A STUDY OF THE OCCURRENCE OF AVIAN SALMONELLOSIS IN SOME FARMS AND A SLAUGHTERHOUSE IN KENYA

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DECLARATION

a) This thesis is my original work and has not been presented for a degree in any other University.

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LIST OF ABBREVIATIONS

BGA	Brilliant green agar
BSA	Bismuth sulphite agar
CFT	Complement fixation test
cm	centimetre(s)
DCA	Desoxycholate citrate agar
gm	gramme(s)
HEA	Hektoen enteric agar
IEP	Immunoelectrophoresis
IHT	Indirect haemagglutination test
KCN	Potassium cyanide
kg	kilogramme(s)
MaAGT	Macroantiglobulin test
MAT	Microagglutination test
MCA	MacConkey agar
mg	milligramme(s)
MiAGT	Microantiglobulin test
MIB	Meat infusion broth
ml	millilitre(s)
mm	millimetre(s)
PBS	Phosphate buffered saline
PRT	Precipitin ring test
PT	Precipitin test
RSPT	Rapid serum plate test
RWBPT	Rapid whole blood plate test
SAT	Slide agglutination test
SB	Selenite broth

sp.	species in singular
spp.	species in plural
SSA	Salmonella-Shigella agar
TAT	Tube agglutination test
TB	Tetrathionate broth
TSIA	Triple sugar iron agar
WT	Wattie test
XLDA	Xylose lysine deoxycholate agar

SUMMARY

Salmonellosis is a zoonotic disease which causes severe gastroenteritis and septicaemia in man and animals. In chickens, the disease causes high losses by death during the first three weeks after hatching, reduced fertility and hatchability, reduced egg-production and stunted growth in chicks.

Many workers have investigated avian salmonellosis. Their findings have indicated that there is a high occurrence of avian salmonellosis in many parts of the world.

In Kenya, S. gallinarum, S. pullorum and S. typhimurium have been isolated from some chickens. However, available information shows that very limited work has been done on avian salmonellosis. Unpublished annual reports (1969 to 1977) from the microbiology diagnostic laboratory of the Kenyatta National Hospital indicate that there is a significant occurrence of human salmonellosis in Kenya. Of the Salmonella bacteria recovered from human patients, S. typhimurium has been the main isolate.

Poultry keeping has become a significant agro-industry and poultry products are an important source of protein. Since salmonellosis is a public health hazard, it was necessary to assess the degree of

salmonellosis in poultry. To achieve this objective, a number of faecal, intestinal-content and tissue specimen from unvaccinated birds from four farms and a slaughterhouse in the neighbourhood of Nairobi were investigated bacteriologically. A serological survey of the same birds was also done. It was found that while the bacteriological isolations indicated an apparent low occurrence of salmonellosis in chickens in Kenya, serologically there was a high occurrence. Salmonella gallinarum was isolated from sick birds while salmonellae in groups B and C were isolated from apparently healthy birds. Poultry infected with such organisms could easily serve as a source of infection in man and probably account for some human salmonellosis as has been observed from the Kenyatta National Hospital.

This study, by its design, has been limited to a few farms and one slaughterhouse. Consequently, one cannot claim that it gives a complete picture of the occurrence of salmonellosis in Kenya. However, these findings indicate that avian salmonellosis is present in Kenya and that it could cause a serious problem. Consequently, further and extensive work needs to be done to assess the degree of salmonellosis in poultry and especially in view of their significance as a source of protein for an increasing number of people in this country.

INTRODUCTION

Salmonellosis is one of the major zoonotic diseases. It causes severe gastroenteritis and septicaemia in man and animals. These conditions may lead to death. As a result of the septicaemia, abortions, polyarthritis, endocarditis, nervous disorders and other conditions may also arise. Avian salmonellosis is economically important to the poultry keeper. The disease may cause high losses by death during the first three weeks after hatching. It can also cause reduced fertility and hatchability. It also may cause reduced egg-production and increased mortality among adult flocks. The most damaging effect of Salmonella infection on young chicks is one of stunted growth to the extent that infected birds are of low quality when they reach market age. The organisms also serve as secondary invaders in many avian diseases. This results in condemnation at the time of slaughter of severely affected carcasses (Van Roekel, 1952).

Poultry salmonellosis is worldwide in distribution. Buxton (1957) found that among the serotypes causing salmonellosis in chickens, Salmonella pullorum and Salmonella gallinarum are the most common. Unpublished annual reports from the

microbiology diagnostic laboratory of the Faculty of Veterinary Medicine, University of Nairobi, show that between 1969 and 1977, eighteen cases of salmonellosis in poultry were recorded. The most common Salmonella bacteria isolated were S. typhimurium and S. pullorum. Another common isolate was S. gallinarum. These findings are contrary to those of the Veterinary Research Laboratories, Kabete, Kenya, which show that the most prevalent Salmonella type in clinical cases of poultry in Kenya is S. gallinarum and that S. pullorum and S. typhimurium were but last reported in 1958 and 1973, respectively (Miringa, 1978).

Many surveys on the occurrence of avian salmonellosis have been done in various countries. To give but a few examples, Brown, Duff, Wilson and Ross (1973) isolated 93 salmonellae out of 7,725 birds surveyed in Scotland. These consisted of 11 serotypes of which S. typhimurium was the predominant one. Basu, Dewan and Suri (1975), working in India, found that out of a total of 744 Salmonella species isolated from poultry during 1958 to 1973, S. typhimurium was the most predominant isolate. Other frequently isolated salmonellae were S. saintpaul and S. gallinarum and/or S. pullorum. Wijffels and Dheedene (1976) observed S. pullorum infection in a large chick-rearing premise in Morocco.

Domestic poultry constitute a large reservoir of Salmonella organisms in nature. Moran, Van Houweling and Ellis (1965) reported that almost 80% of the salmonellae from animals serotyped in the United States were from chickens and turkeys. The natural close association of Salmonella bacteria with domestic poultry and poultry products, the important role that poultry products play in people's daily diets and the application of poultry products to a variety of other types of food products have led to the emphasis on the importance of these poultry products as a source of Salmonella infections in man. Other domestic animals do also serve as potential sources of human salmonellosis. Thus, the control of salmonellosis in animals has a direct bearing on the control of salmonellosis in man. Unpublished annual reports, 1969 to 1977, from the microbiology diagnostic laboratory, Kenyatta National Hospital, indicate that there is a significant occurrence of human salmonellosis in Kenya. Efforts to reduce the number of Salmonella bacteria in animals and animal products could contribute greatly to the reduction of these infections from the human population.

The above discussion shows that not only is salmonellosis important in chickens but it also is a public health hazard. A need to survey the occurrence of the disease in poultry in Kenya was necessary because such work had not been done before and, in addition, information obtained from such a survey would be invaluable in planning the control and/or eradication of salmonellosis.

REVIEW OF LITERATURE

HISTORY

The etiological agent of pullorum disease was discovered by Rettger in 1899 and he described the disease as fatal septicaemia of young chicks in 1900. The disease was also called white diarrhoea and bacillary white diarrhoea successively. However, in 1932, the term pullorum disease came into common usage (Van Roekel, 1952). Pullorum disease was recorded in Kenya (Annual Rep. Dept. Agric. Kenya, 1933), in Tanganyika (Lowe, 1932) and in Uganda (Mettam, 1932).

The etiological agent of fowl typhoid was first isolated in 1888 and was named Bacillus gallinarum by Klein (1889). Moore (1895) investigated the disease in Virginia and Maryland and named it infectious leukemia and the causative agent, Bacillus sanguinarium. Curtice (1902) studied the disease in Rhode Island and named it fowl typhoid. Fowl typhoid was reported to cause heavy losses in Kenya and Tanganyika (Lowe, 1932) and in Uganda (Hall, 1926). Fowl typhoid, also called Kikuyu disease, was first reported in Kenya by Montgomerie (1911 to 1912). Currently, the disease is known to occur throughout the world (Williams, 1972; 1978).

Salmonellae other than S. pullorum and S. gallinarum cause paratyphoid infections. These infections may have existed for many years but the early workers did not have the present-day methods available by which the isolated organisms could be positively identified. Mazza (1899) described a chicken epizootic that raged in various parts of Northern Italy. He isolated the causative organism which may have been a paratyphoid. Other workers, for example, Henning (1939), Buxton (1957), Khan (1970) and Shigidi (1973) described the disease in chickens and showed that paratyphoid infections were common among chickens and had a worldwide distribution.

THE BIOLOGY OF SALMONELLA BACTERIA.

Salmonellae are gram negative rods, closely related morphologically and physiologically to other genera of the family Enterobacteriaceae. They are usually motile, although non-motile forms occur. Most strains produce acid and gas from glucose, maltose, mannitol and sorbitol except S. typhi and S. gallinarum which produce acid only. Anaerogenic strains of normally gas-producing serotypes are found in nature. This is particularly so with S. dublin. Salmonellae do not ferment sucrose and salicin. Although, by definition, salmonellae are non-lactose-fermenters, lactose-fermenting species have been

isolated (Easterling, Johnson, Wohlhieter and Baron, 1969). Salmonellae do not form indole, do not coagulate milk or liquefy gelatin. Although the optimum temperature range for growth of Salmonella is 35 to 37°C, Salmonella senftenberg, strain 77W, possesses an unusual heat-resistant property. To inactivate it, 31 minutes at 57°C is required (Erskine and Margo, 1974).

In the environment, salmonellae can remain viable for a long time. Survival of 87 days in tap water, 115 days in pond water, 120 days in pasture soil, 280 days in garden soil, over 30 months in dried bovine manure, 28 months in naturally infected avian feces and 47 days in manure slurry have been reported (Erskine and Margo, 1974).

ISOLATION AND IDENTIFICATION

The presence of yeasts, moulds, protozoa and many different types of bacteria in the intestinal tracts of man and animals presents a complex problem in isolating salmonellae. Thus, a culture medium inoculated with a loopful of intestinal contents is normally a very mixed culture indeed. For this reason, to ensure that Salmonella bacteria, if present, can be isolated, special media have been devised for their

isolation. In a severe enteric Salmonella infection, it is not necessary that intestinal contents from such an animal be inoculated into a preliminary enrichment medium since it can be assumed that the population of salmonellae in such a specimen is high and that they should be fairly easy to isolate. However, there are times, such as when surveys of the occurrence of Salmonella are carried out, that the number of bacteria in the intestinal tract may be so low as to make it a prerequisite to first inoculate the samples into enrichment media. Media used for this purpose are Selenite Broth (SB) and Tetrathionate Broth (TB). If these are not available, Meat Infusion Broth (MIB) may be used.

Andrews, Wilson, Poelma and Romero (1977) compared methods for the isolation of Salmonella species from frogs' legs. They did not find a significant difference between numbers of salmonellae isolated through SB and TB. Harvey, Price and Morgan (1977) carried out a similar study, but in pigs, and confirmed the findings of Andrews et al. (1977). Jameson (1961) found that a secondary selective enrichment medium inoculated with a relatively large inoculum from the primary enrichment medium, increased the yield of salmonellae.

The commonly used selective media for salmonellae are Salmonella-Shigella Agar (SSA), Bismuth Sulfite Agar (BSA), Desoxycholate Citrate Agar (DCA) and MacConkey Agar (MCA). The selective media are not equally effective in the isolation of Salmonella bacteria. Andrews et al. (1977) compared the effectiveness of SSA, Brilliant Green Agar (BGA), BSA, Xylose Lysine Deoxycholate Agar (XLDA) and Hektoen Enteric Agar (HEA). They found that when SSA was streaked with material from either SB or TB, it gave significantly fewer Salmonella than the other four media. The use of XLDA and HEA as a supplement of SSA, BGA and BSA enhanced the recovery of salmonellae.

On solid media, salmonellae form small, smooth, glistening colonies with entire or slightly undulated edges. On MCA and SSA, the colonies are normally pale in colour, since salmonellae are normally non-lactose fermenters. Owing to the fact that SSA contains ferric ammonium citrate, the colonies may have a black centre due to formation of hydrogen sulfide (H_2S). DCA also contains ferric ammonium citrate, thus, Salmonella colonies are colourless, surrounded by a clear orange-yellow zone of medium and have black centres due to H_2S production. On BSA, salmonellae form typical discrete colonies

which are black and are surrounded by a black zone (due to H_2S production) which may be several times the size of the colony. By reflected light, this zone exhibits a characteristic metallic sheen.

Suspicious colonies are biochemically and immunologically investigated. These tests also aid in pin-pointing the species (Carter, 1975; Merchant and Packer, 1967; Cox and Williams, 1976 and Kauffmann, 1975).

SCREENING OF SERA FOR SALMONELLA ANTIBODIES

The use of the Tube Agglutination Test (TAT) for the detection of carriers of pullorum organisms was widely applied in the field by Jones (1913); Gage, Paige and Hyland (1914) and Rettger, Kirkpatrick and Jones (1914). This test served as a valuable guide in the control of pullorum disease. However, Rettger et al. (1915) tested 107 flocks consisting of 14,617 birds using the TAT. Their findings indicated that a single test was not sufficient to detect all the possible carriers in a flock.

Schaffer, McDonald, Hall and Bunyea (1931) reported the development of the Rapid Whole Blood Plate Test (RWBPT) in which stained antigen was employed. The test was widely used due to its simplicity. Many infected birds were detected.

The Rapid Serum Plate Test (RSPT) for detection of S. pullorum infection in chickens was developed by Runnells, Coon, Farley and Thorp (1927). Bierer and Vickers (1960) found the RSPT more efficient than either the TAT or the RWBPT in detecting S. typhimurium infected turkeys.

Williams and Whittemore (1971) described a Microagglutination Test (MAT) in which tetrazolium stained antigen was employed. This method in comparison to the TAT saved time, space and cost. However, there was no significant difference between results obtained with these two tests. Williams and Whittemore (1972) described the Microantiglobulin Test (MiAGT) for detecting S. typhimurium agglutinins. They found this test 16 times more sensitive than either the TAT or the MAT. Further work by Williams and Whittemore (1973, 1976a) confirmed their original findings.

Williams and Whittemore (1976b) compared five methods of detecting S. typhimurium antibodies in chickens. The most sensitive and reliable was the MiAGT. The TAT, MAT, RWBPT and RSPT were less reliable than the MiAGT. Wray, Morris and Sojka (1975) found that Indirect Haemagglutination Test (IHT) had no advantage over the serum agglutination tests for diagnosis of bovine salmonellosis caused by S. dublin.

The Wattle Test (WT) for carriers of pullorum disease was first applied and reported by Ward and Gallagher (1917). Later, it was extensively investigated by other workers (Bushnell and Brandly, 1929; Edwards and Hull, 1929; Michael and Beach, 1929; Rettger, McAlpine and Warne, 1930). Their findings showed that the method was not sufficiently reliable for practical use in the detection of carriers. However, Stefanov, Ivanov and Sizov (1974) compared the WT with the blood agglutination test and found that the WT, read 24 hours after injection of allergen, detected more infected birds than the blood agglutination test.

Other tests, such as the Precipitin Test (PT) and the Complement Fixation Test (CFT) were found either unreliable or impracticable in the detection of carriers (Edwards and Hull, 1929).

TRANSMISSION

Salmonella organisms can be transmitted in a number of ways. Egg transmission as a result of localisation of the organisms in the ovaries has been reported in S. pullorum and S. gallinarum infections (Snoeyenbos, 19 2; Pomeroy, 1972). Egg transmission

by shell localisation and penetration has been reported (Williams and Whittemore, 1967; Williams and Dillard, 1968a,b; 1969; Williams, Dillard and Gaye, 1968; Chowdhury, Bhattacharyya and Gupta, 1976).

Mallmann (1929) observed that when salmonellae were in organs of chicks, they were nearly always found in the intestinal contents. Hence, the excreta of an infected flock is very infective. Similar findings were reported by Kerr (1930) and Van Roekel, Bullis, Flint and Clarke (1932; 1935). Gwatkin and Mitchell (1944) found that the organisms could be transmitted by flies. Transmission through inhalation was reported by Bunyea and Hall (1929). Moore (1946) reported transmission by rats and turkey buzzards.

EPIDEMIOLOGY

While the chicken appears to be the natural host of S. pullorum and S. gallinarum, other avian species have also exhibited some degree of susceptibility. Mammals are variably susceptible. S. pullorum and S. gallinarum infections in man have been reported (Snoeyenbos, 1972; Pomeroy, 1972).

The paratyphoid organisms have been isolated from chickens as well as other avian species such as turkeys, guinea fowls, canaries, parrots, ducks

and pigeons, and also from rodents, crows, frogs, squabs, turtles, snakes, flies, bats, seagulls, beetles, German cockroaches, oysters and fish (Williams, 1972). The isolation of paratyphoid organisms from wild animals has been reported by Erskine and Margo (1974) and Fay (1972). Paratyphoid organisms have also been recovered from domestic animals (Tanaka, Katsube and Imaizumi, 1976; Amtsberg, Kirpal, Lindfeld, Flasshoff and Bisping, 1977; Sojka, Wray, Shreeve and Benson, 1977). Considering the wide range of hosts and the survival of the organisms in different environmental conditions, it can be appreciated that the control and eradication of such organisms present a difficult task.

MATERIALS AND METHODS

FAECAL SPECIMEN

A total of 906 faecal samples were collected from cloacae of randomly selected chickens in four farms and one slaughterhouse in the neighbourhood of the City of Nairobi. One hundred and ninety samples were collected from Farm I, 213 from Farm II, 100 from Farm III, 25 from Farm IV and 378 from the slaughterhouse. Sterile cotton swabs were used for the collection of the specimen. The swabs were placed in sterile universal bottles containing 15ml of sterile SB. These were immediately transported to the laboratory.

INTESTINAL CONTENTS

One hundred and eighty-nine intestinal-content samples were collected from birds which were being slaughtered in Farm IV and the slaughterhouse. Of these samples, 164 were collected from the slaughterhouse and 25 from Farm IV. The intestinal contents were put directly into sterile universal bottles containing 15ml of sterile SB and immediately transported to the laboratory.

TISSUE SPECIMEN

Some birds in farm IV had acute diarrhoea and were dying. Owing to this fact, full post-mortem examination of 25 birds randomly selected from the farm was done before tissue specimen were taken. The birds were stunned, dissected using sterile scissors and scapel blades and the organs removed aseptically. The specimen included the liver, bile, spleen, ovary, oviduct, heart blood and kidneys. They were placed in separate sterile petri-dishes and immediately transported to the laboratory.

SERA

Out of the 1,070 birds from which faeces and/or intestinal-content samples were obtained, 447 were also bled. These included 187 from farm I, 99 from farm III and 161 from the slaughterhouse. Some of the blood samples were collected by bleeding birds from the wing vein, while other samples were collected from birds being killed at the slaughter-house. The blood samples were incubated at 37^{o}C for 1 hour and subsequently refrigerated for 18 hours at 4^{o}C. The samples were then centrifuged at 750 Xg at room temperature for 10 minutes and each serum collected, put in 5ml vials and stored at -20^{o}C until required.

REAGENTS AND MEDIA

For details on reagents, media and their sources, see Appendix I.

PREPARATION OF BACTERIAL ANTIGENS

Chosen to represent *Salmonella* groups B, C, D, E and I were *S. paratyphi* (B), *S. cholerae-suis* (C), *S. gallinarum* (D), *S. senftenberg* (E) and *S. hull* (I). They were separately placed in nutrient broth and incubated for 24 hours at 37°C and under aerobic conditions. Two and a half millilitres of the broth cultures were then introduced into separate French square bottles containing sterile nutrient agar. The bottles were inverted and incubated aerobically at 37°C for 24 hours. The extra broth culture was poured off and the cells harvested by rolling sterile glass beads on the agar surface and washing with sterile saline (0.15 M sodium chloride solution). To harvest the bacteria, the washings were placed in universal bottles and centrifuged at 5,000 X g for 15 minutes at room temperature. The cells were washed with saline and then centrifuged at 5,000 Xg. This was repeated twice after which the cells were resuspended in a minimum volume of saline solution. They were then rendered nonviable by the addition of 20 volumes of 95% ethyl alcohol (analytical grade)

and heating the suspension at 60°C for 1 hour. The alcohol-treated suspension was harvested and washed 3 times with sterile saline. It was tested for viability by plating it on nutrient agar and incubating it aerobically at 37°C for 24 hours. There was no growth.

A McFarland nephelometer was prepared as shown in Table 1. Corresponding optical densities of the different tubes were determined using a Beckman Model DU-2 Spectrophotometer (Beckman Instruments GMBH, Munich, Germany) at a wavelength of 650 mu and using saline as the blank. A standard curve of optical density versus Salmonella concentration was drawn (Appendix IIa). The optical densities of the alcohol-treated suspensions were read and compared with those of the standard. Thus, their concentrations were determined (Appendix IIb). Each of the suspensions was then diluted to a concentration of 1.3×10^9 per ml, using the formula:

$$\frac{RV}{O}$$

Where R represents the required concentration of bacteria, V represents the volume required and O represents the original concentration of bacteria. The S. gallinarum suspension was used separately while the other suspensions were pooled in equal volumes.

TABLE 1. Preparation of McFarland nephelometer.

Tube number	ml 1% H_2SO_4	ml 1% $BaCl_2$	Approx. No. of cells[a] per ml
1	9.9	0.1	3×10^8
2	9.8	0.2	6×10^8
3	9.7	0.3	9×10^8
4	9.6	0.4	1.2×10^9
5	9.5	0.5	1.5×10^9
6	9.4	0.6	1.8×10^9
7	9.3	0.7	2.1×10^9
8	9.2	0.8	2.4×10^9
9	9.1	0.9	2.7×10^9
10	9.0	1.0	3×10^9

H_2SO_4 represents sulphuric acid.

$BaCl_2$ represents barium chloride.

a indicates that the optical density obtained by the mixture of H_2SO_4 and $BaCl_2$ is equal to the optical density shown by the corresponding number of bacterial cells suspended in saline.

The working dilution of the Salmonella antigen for TAT was determined by titration. Doubling dilutions of the Salmonella antigen were prepared in 6 tubes. The resulting dilutions were 1/5, 1/10, 1/20, 1/40, 1/80 and 1/160. To each tube, an equal volume of known polyvalent "O" Salmonella antiserum was added, thus, doubling the corresponding dilutions. These were mixed and incubated for 24 hours at 37°C. Any agglutination observed was scored following an arbitrary scale of 1+ to 4+. Distinct agglutination was assigned a value of 4+ and weak agglutination a value of 1+. The highest dilution to give an agglutination value of 3+ was taken as the working dilution. On this basis, the working dilution was 1/40.

PREPARATION OF CHICKEN GLOBULIN

Two chickens, shavers 579, obtained from the University of Nairobi, Poultry Unit and weighing 2kg each were used for the preparation of globulin.

The animals were inoculated with pooled Salmonella antigen composed of S. paratyphi B, S. cholerae-suis, S. gallinarum, S. senftenberg and S. hull, at a concentration of 9×10^8 cells per ml in order to boost the globulin fraction in

the serum. The inoculations were given at intervals and volumes as shown in Table 2. The increase in the globulin fraction was monitored by bleeding the chickens before each injection and the antibody titre to the Salmonella antigen screened with the TAT. The animals were bled terminally 8 days after the last injection and the sera collected. The chicken globulin was prepared according to Kabat and Mayer (1967). A rough estimate of the globulin concentration was made by refractometry using an Atago proteinometer and distilled water as the blank. The globulin concentration was approximately 0.5 mg/ml.

PREPARATION OF RABBIT ANTICHICKEN GLOBULIN SERUM

Two adult male New Zealand white rabbits, weighing 2kg each and obtained locally, were used for the preparation of antichicken globulin serum.

An emulsion consisting of 2 volumes of Freund's complete adjuvant (FCA) and 1 volume of undiluted chicken globulin was used. Four sets of injections were made at 10-day intervals. Details of the procedure are given in Table 3. The rabbits were bled just before the fourth set of injections and the presence of antichicken globulin antibodies detected by the Precipitin Ring Test (PRT) and Immuno-electrophoresis (IEP). Terminal bleeding for the

TABLE 2. Schedule of injections of pooled *Salmonella* antigen into chickens.

Day	Volume of antigen	Route of injection
0	0.1 ml	I.V.
2	0.2 ml	I.V.
4	0.4 ml	I.V.
6	0.8 ml	I.V.
8	1.6 ml	I.V.
10	3.2 ml	I.V.

I.V. indicates intravenous.

TABLE 3. Schedule of injections of chicken globulin into rabbits.

Day	Amount and route of injection
0	1 ml into each of the 4 footpads, ½ ml into 4 different sites, I.M.
10	½ ml into 6 different sites, I.D.
20	½ ml into 6 different sites, I.M.
30	½ ml into 6 different sites, I.D.

I.M. indicates intramuscular

I.D. indicates intradermal

preparation of sera was done 10 days after the final injection.

The working dilution of the rabbit antichicken globulin serum for the MaAGT was determined by titration. Chicken serum known to contain antibodies to Salmonella bacteria was analysed with the TAT and the reciprocal of the highest dilution showing agglutination (positive reaction) taken as the titre. The dilutions giving negative reactions probably contained monovalent antibodies. These could be detected by the MaAGT. In this case, the reciprocal of the 1/80 dilution was the titre, while 1/160 and 1/320 were considered negative. A dilution of 1/640 was taken as the sensitising dilution for the Salmonella bacteria.

Two millilitres of the chicken serum diluted 1/320 were mixed with 2ml of the pooled Salmonella antigen diluted 1/40. The final dilution of the chicken serum was 1/640. The mixture was incubated at 37°C for 30 minutes and then 0.5ml of the mixture was put into each of 7 test tubes labelled 1 to 7. The tubes were centrifuged at room temperature for 15 minutes at 5,000 Xg. The pellet which contained the bacteria was saved while the supernatant fluid was discarded. Bacteria were resuspended in 1ml of saline, washed and centrifuged at 5,000 Xg. The

supernatant fluid was discarded and the bacteria washed again with saline. After the third and final centrifugation, the supernatant fluid was discarded and bacteria resuspended in 0.1 ml saline.

Doubling dilutions of the rabbit serum were prepared in 6 tubes. The resulting dilutions were 1/5, 1/10, 1/20, 1/40, 1/80 and 1/160. Small volumes (0.1ml) of the diluted rabbit serum were transferred to corresponding tubes containing the antigen-antiserum mixture prepared earlier. The final mixtures were mixed thoroughly and incubated at 37^{o}C for 24 hours. Presence of agglutination indicated antibody reaction to Salmonella bacteria. The highest dilution to give distinct agglutination was the working dilution for the serum. This was 1/40.

A volume of 0.1 ml saline was added to the 7th tube containing antigen-antiserum mixture and this served as the control.

ISOLATION AND IDENTIFICATION OF SALMONELLA BACTERIA

(a) FAECES

In the laboratory, the bottles containing inoculated SB were incubated aerobically for 24 hours at 37°C before the isolation of the organisms was attempted. Loopfuls of materials from the incubated SB were plated onto MCA and SSA. These were incubated aerobically for 24 hours at 37°C. Then, lactose and non-lactose fermenters observed were gram-stained and those found gram-negative and rodshaped were biochemically investigated. The biochemical tests included reactions in Triple Sugar Iron Agar (TSIA) and BSA; urease production, indole production, methyl red test, Voges-Proskauer test, citrate utilisation, phenylalanine deamination, malonate utilisation and growth in 75% potassium cyanide (KCN). Fermentation tests using glucose, lactose, sucrose, mannitol, maltose and dulcitol broths were also carried out. Media were inoculated, aerobically incubated at 37°C for 18 to 24 hours and the results read according to Cowan and Steel (1965). Organisms which gave reactions characteristic of salmonellae were typed with polyvalent "O" Salmonella antiserum. Bacteria other than Enterobacteriaceae were investigated but not in detail.

(b) INTESTINAL CONTENTS

These were processed in the same manner as the faecal specimen after inoculation into SB.

(c) TISSUES

The tissues were individually processed. They were ground using sterile mortars and pestles and fine sand. This was to rupture the cells so as to release any intracellular organisms. Each of the ground tissues was introduced into sterile universal bottles containing 15 ml of sterile SB. The bile and heart blood were inoculated into SB. The inoculated SB bottles were incubated aerobically at 37°C for 24 hours after which loopfuls of materials from the SB bottles were inoculated into MCA and SSA. Then the same procedure given for isolation and characterisation of Salmonella from faeces was followed.

SEROLOGICAL TESTS

The procedure for the RWBPT was as given by Snoeyenbos (1972). Each bird, with the exception of those from the slaughterhouse and 2 from farm IV, had its blood tested using the RWBPT. One hundred and eighty-eight were from farm I, 213 from farm II, 100 from farm III and 23 from farm IV.

The Slide Agglutination Test (SAT) was carried out following the method given by Carpenter (1975). A total of 447 serum samples were analysed using SAT. One hundred and eighty-seven were from farm I, 99 from farm III and 161 from the slaughterhouse.

The TAT was carried out following the method given by Carpenter (1975). A total of 245 serum samples were analysed using TAT. Ninety-two were from farm I, 46 from farm III and 107 from the slaughterhouse.

The procedure used for the MaAGT was that of Coombs and Stoker (1951). Seven sera which were negative with the TAT and 11 positive with the TAT were randomly selected and analysed using MaAGT for comparative purposes.

PROCEDURE FOR PRECIPITIN RING TEST

The procedure was similar to Lancefield capillary tube test (Swift, Wilson and Lancefield, 1943) with modifications.

The undiluted chicken globulin suspension was drawn into a capillary tube up to half its length. The fluid on the outside of the capillary tube was wiped with tissue paper and the undiluted

rabbit serum drawn into the same tube until the tube was almost full, making sure that the fluid column in the tube was continuous. The capillary tube was placed on a plasticine board in the vertical position and incubated for 1 hour at room temperature. Appearance of a precipitin line around the interphase of globulin and rabbit serum indicated the presence of antichicken globulin activity in the rabbit serum. The control consisted of saline and chicken globulin.

This test was used to detect the presence of antichicken globulin antibodies in the rabbit serum.

PROCEDURE FOR IMMUNOELECTROPHORESIS

The Scheidegger's technique of immunoelectrophoresis as given by Yasuo Yagi (1971) was used with modification.

A 1% agarose solution was prepared by adding 2 gm of agarose in 150 ml distilled water while stirring. The mixture was heated until the agarose was completely dissolved. Then, 50 ml of 4 times concentrated Barb/Tris (Barbituric acid and hydroxymethyl-aminomethylamine) buffer at pH of 8.5 was added and mixed well.

Glass plates, 91 x 83 mm, were washed with soap and water, rinsed with acid-alcohol and dried. Fifteen millilitres of the 1% agarose solution were poured on the plate and evenly distributed. The agarose was then allowed to dry and solidify at room temperature.

Five wells were dug, 1.3 cm apart, in the agar using a well-cutter and a well and trough-directing device (LKB - Produkter AB, Sweden). Five microlitres of a mixture of bromophenol blue and whole rabbit serum in the ratio of 1:5 were put in the first well using a capillary tube and served as the albumin marker. Five-microlitre samples of undiluted chicken globulin were introduced into each of the other 4 wells. The test was run in quadruplicate.

The plate was placed in position in the LKB Bromma Model 1-2117-E01 electrophoresis unit (LKB - Produkter AB, Sweden). The buffer used was 0.5 M barbital acetate, pH 8.4. Power was turned on and maintained at 10 volts per centimetre of gel and a current of 35 to 40 milliamps for a period of 1 hour and 15 minutes.

The plate was removed from the electrophoresis unit and troughs cut 0.65 cm from the well and 1.3 cm apart using a scapel blade and the trough-directing

device. Then, approximately 0.15 ml undiluted rabbit serum was put in all the troughs using a Pasteur pipette. The plate was incubated at room temperature for 14 hours to allow the diffusion of the antiserum through the agar. It was then pressed using different weights ranging from 1 kg to 2.5 kg, starting with the lightest weight and increasing the weights by 0.5 kg successively after every 15 minutes. The 2.5 kg weight was left on the slide for 30 minutes.

After pressing, the plate was placed with the agarose side up in a bowl containing 0.15 M phosphate buffered saline (PBS) and left to wash for 14 hours. The agarose was left to dry and then stained with Ponceau S solution at room temperature for 14 hours. The precipitin lines were stained red. The surface of the plate was rinsed with double-distilled water and the plate placed into a bowl containing 3% glacial acetic acid for destaining. It was left there until the background was clear. The plate was dried at $37^{o}C$.

This test was used to detect the presence of antichicken globulin antibodies in the rabbit serum.

STATISTICAL ANALYSIS

Statistical analysis was done to compare the sensitivity of the different serological tests. In order to compare any two test procedures the 2 x 2 table was chosen. Since the procedures were carried out on the same serum samples, the usual Chi-square test could not be used in the analysis of the data obtained because it requires that the total number of observations of the full table be independent. The blood for the RWBPT was used separately but the same serum from each bird was divided into 3 parts apportioned respectively to each of the other three tests namely, SAT, TAT and MaAGT. Hence, the animal was being sampled thrice, that is, once by each test.

To overcome this problem, the McNemar's test for correlated proportions was used (Remington and Schork, 1970). The data were appropriately recast so as to count each serum only once. If we let the index value 1 denote positive, and 2 denote negative, then the proportion of positives to the first procedure is:

$$\frac{O_{11} + O_{12}}{n}$$

where O denote the observed data, and n the total number of observations. Similarly, the proportion

of positives with respect to the second procedure is:

$$\frac{O_{11} + O_{21}}{n}$$

Thus, the null hypothesis is:

$$\frac{O_{11} + O_{12}}{n} = \frac{O_{11} + O_{21}}{n}$$

and the alternative hypothesis is:

$$\frac{O_{11} + O_{12}}{n} \neq \frac{O_{11} + O_{21}}{n}$$

The test statistic is:

$$\chi^2 = \frac{(O_{12} - O_{21})^2}{O_{12} + O_{21}}$$

with 1 degree of freedom and tested at 5% level of significance.

The results were interpreted by comparing the χ^2 calculated with the χ^2 critical of 3.84. Any values less than 3.84 indicated that there was no significant difference between the two tests at that level of significance. Chi-square calculated values greater than 3.84 indicated a significant difference at that level of significance.

RESULTS

INTRODUCTION

In the present study the term "isolate" refers to the recovery of a particular organism from a particular specimen. For example, if a specimen is inoculated into 3 plates of SSA and 3 plates of MCA and organisms in the genus Salmonella are isolated from all the plates of SSA or MCA or if they are isolated from only 1 or a few of the plates, then they are taken as 1 isolate. If organisms of the same genus are isolated from another specimen then they are considered another isolate.

The percent values for isolates were calculated using the formula:

$$\frac{M}{n} \times 100$$

where M represents the number of isolates and n represents the number of specimens. In most cases, more than 1 organism was isolated from a specimen. Hence, the corresponding percentages add up to more than 100. This must be taken into account when assessing the results in Tables 4 to 15.

In the presentation of results in the text the percent isolates summarised in Tables 4 to 15, are given against each isolate in parenthesis.

In screening blood/sera for the presence of antibodies to S. pullorum and/or S. gallinarum, S. pullorum was used as the antigen for the RWBPT and S. gallinarum as the antigen for the SAT and TAT. In screening sera for the presence of antibodies to other salmonellae, the pooled Salmonella antigen, but without S. gallinarum, was used for SAT and TAT.

FARM I

i) BACTERIA ISOLATED FROM FAECAL SPECIMEN

One hundred and ninety faecal specimen were processed and from these 133 isolates were recovered (Table 4). No salmonellae were isolated. The isolates included E. coli (51.1%), Proteus spp. (6.8%), Aerobacter spp. (2.1%), Streptococcus spp. (5.3%) and Staphylococcus spp. (4.7%). E. coli formed the bulk of the isolates.

In Table 5 are shown bacterial isolates recovered from the 2 breeds in farm I, namely, Superwhites and Shavers 577. From the Superwhites, E. coli (59.5%)

TABLE 4. Bacteria isolated from 190 faecal specimen from farm I.

Genus	Number isolated	% of total
Salmonella	-	-
Escherichia	97	51.1
Proteus	13	6.8
Aerobacter	4	2.1
Streptococcus	10	5.3
Staphylococcus	9	4.7
No growth	55	29.0
Total isolates	133	

- indicates none isolated.

TABLE 5. Bacterial isolations from the different breeds in farm I.

	Breed			
	Super whites		Shavers 577	
Number of specimen	37		140	
Genus	Number isolated	% of total	Number isolated	% of total
Escherichia	22	59.5	75	53.6
Proteus	8	21.6	5	3.6
Aerobacter	-	-	4	2.9
Streptococcus	-	-	10	7.1
Staphylococcus	-	-	9	6.4
No growth	7	18.9	48	34.3
Total isolates	30		103	

- indicates none isolated.

and Proteus spp. (21.6%) were isolated. From Shavers 577, E. coli (53.6%), Proteus spp. (3.6%), Aerobacter spp. (2.9%), Streptococcus spp. (7.1%) and Staphylococcus spp. (6.4%) were isolated.

ii) SEROLOGICAL TESTS

One hundred and eighty-eight birds were tested using the RWBPT. None showed antibody activity to S. pullorum and/or S. gallinarum antigen. One hundred and eighty-seven serum samples were screened with the SAT and from these 4.3% were positive for antibody activity to S. pullorum and/or S. gallinarum. Ninety-two serum samples were analysed using the TAT. Of these, 81.5% showed antibody activity to S. pullorum and/or S. gallinarum.

When the 187 serum samples were tested with the SAT using the pooled Salmonella antigen (without S. gallinarum), 15.5% of the samples showed antibody activity. Of the 92 sera tested with the TAT using the pooled Salmonella antigen, 77.2% showed antibody activity (Table 6).

FARM II

i) BACTERIA ISOLATED FROM FAECAL SPECIMEN

The results of bacterial isolates recovered from faecal specimen obtained from farm II are given in Table 7. As observed in Tables 4 and 5, no salmonellae

TABLE 6. Results of the different serological tests - farm I.

	RWBPT		SAT		TAT	
	Sp/Sg	Other Salm.	Sp/Sg	Other Salm.	Sp/Sg	Other Salm.
Number of sera	188	ND	187	187	92	92
% positive	0	ND	4.3	15.5	81.5	77.2

Sp/Sg indicates S. pullorum and/or S. gallinarum.

Other Salm. means other salmonellae.

ND means "not done".

TABLE 7. Bacteria isolated from 213 faecal specimen from farm II.

Genus	Number isolated	% of total
Salmonella	-	-
Escherichia	98	46
Proteus	58	27.2
Aerobacter	12	5.6
Streptococcus	8	3.8
No growth	44	20.7
Total isolates	176	

- indicates none isolated.

were isolated from the 213 specimen investigated. E. coli (46%), Proteus spp. (27.2%), Aerobacter spp. (5.6%) and Streptococcus spp. (3.8%) were isolated. E. coli formed the bulk of the isolates.

Bacterial isolates recovered from the 3 breeds in farm II, namely, Shavers 577, Stabro and Shavers 579 are given in Table 8. From Shavers 577, E. coli (54.8%), Proteus spp. (5.5%), Aerobacter spp. (6.8%) and Streptococcus spp. (4.1%) were isolated. From Stabro, E. coli (58.6%), Proteus spp. (15.7%), Aerobacter spp. (7.1%) and Streptococcus spp. (4.3%) were isolated. From Shavers 579, E. coli (24.3%), Proteus spp. (61.4%), Aerobacter spp. (2.9%) and Streptococcus spp. (2.9%) were isolated.

Bacterial isolates recovered from male and female birds from farm II are given in Table 9. From the males, E. coli (52.3%), Proteus spp. (18.7%), Aerobacter spp. (8.4%) and Streptococcus spp. (3.7%) were isolated. From the females, E. coli (39.6%), Proteus spp. (35.9%), Aerobacter spp. (2.8%) and Streptococcus spp. (3.8%) were isolated. In Table 10 are shown bacterial isolates recovered from male and female birds of the corresponding breeds in farm II. In Shavers 577, E. coli (59.5%), Proteus spp. (5.4%) and Aerobacter spp. (8.1%) were isolated from the males, while E. coli (50%), Proteus spp. (5.6%), Aerobacter spp. (5.6%) and Streptococcus spp. (8.3%) were isolated from the females. In Stabro,

TABLE 8. Bacteria isolated from the different breeds in farm II.

	Breed					
	Shavers 577		Stabro		Shavers 579	
Number of specimen	73		70		70	
Genus	Number isolated	% of total	Number isolated	% of total	Number isolated	% of total
Escherichia	40	54.8	41	58.6	17	24.3
Proteus	4	5.5	11	15.7	43	61.4
Aerobacter	5	6.8	5	7.1	2	2.9
Streptococcus	3	4.1	3	4.3	2	2.9
No growth	22	30.1	14	20.0	8	11.4
Total isolates	52		60		64	

TABLE 9. Bacteria isolated from male and female birds from farm II.

	Male		Female	
Number of specimen	107		106	
Genus	Number isolated	% of total	Number isolated	% of total
Escherichia	56	52.3	42	39.6
Proteus	20	18.7	38	35.9
Aerobacter	9	8.4	3	2.8
Streptococcus	4	3.7	4	3.8
No growth	20	18.7	24	22.6
Total isolates	89		87	

TABLE 10. Bacteria isolated from male and female birds in the 3 breeds - farm II.

	Breed											
	Shavers 577				Stabro				Shavers 579			
	Male		Female		Male		Female		Male		Female	
Number of specimen	37		36		35		35		35		35	
Genus	Number isolated	% of total	Number isolated	% of total	Number isolated	% of total	Number isolated	% of total	Number isolated	% of total	Number isolated	% of total
Escherichia	22	59.5	18	50.0	21	60.0	20	57.1	14	40.0	3	8.6
Proteus	2	5.4	2	5.6	7	20.0	4	11.4	13	37.1	30	85.7
Aerobacter	3	8.1	2	5.6	4	11.4	1	2.9	2	5.7	-	-
Streptococcus	-	-	3	8.3	2	5.7	1	2.9	2	5.7	-	-
No growth	10	27.0	12	33.3	4	11.4	10	28.6	6	17.1	2	5.7
Total isolates	27		25		34		26		31		33	

- indicates none isolated.

E. coli (60%), Proteus spp. (20%), Aerobacter spp. (11.4%) and Streptococcus spp. (5.7%) were isolated from the males, while E. coli (57.1%), Proteus spp. (11.4%), Aerobacter spp. (2.9%) and Streptococcus spp. (2.9%) were isolated from the females. In Shavers 579, E. coli (40%), Proteus spp. (37.1%), Aerobacter spp. (5.7%) and Streptococcus spp. (5.7%) were isolated from the males, while E. coli (8.6%) and Proteus spp. (85.7%) were isolated from the females.

ii) SEROLOGICAL TESTS

A total of 213 birds were screened with the RWBPT and of these 45.1% were positive for antibody activity to S. pullorum and/or S. gallinarum.

FARM III

i) BACTERIA ISOLATED FROM FAECAL SPECIMEN

One hundred and fifty-three isolates were recovered from 100 faecal specimen (Table 11). No salmonellae were isolated. The isolates included E. coli (66%), Proteus spp. (24%), Aerobacter spp. (2%), Streptococcus spp. (6%), Staphylococcus spp. (8%), Citrobacter spp. (20%) and Pseudomonas spp. (8%).

TABLE 11. Bacteria isolated from 100 faecal specimen from farm III.

Genus	Number isolated	% of total
Salmonella	-	-
Escherichia	66	66
Proteus	24	24
Aerobacter	2	2
Streptococcus	6	6
Staphylococcus	8	8
Citrobacter	20	20
Pseudomonas	8	8
Unclassified	19	19
No growth	3	3
Total isolates	153	

- indicates none isolated.

ii) SEROLOGICAL TESTS

One hundred birds were tested using the RWBPT and from these 80% were positive for antibody activity to S. pullorum and/or S. gallinarum. Ninety-nine serum samples were screened with the SAT and 3% of the samples were positive for antibody activity to S. pullorum and/or S. gallinarum. Forty-six sera were tested using the TAT and of these 65.2% showed antibody activity to S. pullorum and/or S. gallinarum.

When the 99 serum samples were tested with the SAT using the pooled Salmonella antigen (without S. gallinarum), 14.1% of the samples showed antibody activity. Of the 46 sera tested with the TAT using the pooled Salmonella antigen, 71.7% showed antibody activity (Table 12).

FARM IV

i) BACTERIA ISOLATED FROM FAECAL SPECIMEN

Twenty-five faecal specimen were processed and from these 32 isolates were recovered (Table 13). Salmonella gallinarum was isolated from 2 specimen. These constituted 8% of the total specimen studied. Other isolates were E. coli (88%), Proteus spp. (4%), Aerobacter spp. (8%) and Staphylococcus spp. (16%).

TABLE 12. Results of the different serological tests - farm III.

	RWBPT		SAT		TAT	
	Sp/Sg	Other Salm.	Sp/Sg	Other Salm.	Sp/Sg	Other Salm.
Number of sera	100	ND	99	99	46	46
% positive	80	ND	3.0	14.1	65.2	71.7

Sp/Sg indicates S. pullorum and/or S. gallinarum

Other salm. means other salmonellae

ND means "not done"

TABLE 13. Bacteria isolated from 25 faecal specimen from farm IV.

Genus	Number isolated	% of total
Salmonella	2	8
Escherichia	22	88
Proteus	1	4
Aerobacter	2	8
Staphylococcus	4	16
Unclassified	1	4
No growth	2	8
Total isolates	32	

ii) BACTERIA ISOLATED FROM INTESTINAL CONTENTS

The results of bacterial isolates recovered from the 25 intestinal-content samples obtained from farm IV are given in Table 14. Salmonella gallinarum was isolated from 1 specimen. Other isolates included E. coli (60%), Proteus spp. (4%), Aerobacter spp. (12%), Streptococcus spp. (44%) and Staphylococcus spp. (32%).

iii) BACTERIA ISOLATED FROM TISSUES

The results of bacterial isolates recovered from the different tissues obtained from the 25 birds from farm IV are shown in tables 15a and b. Twenty-five of each of the bile, spleen, ovary and liver specimen were studied. From each specimen, 4 S. gallinarum isolates were recovered. This constituted 16% of the total number of tissue samples processed. Five kidneys were investigated and S. gallinarum was isolated from 4. Salmonella gallinarum was also isolated from all the 4 heart-blood and 4 oviduct specimen. Other isolates included E. coli (20%) and Staphylococcus spp. (20%) from bile; E. coli (16%), Proteus spp. (4%) and Staphylococcus spp. (32%) from spleen; E. coli (36%), Proteus spp. (4%), Aerobacter spp. (4%), Streptococcus spp. (4%), Staphylococcus spp. (12%) and Citrobacter spp. (4%) from ovaries; E. coli (60%), Aerobacter spp. (12%) and Staphylococcus spp. (76%) from liver; E. coli (60%), Proteus spp. (20%) and Staphylococcus spp. (40%) from

TABLE 14. Bacteria isolated from 25 intestinal-content samples from farm IV.

Genus	Number isolated	% of total
Salmonella	1	4
Escherichia	15	60
Proteus	1	4
Aerobacter	3	12
Streptococcus	11	44
Staphylococcus	8	32
Unclassified	2	8
No growth	2	8
Total isolates	41	

TABLE 15a. Bacteria isolated from bile, spleen, ovaries and liver - farm IV.

	Bile		Spleen		Ovaries		Liver	
Number of specimen	25		25		25		25	
Genus	Number isolated	% of total	Number isolated	% of total	Number isolated	% of total	Number isolated	% of total
Salmonella	4	16	4	16	4	16	4	16
Escherichia	5	20	4	16	9	36	15	60
Proteus	-	-	1	4	1	4	-	-
Aerobacter	-	-	-	-	1	4	3	12
Streptococcus	-	-	-	-	1	4	-	-
Staphylococcus	5	20	8	32	3	12	19	76
Citrobacter	-	-	-	-	1	4	-	-
Unclassified	1	4	4	16	3	12	-	-
No growth	13	52	12	48	11	44	-	-
Total isolates	15		21		23		41	

- indicates none isolated.

TABLE 15b. Bacteria isolated from kidneys, heart blood and oviducts - farm IV.

	Kidneys		Heart blood		Oviduct	
Number of specimen	5		4		4	
Genus	Number isolated	% of total	Number isolated	% of total	Number isolated	% of total
Salmonella	4	80	4	100	4	100
Escherichia	3	60	2	50	2	50
Proteus	1	20	1	25	1	25
Staphylococcus	2	40	-	-	2	50
Total isolates	10		7		9	

- indicates none isolated.

kidney; E. coli (50%) and Proteus spp. (25%) from heart-blood; E. coli (50%), Proteus spp. (25%) and Staphylococcus spp. (50%) from the oviducts.

iv) SEROLOGICAL TESTS

A total of 23 birds were screened with RWBPT and of these 95.7% were positive for antibody activity to S. pullorum and/or S. gallinarum.

SLAUGHTERHOUSE

i) BACTERIA ISOLATED FROM FAECAL SPECIMEN

Four hundred and nineteen isolates were recovered from 378 faecal specimen investigated (Table 16). Salmonellae were isolated from 2 specimen. These constituted 0.5% of the total number of faecal specimen studied. The isolates belonged to group B. Other isolates included E. coli (81.5%), Proteus spp. (17.7%), Aerobacter spp. (2.1%), Streptococcus spp. (4.2%) and Staphylococcus spp. (4.8%).

ii) BACTERIA ISOLATED FROM INTESTINAL CONTENTS

The results of bacterial isolates recovered from intestinal-content samples obtained from the slaughter-house are given in Table 17. Salmonella bacteria were isolated from 1 specimen. This constituted 0.6% of the total number of intestinal-content specimen studied. Unlike those isolates obtained from faeces, these belonged to group E. Other isolates included E. coli

TABLE 16. Bacteria isolated from 378 faecal specimen from the slaughterhouse.

Genus	Number isolated	% of total
Salmonella	2	0.5
Escherichia	308	81.5
Proteus	67	17.7
Aerobacter.	8	2.1
Streptococcus	16	4.2
Staphylococcus	18	4.8
No growth	14	3.7
Total isolates	419	

TABLE 17. Bacteria isolated from 164 intestinal-content samples from the slaughterhouse.

Genus	Number isolated	% of total
Salmonella	1	0.6
Escherichia	119	72.6
Proteus	80	48.8
Aerobacter	5	3.1
Streptococcus	25	15.2
Staphylococcus	31	18.9
No growth	5	3.1
Total isolates	261	

(72.6%), Proteus spp. (48.8%), Aerobacter spp. (3.1%), Streptococcus spp. (15.2%) and Staphylococcus spp. (18.9%).

iii) SEROLOGICAL TESTS

One hundred and sixty-one serum samples were tested using the SAT and of these 10.6% were positive for antibody activity to S. pullorum and/or S. gallinarum. One hundred and seven serum samples were tested using the TAT and 76.6% of the samples showed antibody activity to S. pullorum and/or S. gallinarum.

When the 161 serum samples were tested with the SAT using the pooled Salmonella antigen (without S. gallinarum), 25.5% of the samples showed antibody activity. Of the 107 sera tested with the TAT using the pooled Salmonella antigen, 66.4% showed antibody activity (Table 18).

In Table 19a are shown the MaAGT titres for the 7 TAT-negative sera. All the sera were positive for Salmonella antibody activity with the MaAGT. Of the 7 sera tested, 4 showed titres of 320, one showed a titre of 640 and the remaining 2 showed titres of 1280 and 20,480, respectively.

In Table 19b, the TAT and MaAGT titres are juxtaposed for the 11 TAT-positive sera. In general, higher titres were obtained with the MaAGT than with

TABLE 18. Results of the different serological tests - slaughterhouse.

	SAT		TAT	
	Sp/Sg	Other Salm.	Sp/Sg	Other Salm.
Number of sera	161	161	107	107
% positive	10.6	25.5	76.6	66.4

Sp/Sg means S. pullorum and/or S. gallinarum

Other salm. means other salmonellae.

TABLE 19a. MaAGT results for the seven TAT-negative sera.

Serum number	MaAGT titre
1	320
2	1280
3	320
4	640
5	20,480
6	320
7	320

TABLE 19b. MaAGT and TAT titres for the eleven TAT-positive sera.

Serum number	TAT titre	MaAGT titre
1	80	320
2	80	2560
3	160	320
4	20	320
5	640	5120
6	80	20,480
7	320	320
8	80	320
9	20	320
10	80	640
11	20	320

Titres are given as reciprocals of the highest dilution which agglutinated.

the TAT. The highest MaAGT titre was 256 times its TAT titre. However, the lowest MaAGT titre was equal to the TAT titre.

GROSS PATHOLOGY OF THE SLAUGHTERED BIRDS FROM FARM IV

Post mortem examination showed that all the chickens had diarrhoea. Their ovaries were pedunculated. Some had ruptured, some were atrophic, others hemorrhagic and a few were well-developed. Some birds had free egg yolk in the abdominal cavity and this led to varying degrees of egg peritonitis. Most of the chickens had well-developed eggs in the oviducts, except three which had atrophic and necrotic ovaries with no signs of egg-laying. Their livers and spleens were slightly enlarged and had petechiae. Some had necrotic foci of varying sizes on their livers and spleens. The kidneys were also enlarged and their air-sacs were cloudy. Their hearts were normal.

STATISTICAL ANALYSIS

The RWBPT, SAT and TAT were compared for their sensitivity using McNemar's test for correlated proportions. This was done by comparing two tests at a time at the 5% level of significance. The χ^2 critical at the 5% level of significance is 3.84. Any values greater than 3.84 indicated a significant difference between the tests compared while values

less than 3.84 indicated that there was not a significant difference between the two tests.

The sensitivities of the three tests are compared in Table 20. When the sera were screened for antibody activity to S. pullorum and/or S. gallinarum, the RWBPT and the TAT detected more positives than the SAT (χ^2 = 52.5 and 160.2, respectively), and the TAT detected more positives than the RWBPT (χ^2 = 54.8). The TAT also detected more positives than the SAT when screening the sera for antibody activity to other salmonellae (χ^2 = 105.6).

TABLE 20. Comparison of the 3 serological tests using McNemar's test for correlated proportions.

A *S. pullorum* and/or *S. gallinarum*

i) RWBPT vs SAT

		RWBPT +	RWBPT -	Total
SAT	+	1	10	11
	-	78	160	238
Total		79	170	249

$\chi^2 = 52.5$

ii) RWBPT vs TAT

		RWBPT +	RWBPT -	Total
TAT	+	18	87	105
	-	13	20	33
Total		31	107	138

$\chi^2 = 54.8$

iii) SAT vs TAT

		SAT +	SAT -	Total
TAT	+	15	169	184
	-	3	66	69
Total		18	235	253

$\chi^2 = 160.2$

B Other salmonellae

i) SAT vs TAT

		SAT		
		+	-	Total
TAT	+	36	152	188
	-	18	51	69
Total		54	203	257

$\chi^2 = 105.6$

D I S C U S S I O N

This study was designed to survey the occurrence of salmonellosis in 4 farms and a slaughterhouse in the neighbourhood of Nairobi. They were surveyed using bacteriological and serological methods. Bacteriological isolations indicated that there was an apparent low occurrence of Salmonella infection in birds from the farms and slaughterhouse, but serologically there was a high occurrence of salmonellosis.

The recovery of 4 Salmonella isolates from the 906 faecal specimen studied indicated an occurrence of 0.4%. However, 37.8% of the 524 sera screened with the RWBPT showed antibody activity to S. pullorum and/or S. gallinarum. Of the sera analysed using SAT and TAT, 6.3% and 76.3% showed antibody activity to S. pullorum and/or S. gallinarum, respectively. However, of the sera analysed using SAT and TAT, 18.8% and 71.4% showed antibody activity to other salmonellae, respectively. This observation is in agreement with the findings of Williams and Whittemore (1976b) who reported that the cloacal-swab method was inadequate for the detection of salmonellae in chickens.

The failure to isolate Salmonella bacteria from the cloacal swabs could be explained by the fact that the excretion of Salmonella from infected birds is intermittent (Magwood and Bigland, 1962; Brownell, Sadler and Fanelli, 1969; Smith, Larkin and Brooksbank, 1972; Brown, Ross and Smith, 1975). Thus, if one does not detect Salmonella bacteria with the cloacal-swab method it does not mean that an animal is completely free from Salmonella. Smith et al. (1972) dosed 12 birds with S. virchow and found that in 2 out of the 12 birds S. virchow was never detected using cloacal-swab monitoring. However, serologically, a continuous increase in antibody activity to S. virchow was detected in the 2 birds. Similar findings were reported by Brown et al. (1975). They experimentally infected 34 cockerels with S. typhimurium and found that in 2 of the cockerels S. typhimurium was never detected using the cloacal-swab method. However, the organism was recovered from tissues of the 2 cockerels at necropsy.

The shedding of salmonellae is influenced by muscular fatigue, cold, heat, wetness, limitation of food and water and concurrent infection (Brownell et al., 1969). This suggests that perhaps the

chickens surveyed in the present work were in their non-shedding state, and since they were not adequately stressed to induce excretion of the organisms, Salmonella bacteria were not present in the faecal swabs. Brown et al. (1975) found that cloacal excretion of S. typhimurium occurred during the first 5 days of infection, after which the excretion rate dropped considerably. Williams and Whittemore (1976b) had similar findings.

The ability of Salmonella bacteria to reside within macrophages, as reviewed by Campbell (1976), offers another possible explanation for the failure to isolate salmonellae from faeces. It is possible that at the time of swabbing most of the organisms were located intracellularly and few free salmonellae were present to be excreted. The fact that Salmonella serotypes react differently to various culture media (Harvey and Price, 1975), could further explain the failure to isolate salmonellae from faeces. However, in a preliminary experiment, inhibition of growth of Salmonella bacteria by selenite broth was found negligible (Appendix III).

Two Salmonella isolates were recovered from the 189 intestinal-content samples studied. This constituted 1.1%. The duodenal loop was investigated. It is interesting to note that, even though there

were only a few Salmonella isolates from the slaughterhouse, those isolated from faeces belonged to group B while those isolated from intestinal contents belonged to group E.

The post-mortem findings in the 25 birds from farm IV were consistent with fowl typhoid infection. This was confirmed by the isolation of S. gallinarum from the organs. However, as in the other farms, isolates from faeces and intestinal contents of these birds were recovered from very few specimen (8% of faecal specimen and 4% of intestinal-content specimen). The isolation of salmonellae from the kidneys suggests that both the kidneys and the gastro-intestinal tract feed the cloaca with the organisms. Indeed, in human beings, salmonellae have been isolated from urine and faeces (Sleigh, 1975).

In general, the TAT was more sensitive than the RWBPT (Table 20). This finding is in agreement with that of Williams and Whittemore (1976b). On the other hand, the RWBPT was more sensitive than the SAT.

The RWBPT is widely used for the detection of antibody activity to S. pullorum and/or S. gallinarum. Smith et al. (1972) infected 12 birds with S. virchow and 12 with S. bredeney and monitored the infection using serological and cloacal-swab methods. They

found that 4 of the 12 birds infected with S. virchow and 1 of the 12 infected with S. bredeney did not show antibody activity to the respective Salmonella sp. when tested with the RWBPT. However, these birds had significant antibody titres when they were tested with the indirect haemagglutination test. In these same birds, Salmonella spp. were isolated from the faeces. In the same study, it was observed that the RWBPT first detected agglutinins between days 8 and 11 post-infection. It subsequently detected positives for approximately 24 days after which its reliability decreased considerably. The reduced reliability was due to low antibody titres.

Farms II, III and IV have a deep-litter system. This creates a favourable environment for Salmonella cross infections should they be present. Thus, the chances of constant Salmonella antigenic stimulation in birds are high. Consequently, birds in farms II, III and IV could have high antibody titres. In contrast to this, in farm I, the wire-mesh system is used. Since droppings do not normally come into contact with the birds, cross infections are minimised. In such a situation, there would be less Salmonella antigenic stimulation in the population. Consequently, the antibody titres in these birds could be low.

Birds with high antibody titres could easily be detected with the RWBPT whereas those with low titres would not be readily detected with this test (Smith et al., 1972). This probably explains why there were a number of birds showing antibody activity to Salmonella bacteria in farms II, III and IV and not in farm I.

In Salmonella infections, ovarial transmission and shell penetration have been reported (Williams and Whittemore, 1967; Williams and Dillard, 1968a, 1968b, 1969; Williams et al.,1968; Snoeyenbos, 1972; Pomeroy, 1972; Chowdhury, Bhattacharyya and Gupta, 1976). Under these conditions, day-old chicks could easily be infected and hence, would carry the organisms to a new premise. In connection with this, it is interesting to note that farm III obtains its day-old chicks from farm II and that birds from both farms showed high Salmonella antibody activity with the RWBPT. However, farm I also obtains some of its birds from farm II and yet these birds did not show Salmonella antibody activity with the RWBPT. It should be emphasised that the majority of these birds gave positive reactions with the TAT (Table 6). It was expected that birds in farm I would have yielded the same RWBPT results as those in farms II and III. The differences in the RWBPT results of farm I and those of farms II and III are probably

due to the housing systems in these farms.

The ability of the TAT to dependably detect infected chickens has been questioned since it may not be able to detect intestinal infections (Yamamoto, Kilian, Babcock and Dickinson, 1962; Olesiuk, Carlson, Snoeyenbos and Smyser, 1969). Smith et al. (1972) found titres of 20 to Salmonella "O" antigen in known non-infected chickens. Similarly, Hall, Jones, Aitken and Parsons (1978), working on experimental S. dublin infection in cattle, found titres upto 80 to somatic and flagellar antigens in uninfected heifers. The possibility of cross-reactivity with other members of the family Enterobacteriaceae cannot be ruled out. Experience showed that polyvalent "O" Salmonella antiserum also agglutinated with E. coli, Proteus spp., Aerobacter spp. and Citrobacter spp. (Appendix IV). This limits its use in the serological identification of Salmonella bacteria. Cowan (1974) reported the presence of common enterobacterial antigens. This could explain the cross-reactivity since it occurred strictly between organisms of the family Enterobacteriaceae. Organisms not in this family, for example Pseudomonas bacteria, did not agglutinate with the antiserum.

Since agglutinating antibody titres in the sera fluctuate considerably (Gordon and Garside, 1944; Karlshoj and Szabo, 1949; Blaxland and Blowers, 1951) lack of agglutination cannot be taken as conclusive evidence that the animals are not infected with Salmonella bacteria. Sera could also show negative antibody activity with the TAT as a result of presence of "incomplete" antibodies (Coombs and Stoker, 1951).

Of the two agglutination tests, namely, the TAT and the SAT, the TAT was more sensitive than the SAT. This was expected since the SAT requires a higher antibody titre to show agglutination than the TAT.

The MaAGT results support those of Williams and Whittemore (1972, 1976a, 1976b) that the MaAGT is superior to the TAT in detecting positives. Coombs and Stoker (1951), working on detection of antibodies in Q-fever, found that the MaAGT could detect titres 64 times as high as those detected by the TAT. In the same study, the majority of patients whose sera did not show antibody activity with the TAT had titres up-to 160 with the MaAGT. In the present work, the observations are similar. However, it is surprising that one serum sample which did

not show any Salmonella antibody activity with the TAT had a titre of 20,480 with the MaAGT. This could be possible if the animal in question had an abnormal synthesis or breakdown of antibodies resulting in many univalent antibody fragments.

As expected, in faeces, E. coli were recovered more often than the other bacteria. Similarly, from intestinal contents and tissues, E. coli were recovered more often than the other organisms. The percentage variations in the isolation of E. coli could have been influenced by environmental conditions. Although E. coli are part of the normal flora of the gastro-intestinal tract of apparently healthy chickens, they can be pathogenic. They cause enteritis, respiratory disease, salpingitis, peritonitis, hepatitis, pericarditis and joint infection in chickens. Following faecal contamination of egg shells, E. coli can infect embryos and may cause death during incubation or during the first week following hatching. Infection of the embryo may lead to stunted growth (Gross and Domermuth, 1975).

Proteus bacteria were isolated in high proportions. The most common species isolated from faeces were P. mirabilis. In a few cases, P. vulgaris were also isolated. Proteus spp. cause urinary and gastro-intestinal infections in animals and man (Carter, 1975).

Aerobacter aerogenes were isolated. They are generally not pathogenic. However, they have been implicated in cystitis in man and dog and in bovine mastitis (Merchant and Packer, 1967).

Staphylococci and streptococci are part of the normal flora of the gastro-intestinal tract and other organs of man and animals (Merchant and Packer, 1967). Staphylococcus aureus causes wound infections, omphalitis, spondylitis, bacterial endocarditis, synovitis and arthritis in chickens. It also causes bumble foot in heavier breeds of chickens. Streptococcus spp. cause septicaemia, arthritis, salpingitis, endocarditis, pericarditis, perihepatitis and misshapen ova in chickens (Domermuth and Gross, 1975).

Citrobacter freundii were isolated from chickens in farm III. The chickens had acute diarrhoea and were dying. However, according to Carter (1975), Citrobacter spp. are not important as pathogens in animals.

Pseudomonas aeruginosa were isolated from farm III. This species causes wound infections in animals and septicaemia in chickens (Carter, 1975).

Poultry and poultry products are a significant source of protein in Kenya. As previously pointed out in this work, poultry carcasses and poultry products may be contaminated with Salmonella bacteria. Unpublished annual reports (1969 to 1977) from the microbiology diagnostic laboratory of the Kenyatta National Hospital show that S. typhimurium constitutes the highest percentage of the total Salmonella isolates recovered from human patients. It is likely that some of the patients had consumed contaminated poultry products and were consequently infected.

This study has indicated the presence of salmonellosis in the farms and slaughterhouse surveyed. Salmonella gallinarum was isolated from sick birds. Salmonellae in groups B and E were isolated from apparently healthy birds. Others (Report, 1969 to 1978; Miringa, 1978; Appel, 1978) isolated salmonellae from clinical specimen from poultry. The main species isolated was S. gallinarum. Salmonella pullorum and S. typhimurium were also isolated.

In the present work, bacteriological studies have indicated that there is an apparent low occurrence of Salmonella in chickens in Kenya. However, serological results indicate that there is a high occurrence of salmonellosis. This study, by its design, has been limited to a few farms and one slaughterhouse.

Consequently, one cannot claim that it gives a complete picture of the occurrence of salmonellosis in Kenya. Many more birds from representative areas need to be surveyed.

APPENDIX I

Materials and Sources

A. Chemicals and other reagents

i) The following chemicals of reagent grade and prepared by the British Drug House (B.D.H.) Ltd., England, were obtained from Howse and McGeorge Ltd., Nairobi:

Alpha-naphthol
Amyl alcohol
4-dimethylamino benzaldehyde
Ethyl alcohol
Ferric chloride
Glacial acetic acid
Hydrochloric acid
Methyl red indicator powder
Phenol red
Potassium cyanide
Potassium dihydrogen phosphate
Potassium hydroxide
Ponceau S
Sodium azide
Sodium chloride

ii) Bromophenol blue was obtained from Sigma Chemical Co., St. Louis, U.S.A.

iii) The following chemicals were obtained from Koch-Light Laboratories Ltd., Coinbrook, England:

Barbituric acid

Hydroxymethyl-methylamine (Tris)

Sodium acetate

Sodium barbital

iv) Agarose was obtained from Litex-Denmark.

B. Media

i) The following media prepared by Oxoid Ltd., London, were obtained from E.T. Monks Ltd., Nairobi:

Agar

Bismuth sulphite agar

Glucose phosphate peptone water

MacConkey agar

Nutrient agar

Salmonella-Shigella agar

Selenite broth

Simmon's citrate medium

Triple sugar iron agar

ii) The following media prepared by Difco Laboratories, U.S.A., were obtained from E.T. Monks Ltd., Nairobi:

Malonate broth

Phenylalanine agar

Potassium cyanide broth base

iii) The following media prepared by B.D.H. Laboratories, England, were obtained from Howse and McGeorge Ltd., Nairobi:

D-glucose
Dulcitol
Lactose
Maltose
Mannitol
Peptone
Sucrose

C. Sera

The following sera prepared by Wellcome Laboratories, England, were obtained from E.T. Monks Ltd., Nairobi:

Salmonella polyvalent "O" antiserum
Salmonella factor sera including factors:
2-0; 3, 10, 15, 19-0; 4-0; 5-0;
6, 7-0; 8-0; 9-0.
Salmonella polyvalent "H" antiserum
(specific and non-specific)

D. Other Materials

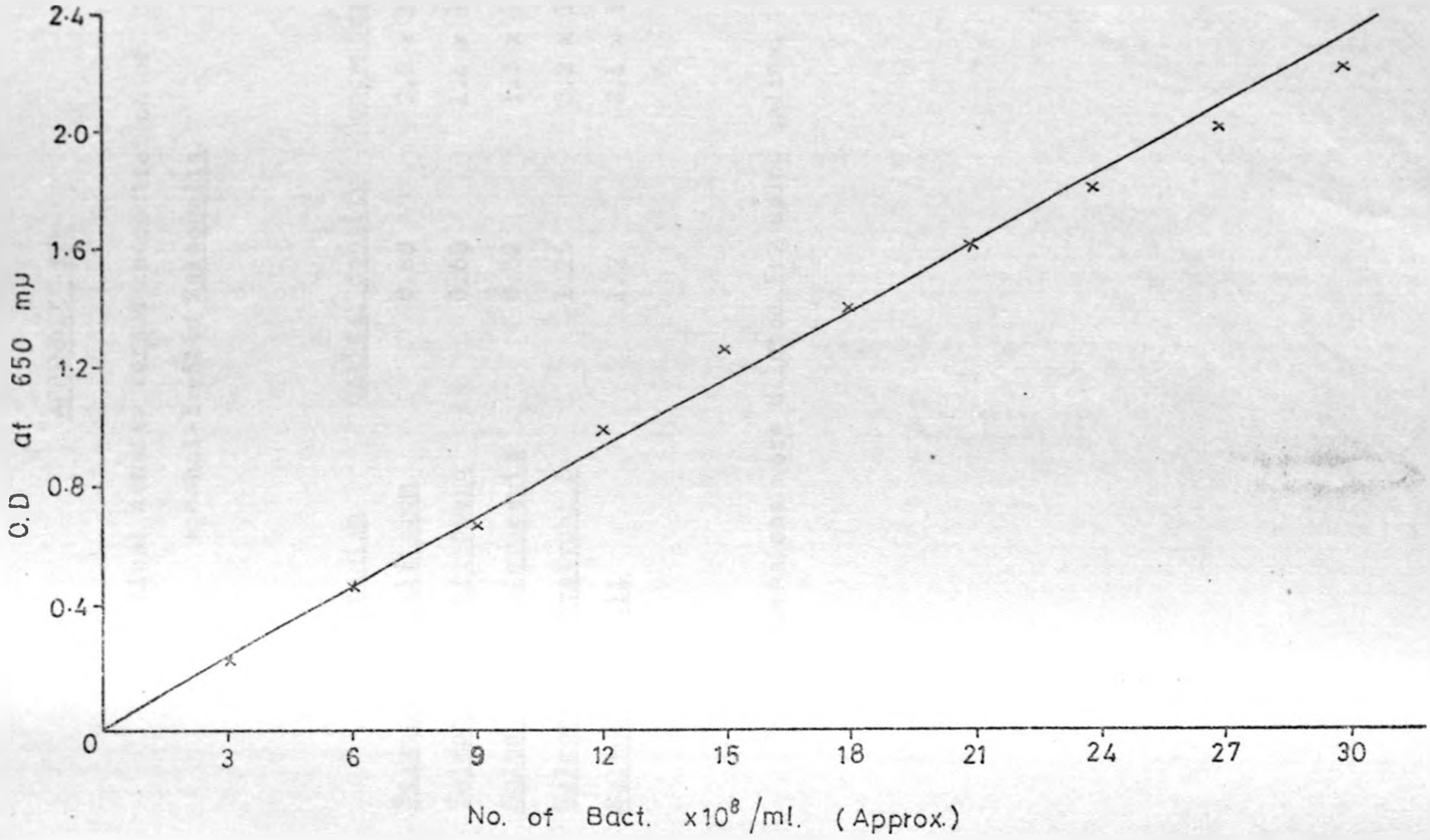
i) Plastic petri dishes made by CHR. Bardram, Hospital and Laboratory Supplies, Birkerod, Denmark, were obtained from Howse and McGeorge Ltd., Nairobi.

ii) Scapel blades Model Swann-Morton B.S. 2982 made by W.R. Swann and Co. Ltd., England, were obtained from Howse and McGeorge Ltd., Nairobi.

iii) Capillary tubes Model 3017-A50, 75 mm x 1.3 to 1.5 mm made by Arthur H. Thomas Co., Philadelphia, U.S.A. were obtained from Howse and McGeorge Ltd., Nairobi.

APPENDIX IIa

Standard Curve (McFarland Nepheiometer).



APPENDIX IIb

Optical density versus concentration of alcohol-treated *Salmonella*.

Organism	Optical density	Concentration
Salmonella gallinarum	0.88	2.3×10^9
Salmonella senftenberg	0.68	1.6×10^9
Salmonella choleraesuis	0.52	1.3×10^9
Salmonella paratyphi B	1.25	3.2×10^9
Salmonella hull	1.2	3.1×10^9

The suspensions were diluted 1:2 using saline.

APPENDIX III

Comparison of direct plating versus pre-enrichment through selenite broth - Farm IV.

	Swab		Intestinal contents		Bile		Spleen		Ovaries		Liver		Kidneys		Heart blood		Oviduct	
	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S	D	S
Salmonella	-	2	2	2	4	4	4	4	4	4	4	4	4	3	4	4	4	4
Escherichia	12	20	14	14	4	5	4	2	4	7	10	14	2	1	1	1	2	2
Proteus	1	1	-	1	-	-	-	1	-	1	-	-	-	1	-	-	-	1
Aerobacter	-	2	1	2	-	-	-	-	1	-	-	3	-	-	-	-	-	-
Streptococcus	-	-	2	10	-	1	-	-	1	-	-	-	-	-	-	-	-	-
Staphylococcus	-	4	6	3	5	-	8	5	2	2	18	7	2	2	1	-	1	1
Citrobacter	-	-	-	-	-	-	-	-	1	1	-	-	-	-	-	-	-	-

D represents direct plating
S represents selenite enrichment
- indicates none isolated.

APPENDIX IV

Cross-agglutination of Enterobacteriaceae with polyvalent "O" *Salmonella* antiserum.

	Poly "O" reaction					
	+		-		±	
Genus	No.	%	No.	%	No.	%
Salmonella	3	100	0	0	0	0
Escherichia	24	58.5	16	39.0	1	2.4
Proteus	2	40.0	3	60.0	0	0
Aerobacter	2	100	0	0	0	0
Citrobacter	29	80.6	5	13.9	2	5.6
Pseudomonas	0	0	6	100	0	0

LITERATURE CITED

Amtsberg, G., Kirpal, G., Lindfeld, C.A., Flasshoff, F.G. and Bisping, W. (1977): Studies on the distribution of Salmonella infections in calves in a district of Northwest Germany. Vet. Bull. 47: 670.

Andrews, W.H., Wilson, C.R., Poelma, P.L. and Romero, A. (1977): Comparison of methods for the isolation of Salmonella from imported frog's legs. Appl. and Environ. Microbiol. 33: 65.

Ann. Rep. Dept. Agric., Kenya (1933): p. 66.

Appel, G. (1978): Personal communication.

Basu, S., Dewan, M.L. and Suri, J.C. (1975): Salmonellosis in poultry in India during 1958 to 1973. Ind. J. Med. Res. 63: 1489.

Bierer, B.W. and Vickers, C.L. (1960): Nitrofuran medication for experimental Salmonella typhimurium infection in pouits. Avian Dis. 4: 22.

Blaxland, J.D. and Blowers, A.J. (1951): Salmonella typhimurium infection in duck eggs as a cause of human food poisoning. Vet. Rec. 63: 56.

Brown, D.D., Duff, R.H., Wilson, J.E. and Ross, J.G. (1973): A survey of the incidence of infections with salmonellae in broilers and broiler breeders in Scotland. Brit. Vet. J. 129: 493.

Brown, D.D., Ross, J.G. and Smith, A.F.G. (1975): Experimental infection of cockerels with Salmonella typhimurium. Res. Vet. Sci. 18: 169.

Brownell, J.R., Sadler, W.W. and Fanelli, M.J. (1969): Factors influencing the intestinal infection of chickens with Salmonella typhimurium. Avian Dis. 13: 804.

Bunyea, H. and Hall, W.J. (1929): Some observations on the pathology of bacillary white diarrhoea in baby chicks. J.A.V.M.A. 75: 581.

Bushnell, L.D. and Brandly, C.A. (1929): Some experiments on the control of bacillary white diarrhoea. J.A.V.M.A. 74: 444.

Buxton, A. (1957): cited by Shigidi, M.A. (1973) in Bull. Epiz. Dis. Afr. 21: 159.

Campbell, P.A. (1976): Immunocompetent cells in resistance to bacterial infections. Bact. Rev. 40: 284.

Carpenter, P.L. (1975): pp. 183 and 192. In Immunology and Serology, 3rd edition, W.B. Saunders Co., Philadelphia, London, Toronto.

Carter, G.R. (1975): pp. 31, 48, 53-55. In Diagnostic procedures in vet. microbiology, 2nd edition, Charles C. Thomas, Publisher, Springfield, Illinois, U.S.A.

Chowdhury, S.P.C., Bhattacharyya, A.K. and Gupta, P.D. (1976): Studies on the incidence of Salmonella in egg shell of duck. Ind. J. Anim. Hlth. 15: 169.

Coombs, R.R.A. and Stoker, M.G.P. (1951): Detection of Q-fever antibodies by the antiglobulin sensitisation test. The Lancet 2: 15.

Cowan, S.T. (1974): Gram-negative facultatively anaerobic rods, pp. 297-298. In R.E. Buchanan and N.E. Gibbons (eds.), Bergeys Manual of Determinative Bacteriology, 8th edition, The Williams and Wilkins Company, Baltimore.

Cowan, S.T. and Steel, K.J. (1965): Characterisation, pp. 17-39. In The Manual of identification of medical bacteria, Cambridge University Press.

Cox, N.A. and Williams, J.E. (1976): A simplified biochemical system to screen Salmonella isolates from poultry for serotyping. Poultry Sci.55: 1968.

Curtice, C. (1902): cited by Pomeroy, B.S. (1972) in M.S. Hofstad and B.W. Calnek (eds.), Diseases of poultry, 6th edition, Iowa State University Press, Ames, U.S.A., p. 115.

Domermuth, C.H. and Gross, W.B. (1975), pp. 84 and 88. In S.B. Hitchner, C.H. Domermuth, H.G. Purchase and J.E. Williams (eds.), Isolation and Identification of avian pathogens, 3rd edition, Arnold Printing Corporation, Ithaca, New York.

Easterling, S.B., Johnson, E.M., Wohlhieter, J.A. and Baron, L.S. (1969): Nature of lactose-fermenting Salmonella strains obtained from clinical sources. J. Bact. 100: 35.

Edwards, P.R. and Hull, F.E. (1929): Bacillary white diarrhoea and related diseases of chickens. Ky. Agr. Exp. Sta. Bull. 296: 237.

Erskine, V.M. and Margo, A.D. (1974): Salmonellosis: An environmental health problem. J.A.V.M.A. 165: 1015.

Fay, L.D. (1972): Wildlife disease research, Food and Agriculture Organisation of the United Nations, Rome, F.A.O. No. TA 3049. pp. 5 and 20.

Gage, G.E., Paige, B.H. and Hyland, H.W. (1914): On the diagnosis of infection with Bacterium pullorum in the domestic fowl. Mass. Agr. Exp. Sta. Bull. 148: 1.

Gordon, R.F. and Garside, J.S. (1944): Salmonella infections of ducks and ducklings. J. Comp. Path. 54: 61.

Gross, W.B. and Domermuth, C.H. (1975): Colibacillosis, pp. 34-37. In S.B. Hitchner, G.H. Domermuth, H.G. Purchase and J.E. Williams (eds.), Isolation and identification of avian pathogens, 3rd edition, Arnold Printing Corporation, Ithaca, New York.

Gwatkin, R. and Mitchell, C.A. (1944): Transmission of Salmonella pullorum by flies. Can. J. Pub. Hlth. 35: 381.

Hall, G.A., Jones, P.W., Aitken, M.M. and Parsons, K.R. (1978): The serology of experimental Salmonella dublin infections in cattle. J. Hyg. Camb. 81: 31.

Hall, G.N. (1926) cited by Khan, A.Q. (1970) in Bull. Epiz. Dis. Afr. 18: 207.

Harvey, R.W.S. and Price, T.H. (1975): Studies on the isolation of S. dublin.
J. Hyg. Camb. 74: 369.

Harvey, R.W.S., Price, T.H. and Morgan, J. (1977): Salmonella surveillance with reference to pigs - Cardiff Abattoir, 1968-1975.
J. Hyg. Camb. 78: 439.

Henning, M.W. (1939): The antigenic structure of salmonellae obtained from domestic animals and birds in South Africa.
Onderstepoort J. Vet. Res. 13: 77.

Jameson, J.E. (1961): A study of tetrathionate enrichment techniques, with particular reference to two new tetrathionate modifications used in isolating salmonellae from sewer swabs.
J. Hyg. Camb. 59: 1.

Jones, F.S. (1913): The value of the macroscopic agglutination test in detecting fowls that are harbouring Bacterium pullorum.
J. Med. Res. 27: 481.

Kabat, E.A. and Mayer, M.M. (1967): Ammonium sulphate fractionation, pp. 762-763. In Experimental immunochemistry, 2nd edition, U.S.A.

Karlshoj, K. and Szabo, L. (1949): Salmonella infections in eggs of ducks. Amer. J. Vet. Res. 10: 388.

Kauffmann, F. (1975): The classification of Salmonella-species, pp. 15-128. In Classification of bacteria: a realistic scheme with special reference to the classification of Salmonella and Escherichia species, State Serum Institute, Copenhagen.

Kerr, W.R. (1930): Selective media for the cultivation of Bacillus pullorum and Bacillus sanguinarium. J. Comp. Path. Therap. 43: 77.

Khan, A.Q. (1970): Salmonella infections in birds in the Sudan. Bull. Epiz. Dis. Afr. 18: 207.

Klein, E. (1889): cited by Pomeroy, B.S. (1972), in M.S. Hofstad and B.W. Calnek (eds.), Diseases of poultry, 6th edition, Iowa State University Press, Ames, U.S.A., p. 115.

Lowe, H.J. (1932): Disease Control. Ann. Rep., Dept. Vet. Sci. and Anim. Husb., Tanganyika Territory, pp. 2-13.

Magwood, S.E. and Bigland, C.H. (1962): Salmonellosis in turkeys: Evaluation of bacteriological and serological evidence of infection. Can. J. Comp. Med. 26: 151.

Mallman, W.L. (1929): Salmonella pullorum in the intestinal contents of baby chicks. J. Inf. Dis. 44: 16.

Mazza (1899): cited by Van Roekel, H. (1952) in H.E. Biester and L.H. Schwarte (eds.), Diseases of poultry, 3rd edition, Iowa State University Press, Ames, U.S.A., p.268.

Merchant, I.A. and Packer, R.A. (1967): pp. 211, 243, 278, 288. In Veterinary Bacteriology and Virology, 7th edition, Iowa State University Press, Ames, U.S.A.

Mettam, R.W.M. (1932): Ann. Rep. Vet. Dept., Uganda Protectorate. pp. 21-37.

Michael, S.T. and Beach, J.R. (1929): An experimental study of tests for the detection of carriers of Bacterium pullorum. Hilgardia 4: 185.

Miringa, E.N. (1978): Personal communication.

Montgomerie (1911 - 1912): cited by Hudson, J.R. (1934) in Vet. J. 90: 345.

Moore, E.N. (1946): Fowl typhoid transmission. Del. Agr. Exp. Sta. Bull. 262: 21.

Moore, V.A. (1895): cited by Pomeroy, B.S. (1972) in M.S. Hofstad and B.W. Calnek (eds.), Diseases of Poultry, 6th edition, Iowa State University Press, Ames, U.S.A., p. 115.

Moran, A.B., Van Houweling, C.D. and Ellis, E.M. (1965): The results of typing Salmonella from animal sources in the United States. Proc. Natl. Conf. on salmonellosis, U.S. Dept. of HEW, Public Health Serv., N.C.D.C., Antlanta, Ga., pp. 33 - 37.

Olesiuk, O.M., Carlson, V.L., Snoeyenbos, G.H. and Smyser, C.F. (1969): Experimental Salmonella typhimurium infection in two chicken flocks. Avian Dis. 13: 500.

Pomeroy, B.S. (1972): Fowl typhoid, pp. 114-135. In M.S. Hofstad and B.W. Calnek (eds.), Diseases of poultry, 6th edition, Iowa State University Press, Ames, U.S.A.

Remington, R.D. and Schork, M.A. (1970): Tests for association in contingency tables, pp. 235-244. In Statistics with applications to the biological and health sciences, Prentice-Hall Inc.

Report (1969 - 1978): Unpublished annual reports from the microbiology diagnostic laboratory of the Faculty of Veterinary Medicine, University of Nairobi.

Rettger, L.F. (1900): Septicaemia among young chickens. N.Y. Med. J. 71: 803.

Rettger, L.F., Kirkpatrick, W.F. and Jones, R.E. (1914): Bacillary white diarrhoea of young chicks. Storrs Agr. Exp. Sta. Bull. 77: 259.

Rettger, L.F., Kirkpatrick, W.F. and Jones, R.E. (1915): Bacillary white diarrhoea of young chicks: its eradication by elimination of infected breeding stock. Storrs Agr. Exp. Sta. Bull. 85: 149.

Rettger, L.F., McAlpine, J.G. and Warne, D.E. (1930): A comparative study of the routine macroscopic agglutination and the intracutaneous (wattle) tests for Bacterium pullorum infection in poultry breeding stock. J.A.V.M.A. 77: 47.

Runnells, R.A., Coon, C.J., Farley, H. and Thorp. F. (1927): An application of the rapid method agglutination test to the diagnosis of bacillary white diarrhoea infection. J.A.V.M.A. 70: 660.

Schaffer, J.M., McDonald, A.D., Hall, W.J. and Bunyea, H. (1931): A stained antigen for the rapid whole blood test for pullorum disease. J.A.V.M.A. 79: 236.

Shigidi, M.A. (1973): Salmonella wien and Salmonella muenchen infection in fowls in Sudan. Bull. Epiz. Dis. Afr. 21: 159.

Sleigh, J.D. (1975): The Enterobacteriaceae: Salmonella, pp. 403-419. In R. Cruinkshank, J.P. Duguid, B.P. Marmion and R.H.A. Swain (eds.), Medical Microbiology, the practice of medical microbiology, 12th edition, Vol.II, Churchill Livingstone, Edinburgh, London and New York.

Smith, P.J., Larkin, M. and Brooksbank, N.H. (1972): Bacteriological and serological diagnosis of salmonellosis of fowls. Res. Vet. Sci. 13: 460.

Snoeyenbos, G.H. (1972): Pullorum disease, pp. 83-114. In M.S. Hofstad and B.W. Calnek (eds.), Diseases of poultry, 6th edition, Iowa State University Press, Ames, U.S.A.

Sojka, W.J., Wray, C., Shreeve, J. and Benson, A.J. (1977): Incidence of Salmonella infection in animals in England and Wales, 1968-1974. J. Hyg. Camb. 78: 43.

Stefanov, V., Ivanov, P. and Sizov, I. (1974): Diagnosis of fowl typhoid and pullorum disease in live poultry (wattle test with allergen). Poultry Abs. 1: 144.

Swift, H.F., Wilson, A.T. and Lancefield, R.C. (1943): Lancefield capillary tube test, pp. 36-37. In C.A. Williams and M.W. Chase (eds.), Methods in immunology and immunochemistry, Vol.III (1971), Academic Press, New York and London.

Tanaka, Y., Katsube, Y. and Imaizumi, K. (1976): Serological investigation of Salmonella carrier-dogs. Jap. J. Vet. Sci. 38: 513.

Van Roekel, H. (1952): Pullorum disease, pp. 215-260. In H.E. Biester and L.H. Schwarte (eds.), Diseases of poultry, 3rd edition, Iowa State University Press, Ames, U.S.A.

Van Roekel, H., Bullis, K.L., Flint, O.S. and Clarke, M.K. (1932): Twelfth annual report on eradication of pullorum disease in Massachusetts. Mass. Agr. Exp. Sta. Contr. Ser. Bull. 63.

Van Roekel, H., Bullis, K.L., Flint, O.S. and Clarke, M.K. (1935): Fifteenth annual report on eradication of pullorum disease in Massachusetts. Mass. Agr. Exp. Sta. Contr. Ser. Bull. 78.

Ward, A.R. and Gallagher, B.A. (1917): An intradermal test for Bacterium pullorum infection in fowls. U.S.D.A. Bull. 517.

Wijffels, R.F. and Dheedene, J.O. (1976): Salmonella pullorum infection in a large chick-rearing premises in Morocco. Vet. Bull. 47: 669.

Williams, J.E. (1972): Paratyphoid infections, pp. 81-82 and 135-202. In M.S. Hofstad and B.W. Calnek (eds.), Diseases of poultry, 6th edition, Iowa State University Press, Ames, U.S.A.

Williams, J.E. (1978): Recent literature on detection of salmonellae in poultry production flocks: A critical review. Avian Path. 7: 1.

Williams, J.E. and Dillard, L.H. (1968a): Salmonella penetration of fertile and infertile chicken eggs at progressive stages of incubation. Avian Dis. 12: 629.

Williams, J.E. and Dillard, L.H. (1968b): Penetration of chicken egg shells by members of the Arizona group. Avian Dis. 12: 645.

Williams, J.E. and Dillard, L.H. (1969): Salmonella penetration of the outer structures of white- and speckled-shell turkey eggs. Avian Dis. 13: 203.

Williams, J.E., Dillard, L.H. and Gaye, O.H. (1968): The penetration patterns of Salmonella typhimurium through the outer structures of chicken eggs. Avian Dis. 12: 445.

Williams, J.E. and Whittemore, A.D. (1967): A method of studying microbial penetration through the outer structures of the avian egg. Avian Dis. 11: 467.

Williams, J.E. and Whittemore, A.D. (1971): Serological diagnosis of pullorum disease with microagglutination system. Appl. Microbiol. 21: 394.

Williams, J.E. and Whittemore, A.D. (1972): Microantiglobulin test for detecting Salmonella typhimurium agglutinins. Appl. Microbiol. 23: 931.

Williams, J.E. and Whittemore, A.D. (1973): Microtesting for avian salmonellosis. Proceedings 77th Annual Meeting U.S., Animal Health Association.

Williams, J.E. and Whittemore, A.D. (1976a): Field applications of MA and MAG tests for detection of avian salmonellosis. United States Animal Health Association's 80th Annual Meeting, Miami Beach, Florida.

Williams, J.E. and Whittemore, A.D. (1976b): Comparison of six methods of detecting Salmonella typhimurium infection of chickens. Avian Dis. 20: 728.

Wray, C., Morris, J.A. and Sojka, W.J. (1975): A comparison of indirect hemagglutination tests and serum agglutination tests for diagnosis of Salmonella dublin infection in calves. Brit. Vet. J. 131: 727.

Yamamoto, R., Kilian, J.G., Babcock, W.E. and Dickinson, E.M. (1962): Some observations on serological testing for Salmonella typhimurium in breeder turkeys. Avian Dis. 6: 444.

Yasuo Yagi (1971): Identification of multiple antibody components by radioimmuno-electrophoresis and radioimmunodiffusion, pp. 468-473. In C.A. Williams and M.W. Chase (eds.), Methods in immunology and immunochemistry, Vol. III, Academic Press, New York and London.