the sanitary quality of the slaughterhouses, the sensitivity of the methods used in the studies, the age of the animals examined and possibly several others.

The 59 strains whose identity was properly confirmed were classified to 5 known serogroups, viz., B, C_1, C_2, D and E (See Table II). This finding is in agreement with earlier work which also shows that the majority (well over 90%) of isolates from cattle, and also other animals, were from these groups (Le Minor et al., 1971 and in press; Prost and Riemann, 1967; Ministere de L'Agriculture, 1978 and 1980; Report, 1970; Buxton and Fraser, 1977; Basu et al., 1975; Tiwary and Prasad, 1972; Abdel Galil et al., 1972; Kheifets et et al., 1973; Baglivi et al., 1979; Saxena et al., 1980;Samuel et al., 1980 and Samuel et al., 1981). The above being the general trend, the relative incidence of the serogroups may vary from place to place. A comparison of the serogroups isolated from both slaughterhouses in this country shows that group D with a total of 24(40.7%) strains ranks first. With 13(22%) strains, group C_1 is the next most frequent serogroup. With 11(18.6%) strains, group B takes the 3^{rd} place to be followed by group C_2 with 6(10.2%) and E with 5(8.5%) strains. Several other studies of cattle also show that group D is the most frequent serogroup. For example, Hummel (1969) reported group D to be the most common serogroup isolated from specimens taken from cattle in Tanzania. (For a complete reference see the above-quoted authors).

However, the relative importance of the other serogroups may vary from place to place. This variation was observed in the findings at the two slaughterhouses in this country. The variation of the relative frequency of the 5 most prevalent serogroups has also been reported in the works quoted earlier. In one of these it was indicated that B with 47.1% ranks first to be followed by D_1 (23.7%), C_1 (13.3%). C_2(7.1%) and E (4.4%) (Report, 1970). Le Minor et al., (1971) also show the relative importance of the serogroups isolated from a variety of sources as follows: B(39%), D(33%), C(12.8%) E (5.3%) and A (0.6%). In the report of the Ministere de L'Agriculture (1980) the incidence of the serogroups is given as follows: B (57.25%), C (18.24) D (13.19%) and E(10.86%). From the literature it can be seen that the relative importance of the serogroups may vary from place to place.

The relative distribution of Salmonella serogroups in the different organs examined at the two slaughterhouses does not follow the same trend with two of the organs (Table IV). Equally, there seems to be no agreement in the literature on which of the organs yield more Salmonellae, primarily because in many of them the same organs were not examined. Therefore, some state the recovery of more Salmonellae from mesenteric lymph nodes than other organs and some state otherwise. For example, Khan (1970) recovered more Salmonellae from mesenteric lymph nodes than intestinal contents or bile.

Kheifets et al., (1973) recovered more of the organisms from livers than Kidneys, from different groups of domestic animals including cattle. Hummel (1974) also stated isolating more strains of Salmonellae from mesenteric lymph nodes than small intestines. The work of Baglivi et al (1979) showed that there was a significantly higher recovery rate of the organisms from lymph nodes than from feces. Samuel et al., (1980), on the other hand, reported recovering more organisms from ruminal contents than mesenteric lymph nodes or other sources. In a report of relatively recent work on Salmonella in the intestinal tract and associated lymph nodes of sheep and cattle, Samuel et al., (1981) have shown that in cattle Salmonellae were frequently present, usually in large numbers, in the lymph nodes draining the ileum, caecum, and colon, but rarely in the ruminal and abomasal nodes. From the above , it is quite difficult to say which organ would be the best site for recovering Salmonellae in carrier animals. But it seems as if a few agree on the mesenteric lymph nodes. The work of Carter and Collins (1974), quoted by Samuel et al. (1981), clearly shows that the closer the infected organ is to the intestine, the more recent is the infection. Therefore, the infection rate of the GIT and the associated organs depends on the duration of infection. Hence, it should not be surprising if different workers produce results that are different.

One of the factors that may contribute to the discrepancies in the findings of several workers is the difference in the methods employed, of which the culture media used take the lion's share. Most workers used the media that they are well familiar with, or those that were available. Therefore, the set of media used by different investigators is different. However, in most cases, because there is not one medium that supports the growth of all serotypes of Salmonellae, more than one culture medium is employed. This method was adopted in this work and the findings support what is mentioned above (Tables V and VI).

A total of 79 strains of Salmonellae were isolated on either one or both of the two culture media. An overall comparison of BGM and DCA shows that 44 (55.6%) strains were recovered on the latter while 35 (44.3%) strains were isolated from the former. This shows that DCA is a bit superior to BGM. All of the serogroups were recovered on both media with the exception of a few that were more frequently recovered on DCA. A total of 40 (50.6%) strains belonging to serogroups B, C_1, C_2, D and E were recovered on both media. Out of the remaining isolates 15 (18.9%) were isolated on BGM alone and 24 (30.4%) strains on DCA alone. A comparison of the rate of recovery of the different serogroups from one or both of the two media shows that out of the total of 17 strains belonging to group B, 12

(70.6%) were isolated on both, whereas only 2 (11.8%) and 3(17.6%) were recovered on BGM and DCA alone, respectively. Strains belonging to group C_1, C_2 and D also follow the same trend. But strains belonging to serogroup E seem to grow better on DCA, because from Table VI it can be seen that of the 6 strains isolated, 2 were simultaneously recovered on both media, none on BGM alone and the remaining 4(66.7%) on DCA alone. In general, it can be concluded that DCA seems to be a better culture medium, in that it supports the growth of all of the serogroups encountered in this work, and secondly the importance of using more than one medium.

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