Salmonella virchow in a Chicken-packing Station and Associated Rearing Units

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Summary: In tracing the source of an outbreak of food-poisoning with Salmonella virchow a chicken-packing station and associated rearing farms were investigated. The serotype was found in chickens in 9 of the 14 rearing farms investigated and in the hatchery, but not in the breeding flocks supplying the hatchery. Several personnel on the farms were affected. The infection was most likely to have been introduced by contaminated feeding-stuffs.

Introduction

On 8 July 1968 it became apparent that frozen chickens from a packing-station in Cheshire were contaminated with *Salmonella virchow* and were implicated in an extensive outbreak of food-poisoning in Liverpool (Semple, Turner, and Lowry, 1968).

The packing-station was the centre of a farming co-operative consisting of seven breeding flocks, whose eggs went to one hatchery, which in turn sent day-old chicks to 16 rearing farms. These birds were kept in deep litter units for nine weeks and then sent to slaughter at the packing-station.

Three types of product were prepared: "New York dressed," which were plucked but not eviscerated; "oven-ready," which were plucked and eviscerated; and "deep frozen," which after plucking and evisceration were placed at $-30°$ C. for one hour and then stored at $-10°$ C. until sold. The New York dressed and oven-ready birds were chilled and distributed in refrigerated vans to wholesale and retail outlets the morning after slaughter.

Methods

The investigation was directed to the isolation of salmonella species only. All materials were inoculated into selenite F medium (Hobbs and Allison, 1945), which, after overnight incubation at 37° C., was subcultured to desoxycholate citrate agar. Suspected colonies on this medium after 24 hours' incubation were identified by the usual biochemical and serological reactions. All primary cultures in selenite F were reincubated for a further 24 hours and again subcultured to desoxycholate citrate agar if the first subcultures were negative. In the Veterinary Investigation Laboratory all primary cultures were incubated for 48 hours before subculture. Material from chickens, environmental swabs, and feeding-stuffs were subjected to the double enrichment technique of Jameson (1962). This technique was found to be very effective, particularly if the second culture in selenite F was incubated at 42° C.

Eviscerated Carcasses.—These were either deep frozen or chilled when taken from the processing plant. They were placed in an incubator at 37° C. for 18 hours. The abdominal cavity was then rinsed with approximately 20 ml. of selenite F medium (single strength) and the free fluid withdrawn for incubation.

Unplucked and/or Uneviscerated Birds.—The abdominal cavity was opened with sterile instruments and portions of the liver, spleen, gall bladder, kidney, and bowel were taken into 100-ml. volumes of single-strength selenite F.

Giblets and Pluck.—The giblets and pluck of 95 birds killed and eviscerated at the processing plant were sent to the laboratory in separate plastic bags, the contents of which were transferred to 100 ml. of selenite F in 1-lb. (450-g.) honey jars.

Faeces.—Human faeces were plated direct on to desoxycholate citrate agar and inoculated into 10 ml. of selenite F. The hut droppings, dust, and cloacal swabs were placed in 10 ml. of selenite F.

Eggs.—Egg shells and yolk-sac fluid from dead-in-shell chicks and day-old culls were added to 10 ml. of selenite F broth.

Swabs.—Swabs from working surfaces within the plant were placed direct into 10 ml. of selenite F.

Feeding-stuff.—A 25-ml. volume of feeding-stuff, either pellets or meal, was added to each of 100 ml. of selenite F and balanced tetrathionate broth (Rolfe, 1946).

Water.—Chlorinated water from the chill tanks was taken up into 120-ml. bottles containing sodium thiosulphate and then diluted with an equal volume of double-strength selenite F.

Sewage.—An alginate sewer swab (Robinson, 1958) was left in the main effluent of the packing-station for 24 hours. It was dissolved in 20 ml. of sodium hexametaphosphate and four tenfold dilutions were made in selenite F.

Results

Processing Plant.—The results of the initial investigation at the plant are given in Table I. Six of 12 eviscerated carcasses yielded *S. virchow*. There was no evidence of gross contamination within the plant and the standard of general hygiene was adequate. None of the staff were carriers.

TABLE I.—*Isolations of S. virchow from Packing Station*

	No. Examined	No. Positive
Fresh eviscerated carcasses	6	6
Fresh giblets	1	1
Frozen eviscerated carcasses	6	0
Frozen giblets	1	0
Chill tanks	12	1
Swabs from working surfaces and inside of distribution vans	10	0
Sewer swab	1	1
Staff—faeces	85	0

Rearing Units.—In order that production might continue at the plant after it had been thoroughly cleansed, and also to trace the source of infection further, it was decided to screen the rearing farms which had chickens due to come to slaughter in the next week or so. Public health inspectors visited 12 of the 16 farms on 13 July—the remaining four were either empty or out of the area. Samples were taken of faeces from the deep litter huts, broiler feed, and, in some instances, fowls. The results are given in Table II. Of the 12 farms sampled eight had infected birds. From one unit (farm 1 in Table II) 95

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birds were killed at the processing plant and only their giblets were sent to the laboratory; 49 (52%) were positive. The presence of *S. virchow* in the rearing units having been confirmed, arrangements were made that one week before the birds in each unit reached slaughtering age a representative sample (1%) of cloacal swabs would be submitted for culture. *S. virchow* was cultured from 7 of the 11 farms so tested. In addition there was one unit on farm 10 where the droppings of 3–4-day-old chicks had been found to be positive. Hut droppings were collected at weekly intervals for six weeks, and on each occasion the cultures were positive. People working on the farms were asked to submit faecal samples, and one was found to be an excreter of *S. virchow*.

TABLE II.—*Isolations of S. virchow from Rearing Units*

Farm No.	Specimens	Specimens taken 12 July 1968		Subsequent Investigations	
		No. of Samples	No. Positive	No. of Samples	No. Positive
1	Meal	1	0		
	Hut droppings	13	0		
	Plucked chickens	10	5		
	Giblets	95	49		
	Cloacal swabs			430	120
2	Pellets	1	0		
	Unplucked chickens	1	0		
	Hut droppings	3	0		
	Cloacal swabs			130	0
3	Pellets	1	0		
	Hut droppings	8	4		
	Cloacal swabs			574	130
4	Pellets	1	0		
	Hut droppings	3	0		
	Unplucked chickens	3	0		
	Cloacal swabs			270	0
5	Meal	2	0		
	Hut droppings	3	0		
	Cloacal swabs			310	0
6	Pellets	4	0		
	Hut droppings	6	3		
	Cloacal swabs			1,522	310
7	Pellets	1	0		
	Hut droppings	6	4		
	Cloacal swabs			1,286	240
8	Pellets	1	0		
	Hut droppings	3	2		
	Cloacal swabs			696	180
9	Pellets	1	0		
	Hut droppings	6	6		
	Cloacal swabs			1,197	344
10	Pellets	1	1	4	1
	Hut droppings	2	1	6	6
11	Meal	1	0		
	Hut droppings			8	0
12	Meal	1	0		
	Hut droppings	3	1		
13	Cloacal swabs			99	0
14	Cloacal swabs			100	10
15, 16	Not investigated				

Hatchery.—Samples of dust were positive from three of the four major rooms at the hatchery, but after disinfection the swab samples became negative. For a period of three weeks all dead-in-shell chicks, shells, and newly hatched culls were submitted for examination; 1,741 specimens were cultured with negative results. Paper trays holding faecal droppings were also cultured but no salmonellae were isolated. Four people working at the hatchery were found to be carriers of *S. virchow*.

Breeder Flocks.—Over a period of three weeks samples of unfumigated eggs, litter, food, and any pullets which died were collected and cultured. At one farm where the breeding birds had reached the end of their productive life rectal swabs, faecal samples, swabs of nesting boxes, and dust were collected. *S. virchow* was not isolated from any of these specimens. A man working on one of the breeding units was a carrier of the serotype; he had also been associated with a rearing unit which was known to be infected.

Discussion

Salmonella food-poisoning in man from the ingestion of infected poultry products has often been described and has been reviewed by Kampelmacher (1963) and Taylor (1967). Surveys in this country (Dixon and Pooley, 1961), the Netherlands (Van Schothorst, Guinée, Kampelmacher, and Keulen, 1965), and the U.S.A. (Wilson, Peffenborger, Foster, and Lewis, 1961) have shown that the proportion of contaminated poultry emanating from packing-plants lies somewhere between 10 and 20%. Recently Tucker and Gordon (1968) reported that only four isolations were made during the examination of nearly 17,000 birds (0.024%) at 10 packing-stations in England. They expressed the optimistic view that salmonellosis in poultry is on the decline.

Laboratory work in connexion with an outbreak of food-poisoning in Liverpool showed that *S. virchow* was widely disseminated throughout a broiler production unit, so that at one point about half of the birds being slaughtered were carriers of the serotype. The ultimate source of the infection was not established, but the most likely explanation was that the breeding flock became infected through contaminated feeding-stuffs and that this in turn led to contamination of the hatchery. That the breeding flocks appeared to be free of the infection is not remarkable, for adult birds harbour salmonella organisms much less readily than chickens (Snoeyenbos, Carlson, McKie, and Smyser, 1967).

S. virchow has been isolated from the materials which are used as protein supplements for poultry foods (Vernon, 1967). *S. virchow* was isolated from 2 out of 398 samples of meat and meat-and-bone-meal in February 1967; and from 2 out of 81 samples of poultry offal meal in September and November of the same year (J. H. McCoy, personal communication). Two samples of food supplied to the rearing units were positive, but, as both were from open containers, external contamination was a possibility. However, one of these samples was collected immediately before the introduction of another batch of day-old chicks, which indicated that the infection could have been introduced during the preceding three-monthly cycle or even earlier. At the time of the present investigation milking-cows on a farm unrelated to any of the poultry group became ill, and were found to be infected with *S. virchow*. They had been grazing on land which had been manured with litter from two units, one of which was subsequently shown to be infected. This, too, suggested that the infection had been present for some months.

A number of human cases of food-poisoning with *S. virchow* occurred in the north-western area of the country in the period October to December 1967 (Semple *et al.*, 1968). There was also an infection with the same serotype at a poultry-packing station in Lancashire at this time, which further suggests that infected foodstuffs were available to farm stock in the north-west in the autumn of 1967.

The breeding units and hatchery were supervised by the Animal Health Division of the Ministry of Agriculture under a poultry health scheme. This scheme primarily controls infections with *S. pullorum* and *S. gallinarium*, but it includes other salmonellae which might cause mortality in poultry. *S. virchow* has not been associated with disease of poultry in this country and appears to be an intestinal commensal. It was ascertained that there had been no abnormal losses from disease on the rearing farms and nothing unusual found on routine inspection of carcasses at the packing-station. Some salmonellae—for example, *S. typhimurium* (Wilson, 1945) and *S. menston* (Gordon and Tucker, 1965) can be transmitted through the ovary, and it is possible that *S. virchow* may have been transmitted from a breeding flock to the hatchery by this route before our investigation was undertaken. Passage on the outer surface of the eggs was unlikely, since the eggs were fumigated with formalin at the breeding units before dispatch and again at the hatchery on arrival.

The possibility that a human carrier may have introduced the disease cannot be excluded. Four workers at the hatchery were found to be infected, but two of these were known to have been employed for only a very short period before sampling.

Conclusions

There is no routine sampling of live birds or carcasses in chicken-packing stations in this country, and in view of the large number of birds slaughtered weekly such checks would be impracticable with existing facilities. However, there seems to be a case for more rigorous control of general hygiene throughout broiler production units and bacteriological control of animal feeding-stuffs. Outbreaks of food-poisoning can be more easily prevented if the proportion of infected birds is reduced to the level found by Tucker and Gordon (1968). The popularity of New York dressed birds is of particular concern. Such birds carrying food-poisoning organisms are potentially dangerous in shops and the kitchen.

The dispersal of infected litter to grazing land presents hazards to livestock which can in turn infect humans by direct contact or by contaminating milk. Measures which control disposal of litter from rearing units are worth further consideration.

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Red Cell Survival after Heterograft Valve Surgery

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Summary: Intravascular haemolysis was studied in 24 patients three to nine months after calf or pig valve heterografts had been inserted for severe valvular heart disease. No patient had haemolytic anaemia. In five of the 24 patients there was subclinical haemolysis, and in these five the haemolysis appeared to be related to residual aortic regurgitation or to the presence of other foreign material such as a Dacron aortic graft. The extent of postoperative haemolysis in these five patients was comparable to that observed preoperatively in patients with valvular heart disease.

The results support the belief that, in contrast to artificial valve prostheses, heterograft valves behave similarly to human valves as regards haemolysis.

Introduction

Intravascular haemolysis is a frequent complication of prosthetic valve replacement (Brodeur *et al.*, 1965; Rubinson *et al.*, 1966; Bell *et al.*, 1967), and in a significant proportion of cases frank haemolytic anaemia occurs, particularly with incompetent prostheses (Sayed *et al.*, 1961; Marsh, 1964; Stevenson and Baker, 1964). Random destruction of red cells due to valve turbulence and shearing forces created by a regurgitant flow of blood form the most likely basis for the haemolysis, and this theory has recently received experimental support (Nevaril *et al.*, 1968). Such mechanical trauma to the red cells is accentuated by contact with the foreign materials used in artificial prostheses. An immune basis in some patients has been suggested by the development of a positive Coombs antiglobulin test and the fact that the haemolytic anaemia sometimes responds to treatment with corticosteroids (Pirofsky *et al.*, 1965).

Since 1966 formaldehyde-preserved calf and pig heterograft valves have been used to replace diseased valves and defective prostheses (O'Brien and Clarebrough, 1966, 1967). As these valves produce little turbulence (O'Brien and Clarebrough, 1966), and thus less mechanical damage to the red cells than artificial prostheses, it might be expected that intravascular haemolysis is uncommon in patients with heterograft valves. Though of obvious practical significance, it seems that this point had not been studied previously.

Methods

We have studied 24 patients who had calf or pig valve heterografts inserted at the cardiac unit of the Chermside Hospital, Brisbane. All had severe valvular heart disease, verified by preoperative haemodynamic studies. The haematological investigations were made three to nine months postoperatively. In addition, five of the patients were studied preoperatively. Red cell survival was investigated with the ^{51}Cr technique after labelling a 10–15 ml. sample of the patient's red cells in vitro with 50–100 μCi ^{51}Cr (Veall and Vetter, 1958). The lower limit of normal for the $T\frac{1}{2}$ ^{51}Cr-labelled red cells in this laboratory is 25 days. As further evidence of intravascular haemolysis, plasma haemoglobin was estimated in all patients. For red cell haemoglobin concentration and reticulocyte counts standard methods were used. The serum iron level and the iron-binding capacity of the serum were estimated by the method of Trinder (1956) adapted to the autoanalyser.

Results

Haemodynamic.—Three patients (Cases 1, 2, and 3) had significant residual aortic incompetence, but only one (Case 2)

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