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The detection of a bovine carrier of *Salmonella heidelberg*

By E. T. DAVIES and J. A. J. VENN
Ministry of Agriculture, Fisheries & Food, Veterinary Investigation Centre, Langford, Bristol

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INTRODUCTION

An outbreak of food poisoning in human beings, due to *Salmonella heidelberg*, occurred in Cirencester and the surrounding rural area in November 1961 (Knox and others, in preparation). A high proportion of the affected people were found to have consumed unpasteurized Tuberculin Tested milk bottled at a dairy in the town, and on 13 November the organism was isolated by the Public Health Laboratory Service from samples of milk taken at the dairy from churns which had all come from a single farm.

The Animal Health Division of the Ministry of Agriculture, Fisheries & Food was asked to determine the source of the infection on the farm and this paper describes the methods used and the results obtained.

DESCRIPTION OF THE INFECTED FARM

The premises were in three parts, each separated from the others by two main roads. In the upper part the only building was a hay barn and the fields carried dry cows, in-calf heifers and sheep. The middle part contained the stockmen's cottages, cow-shed, dairy, bull-pens and some calf sheds. The fields in this part had been grazed by the milking cattle during the previous 2 months. The lower part contained the owner's house, stables, more calf pens, a poultry house in a loft over the stables, and paddocks for the horses.

The buildings for the stock were in good condition and drains, with one exception, ran into septic tanks. These were satisfactory but sometimes tended to overflow if large amounts of water were used in the buildings. The calf-pen drain ended in a soak-away just outside the pen. The general management on the farm was satisfactory.

Stock on the premises consisted of a Guernsey herd of 44 cows, 21 heifers, 9 calves and 1 bull; 4 hunters; approximately 80 sheep and about 100 poultry, some of which were 2-week-old chicks. Milking took place in the cowshed with two regular cowmen, although a relief milker was employed every 2 or 3 weeks. The cows' udders were washed with warm water and detergent, using one cloth for all the cows. The machine used was a bucket unit type and each bucket, when full, was emptied through a strainer into a churn standing in the centre passage of the cow-shed. The churns were then put in the water tank of the refrigerated recirculating in-churn cooler in the dairy and the cooler head with paddle was placed in the churn.

METHOD OF INVESTIGATION

Cultures were made of rectal and faecal swabs from the cattle, horses, sheep and poultry. Milk samples from the cows were also cultured and blood from the cattle tested for *Salmonella heidelberg* agglutinins. Moore sewer swabs (Moore, 1948) were used to check the drains and also the water in the refrigerated cooling tank.

A bovine foetus aborted on 15 December 1961 and a mouse caught in the cowshed food store on 24 November 1961 were also examined bacteriologically.

Collection of samples

Rectal and faecal samples were collected in the morning, brought to the laboratory and examination commenced the same day. Moore swabs were left in the drains or in the water tank for 5–7 days before being brought to the laboratory.

The first set of milk samples were 100 ml. quantities, each of which was a bulk sample from 2 or 3 cows, collected about midday.

The second milk samples were collected at a 6·00 a.m. milking. The cowman washed his hands with soap in a bucket of water containing approximately 5% chloroxylenol. Then he washed the udder of one cow with a cloth and warm water containing detergent. One of us immediately wiped the teats with cotton wool pads soaked in 1% Cetavlon and then collected approximately 12 ml. of milk per quarter in two sterile Universal bottles—the right side into one bottle and the left into the other. This procedure was used with each cow.

Bacteriological techniques

Rectal swabs and faeces samples were inoculated into selenite F broth and incubated at 37° for 24 hr., subcultured on to deoxycholate citrate (DCA) and MacConkey agar (McCA) and these plates were read after 24 and 48 hr. incubation.

Sewer swabs were cut into six pieces and each was placed in 100 ml. selenite F, incubated at 37° C. and after both 24 and 48 hr. plated on to DCA and McCA. These plates were read at 24 and 48 hr.

To each bulk milk sample was added an equal volume of double strength selenite F broth which was incubated and then plated out in the same way.

The individual milk samples were examined at 10.00 a.m. on the morning they had been collected. 0·1 ml. was sown directly on to DCA and McCA. The residue of the sample was then centrifuged in its original bottle. The middle portion was removed by pipette and discarded while the cream and any deposit were mixed. Two large loopfuls (approx. 1 ml.) were each placed on DCA and McCA and spread by means of sterile steel spreaders. To the residue in the bottle was added 20 ml. selenite F broth. All inoculated media were treated as described above.

RESULTS

Cultures

On 20 November 1961 a positive rectal swab was obtained from a 2-week-old calf which had been born since the outbreak commenced. It was fed uncooled milk taken from the last or last-but-one churn filled at each milking. It was found

to have a greyish diarrhoea, but its temperature was normal, it had a good appetite and seemed very lively. All calves on the farm were reswabbed 4 days later with negative results. No treatment had been given and all subsequent rectal swabs from the herd were negative. However, as a precaution all calves in the pen were dosed from 25 November 1961 to 4 December 1961 with furazolidone. The calf-pen drain gave a positive culture on 24 November 1961 and 8 December 1961—that is, 15 days after the rectal swab taken from the calf was negative and in spite of liberal applications of approved disinfectants into the soak-away pit.

Table 1

Material	Positive culture	Negative culture
Rectal swabs	20. xi. 61	24. xi. 61
		27. xi. 61
		8. xii. 61
Calf-pen drain	24. xi. 61	11. i. 62
	8. xii. 61	5. ii. 62
		13. ii. 62
Individual and grouped milk samples	6. xii. 61	20. xi. 61
		5. iii. 62

All other specimens collected gave negative results on every occasion tested.

Cultures of the in-churn cooler water and of a swab left seven days in the water tank gave negative results on 24 November 1961 and 30 November 1961. On 7 December 1961 cultures of individual milk samples collected the previous day showed that one cow—'Flash 12'—was excreting *Salmonella heidelberg* from the right side of the udder. This cow's milk normally went into the last or last-but-one churn of the morning milking and into either the first or second churn at the evening milking. It had been observed when sampling that the milk was watery and the cowman had confirmed that she had not given as much milk as expected since calving on 28 October 1961. The udder and lymph glands felt normal and no other symptoms could be observed. This cow was home-bred, $5\frac{1}{2}$ years old and had calved four times—on the last occasion producing twins. Both calves had been sent to market and were slaughtered when less than 2 weeks old.

'Flash 12' was slaughtered on 8 December 1961 and samples of blood and milk were collected at the same time. The blood serum tested showed a higher titre (see below) to both O and H antigens than any of the other stock. The milk was cultured and only the samples from the right hind-quarter were found to be infected.

Serum agglutination tests

Fifty-two samples were examined; thirty-eight samples did not react. The end titres of the fourteen samples which reacted are shown in Table 2.

Post-mortem of 'Flash 12'

The cow was in good condition. There was an area of pneumonia and pleurisy with adhesions on the right side of the thorax adjacent to a callus on a rib,

probably resulting from a previous fracture. The lesion was of long standing. The pancreas was congested but the other viscera appeared normal. The supramammary lymph nodes on the right side were enlarged and oedematous but on the left side were normal. The right hind-quarter of the udder contained numerous haemorrhages extending over an area 10–15 cm. in diameter. The milk of the quarter was slightly blood-stained but this had not been noticed when the cow was alive.

Table 2

	8. xii. 61		5. ii. 62	
Animal identification	O	H	O	H
Nellie 2	1/20	1/40	1/20	1/40
Nellie 9	1/10	1/40	1/20	1/40
Dolly 3	1/10	1/40	—	1/10
Starlight 19	—	1/20	—	1/20
Trixie II	—	1/10	1/10	1/20
Nellie 10	—	1/10	1/10	1/20
Flash 12	1/80	1/640	Killed 8. xii. 61	
Starlight 14	—	1/10	1/10	1/20
May 3	1/10	1/20	Sold	
Nellie 13	—	1/10	—	1/10
Nellie 3	1/10	1/40	—	1/10
Dolly 8	1/10	1/10	—	1/20
May 6	—	1/10	1/10	1/40
May 7	—	1/20	1/10	1/20

Salmonella heidelberg was isolated only from the tissue of the right hind-quarter. *Streptococcus uberis* was recovered from the other three quarters. No significant bacteria were isolated from the supramammary lymph nodes, two portions of the small intestine, mesenteric lymph nodes, liver, gall bladder, spleen, kidneys and uterus.

Histological preparations were made of all the tissues cultured. No significant changes were observed in the viscera. All four quarters of the udder showed varying degrees of chronic interstitial mastitis with some fibrosis. These lesions resembled those associated with chronic streptococcal mastitis. In addition in the right hind-quarter there were numerous haemorrhages with blood filling the acini and ducts in many foci throughout the sections. No inflammatory reactions could be seen around these haemorrhagic areas and no bacteria could be demonstrated in any of the udder sections.

DISCUSSION

The first series of milk samples collected were negative on culture and although it is realised that this result may have been due to intermittent excretion, the different techniques employed seem to have been more important. Plating out directly 0·1 ml. of milk from the right side-quarters of 'Flash 12' produced only 12 colonies of *Salmonella heidelberg* per plate, whereas the centrifuge deposit and cream plated out as a thick layer of about 1 ml. produced over 100 colonies per plate. The first milk samples were from groups of three cows so that the infection of the final sample would result in even less than 12 colonies per plate on direct

plating and possibly even produce a negative result. In spite of this it is also realised that our cultures via selenite broth should not have had this handicap.

During the course of the investigation, churn samples were examined by the Public Health Laboratory Service and at one time it seemed that more than one cow was excreting because positive cultures were obtained from several churns on each occasion. There are at least three possible explanations for this result. During milking if the milk from one cow was more than enough to fill a churn then the surplus would be poured into the next empty churn. Churns were sometimes topped up by the cowman in the dairy resulting in further mixing of the milk. Also although churns were filled in sequence from the cows as they were milked in the shed, this sequence in the evening was the reverse of that in the morning so that the first cow's milk went into the first churn of one milking and the last churn of the next.

The blood agglutination test results checked well with the cultural examinations. They confirmed that only one cow was infected because no other cow had a titre approaching the level of 'Flash 12'. The low titre reactions were probably not specific to the present *S. heidelberg* infection as they had not significantly changed when the cows were retested 59 days later.

The blood test unfortunately did not confirm the infection in the 5-week-old calf no. 11, which was the only animal on the farm excreting the organism in the faeces.

In spite of this it would appear that blood testing alone would have quickly revealed the affected cow but in this type of problem it was essential to establish definite evidence of the source of the organism and thus it was considered more desirable to rely on milk and faecal sampling.

There are many references to salmonellae having been recovered from cows milk (Kinlock, Smith & Taylor, 1926; Lehr, 1927; Rudolf, 1928; Clarenburg, 1931; Poppe, 1931; Stanfuss, Wilken & Sorrensen, 1932; Conybeare & Thornton, 1938; Tulloch, 1939; Pullinger & Scott Millar, 1945; Lewin & Roux, 1945; Field, 1948), and whilst it is well recognized that they can be excreted by the udder of a cow suffering from septicaemic salmonellosis (Kinlock *et al.* 1926; Lehr, 1927; Stanfuss *et al.* 1932; Tulloch, 1939; Lewin & Roux, 1945; Field, 1948), no reports of conclusive evidence of excretion from the udder of a clinically normal animal have been found. Both Stanfuss *et al.* (1932) and Conybeare & Thornton (1938) recorded the isolation of salmonellae from the milk of normal cows but in each instance the milk could easily have been contaminated with infected faeces. Although Rudolf (1928) reported that of 29 cases of acute mastitis he investigated, 5 were due to salmonellae, our laboratory records show that over $4\frac{1}{2}$ years 12,017 samples of milk were examined from clinical cases of mastitis and only once was *Salmonella dublin* isolated. It is conceivable that Rudolf's cases had been selected from cows that were dying from mastitis and this would tend to explain his rather high incidence of salmonella infection.

The lesions seen in the affected quarter cannot be regarded as pathognomonic of salmonella since they resemble the changes seen in any chronic bacterial udder infection.

How the cow 'Flash 12' acquired the infection is difficult to establish. If she had ingested the organism with contaminated feeding-stuffs it might have been expected that a systemic illness with symptoms associated with a digestive disorder would have occurred—such as indeed was observed in the young milk-fed calf. In view of the absence of such a history and our failure to demonstrate significant lesions and infections in any part of the carcass except one quarter of the udder, it appears possible that infection entered via the teat canal. The cow was infused via the teats with intramammary penicillin (as a routine procedure when drying off) one month before calving and this was the only occasion when the teats were handled by the stockmen in a way likely to introduce infection.

SUMMARY

1. *Salmonella heidelberg* infection in humans was traced to one cow excreting the organism in milk.

2. The cow was clinically normal and infection was confined to the right hindquarter of the udder.

3. The affected quarter had numerous haemorrhagic foci but there was no inflammatory response to the infection.

4. Histological preparations suggested that *Streptococcus uberis* was causing a chronic mastitis in all four quarters.

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