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Transmission of *Salmonella mbandaka* to cattle from contaminated feed

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SUMMARY

Salmonella mbandaka was isolated from cattle on three dairy farms. The duration of infection was less than four weeks and none of the animals became clinically ill. The animals had all consumed a diet containing a vegetable fat supplement contaminated with *S. mbandaka* and this was shown to be the source of the infections. It is significant that a feed containing purely vegetable components was incriminated.

INTRODUCTION

Feedstuffs are recognized as a source of salmonellas for animals and it has been suggested that the increase in bovine salmonellosis caused by 'exotic' serotypes during the last decade might be due to the increased use of contaminated imported feed (Williams, 1975; Wray and Sojka, 1977). Richardson (1975) found strong circumstantial evidence that dairy cake was the source of infection in outbreaks of salmonellosis on four farms, but there is a lack of direct evidence from outbreaks to implicate contaminated feed as a source of infection in cattle.

This communication reports the infection of cattle with *Salmonella mbandaka* which was directly attributable to their consumption of contaminated feed. The infection, which occurred at three dairy farms, was first diagnosed as the result of bacteriological examination of animals at two of the farms during investigations into an outbreak of salmonellosis due to *S. saint-paul* (P. W. Jones, 1981, unpublished).

MATERIALS AND METHODS

Dairy farms. Three dairy farms (A, B and C) were involved, on each of which approximately 100 Friesian cows were being milked. In addition Farm A housed 40 calves and 1 bull, Farm B 20 calves and Farm C 40 calves, 1 bull and 234 heifers, steers and dry cows. During the period of this report cows and calves at all three farms were continuously housed. A group of steers at Farm C were at pasture.

Feed. All calves were fed on proprietary reconstituted milk substitute and calf

pellets. Cows at Farms A and B were fed a 'complete diet' composed of lucerne silage, maize silage, soya bean meal, maize meal, chopped hay, dairy minerals, calcined magnesite, barley, sugar beet pulp, lucerne meal and vegetable fat. Cows at Farm C were fed a diet of hay and lucerne silage supplemented by dairy cake containing barley, soya bean meal, maize, fish meal, vegetable fat and dairy minerals. Dry cows and steers were fed a diet which did not contain vegetable fat.

All rations were compounded at a feed mill situated approximately equidistant from the three farms. The mill was also used to prepare feed for a pig herd of approximately 110 sows and gilts, 10 boars and 1000 piglets.

The vegetable fat which was used in the 'complete diet' at Farms A and B, the dairy cake at Farm C and the pig feed were obtained from a commercial manufacturer. The fat contained palm oil, with palm kernel and ground straw as carrier base.

Sampling procedures. Milking cows and calves at Farms A and B were sampled by rectal swabbing at intervals of approximately 14 days as part of a study of a natural outbreak of salmonellosis due to *S. saint-paul.* Following the discovery of *S. mbandaka* the frequency of sampling was increased and cattle at Farm C were also included. Sampling was continued until the organism could no longer be isolated. Rectal swabs were also taken from human contacts. In addition milk filters from the three farms, together with wild life including 9 mice (*Mus musculus*), 41 birds [namely, 26 starlings (*Sternus vulgaris*), 7 rooks (*Corvus frugilegus*), 5 crows (*Corvus brachyrhynchos*) and 3 wood pigeons (*Columba palumbus*)], 16 samples of slurry from the piggery and 59 samples of feed ingredients were examined.

Isolation of salmonellas from feed. Salmonellas were isolated by direct enrichment of 10 g of feed in 100 ml volumes of Rappaport broth (RAP: Rappaport, Konforti & Navon, 1956) and selenite brilliant green broth (SBG: Difco). In addition salmonellas were isolated by pre-enrichment of 10 g of feed in 100 ml buffered peptone water (peptone, Difco B118, 10 g, sodium chloride 5 g, disodium hydrogen orthophosphate . $12H_2O$ 9 g, potassium dihydrogen orthophosphate 1·5 g, distilled water 1000 ml, pH 7·2) for 18 h at 37 °C followed by enrichment of 10 ml of the buffered peptone water in Müller–Kauffmann tetrathionate broth (MKT: Oxoid CM343). The RAP cultures were incubated at 37 °C and the SBG and MKT at 43 °C. After 24 and 48 h incubation all enrichment broths were inoculated on modified brilliant green agar (Oxoid CM 329) with the addition of 120 mg/l sulphadiazine (BGSD). Plates were incubated at 37 °C and examined after 24 and 48 h. Non-lactose and non-sucrose fermenting bacteria, resembling salmonellas in colony morphology, were identified biochemically according to the method of Buchanan & Gibbons (1974) and serologically according to the method of Kauffmann (1972).

The concentration of salmonellas in feed was estimated by the 'Most Probable Number' technique (Taras *et al.* 1971). Samples were inoculated into buffered peptone water and then enriched in MKT. The technique used was a three-dilution test with three tubes per dilution each containing 10, 1 and 0·1 ml of sample respectively.

Isolation of salmonellas from cattle. Salmonellas were isolated from cattle by

enrichment culture of rectal swabs (two from each animal) in 10 ml volumes of RAP and SBG. The concentration of *S. mbandaka* in cattle faeces was determined by diluting 1 g of faeces in 0·85% (w/v) saline and spreading appropriate dilutions in saline over the surface of BGSD. Three replicate plates at each dilution were incubated for 24 h and the number of colonies agglutinating in O6, 7 antiserum (Wellcome Laboratories) were counted.

Isolation of salmonellas from slurry and milk filters. Salmonellas were isolated from slurry by enrichment of 10 g of sample in 100 ml volumes of SBG and RAP and from milk filters by enrichment of approximately half of each filter in 100 ml volumes of SBG and RAP.

Isolation of salmonellas from mice and birds. Salmonellas were isolated from mice as described by Jones & Twigg (1976) and from birds by enrichment of throat swabs, cloacal swabs and feet in 10 ml volumes of SBG and RAP.

Virulence of Salmonella mbandaka. Inocula of *S. mbandaka* for administration to mice, rats and calves were prepared by a method similar to that described by Hall & Jones (1976) for *S. dublin*. A single colony of *S. mbandaka* from BGSD inoculated from RAP was suspended in 0·85% (w/v) saline and spread over the surface of nutrient agar (Oxoid CM67 25 g, Bacto-Agar 18 g, distilled water 1 l, pH 7·5). After incubation at 37 °C for 24 h the bacterial growth was removed and a washed suspension in 0·85% (w/v) saline was prepared. Vials containing 0·1 ml of suspension were stored at −70 °C and thawed at room temperature as required. Bacterial suspensions for infecting animals were produced by inoculation of bacto-tryptose broth (Jones & Matthews, 1975) from the contents of vials using a standard loop. The bacto-tryptose broth was incubated at 37 °C for 18 h, after which time the *S. mbandaka* had multiplied to a concentration of approximately 10^9/ml. The concentration of bacteria in inocula was determined by spreading 0·1 ml volumes of appropriate dilutions in 0·85% (w/v) saline over the surface of BGSD.

Suspensions of *S. mbandaka* containing $2{\cdot}0 \times 10^9$, $2{\cdot}0 \times 10^7$, and $2{\cdot}0 \times 10^5$ organisms/ml were prepared in 0·85% (w/v) saline and 0·1 ml volumes were inoculated intraperitoneally into three groups of 10 mice (Compton White, male, 5–6 weeks).

Similar suspensions containing $1{\cdot}2 \times 10^9$ and $1{\cdot}2 \times 10^8$ organisms/ml were used to inoculate two groups of rats (PVG, male, 7–8 weeks). Each rat received 1 ml intraperitoneally; 12 rats were inoculated with $1{\cdot}2 \times 10^9$ organisms and 10 rats with $1{\cdot}2 \times 10^8$ organisms. Two further groups of 12 and 10 rats respectively were inoculated for purposes of comparison with $1{\cdot}0 \times 10^9$ or $1{\cdot}0 \times 10^8$ *S. dublin* (3246) as described previously (Aitken *et al.*, 1978).

Suspensions for infection of calves were prepared by dilution of an 18 h bacto-tryptose broth in 20 ml of an antacid mixture (sodium bicarbonate 5 g, magnesium carbonate, (light) 5 g, magnesium trisilicate 5 g, distilled water 100 ml). Two calves (Friesian, male, 4 weeks) received $1{\cdot}1 \times 10^8$ *S. mbandaka* orally and two more (Friesian, male, 4–6 weeks) $1{\cdot}9 \times 10^9$ orally. Rectal temperatures were taken twice daily and average temperatures calculated, and the calves were examined each morning when blood and faeces samples were taken and mouth

swabs prepared. These procedures were commenced on the day of infection and continued until the animals were killed and necropsied. Methods for the isolation of salmonellas from blood, faeces and mouth swabs have been described previously (Hall, Jones & Aitken, 1978).

The calves given 10^8 *S. mbandaka* were killed 22 days after infection and those given 10^9 16 days after infection by the injection of 20 ml of Euthatal (May & Baker) intravenously. At necropsy 38 tissues and body fluids were removed and examined for the presence of salmonellas as described by Hall & Jones (1977). The tissues samples were liver, spleen, lung, kidney, adrenal, heart muscle, rectum, colon, caecum, ileum, abomasum, omasum, rumen, bladder, gall bladder, tonsil and salivary gland. The lymph nodes sampled were prescapular, mandibular, retropharyngeal, bronchial, hepatic, mesenteric, colonic, caecal, abomasal, omasal, rumenal and internal iliac. Samples were also removed from contents of rumen, omasum, abomasum, ileum, caecum, colon, rectum, gall bladder and bladder.

RESULTS

Isolation of Salmonella mbandaka *from cattle. S. mbandaka* was first isolated from nine cows at Farm A on 4 March 1981. The number of animals from which isolations were subsequently made on each farm is shown in Table 1. Small numbers of animals were involved and the excretion did not persist beyond one month. The maximum number of salmonellas excreted by the animals examined was $3{\cdot}1 \times 10^3$/g faeces. With the exception of three isolations from steers at Farm C all isolations were made from milking cows. Animals at Farms A and B have been regularly examined for six months following the last isolation of *S. mbandaka* and the organism has not been detected.

Isolation of salmonellas from other sources. Salmonellas were not isolated from human contacts or wildlife. *S. anatum* was isolated from seven samples of pig slurry but *S. mbandaka* was not detected. *S. mbandaka* was isolated from milk filters on two of the three farms (Table 2). The isolations may however have been limited on Farms A and B by the isolation of *S. saint-paul* from the filters. Filters have been examined for a further six months since the last isolation was recorded and *S. mbandaka* has not been detected.

Isolation of Salmonella mbandaka *from feed. S. mbandaka* was first isolated from a sample of 'complete mix' on 9 March 1981. This was a component of the 'complete diet' used at Farms A and B and contained soya bean meal, sugar beet, maize meal, barley, calcined magnesite, lucerne meal, dairy minerals and vegetable fat. The organism was not isolated from any of the ingredients of the 'complete diet' when these were examined on the same date. These included the components of the 'complete mix' with the addition of lucerne silage, maize silage and chopped hay. *S. mbandaka* was similarly not isolated on subsequent sampling from fish meal, flaked maize, three batches of dairy and pig mineral supplements, four samples of pig feed, four batches of 'complete diet' or dairy pellets used at Farm C. Bin and conveyor scrapings from the feed mill and samples taken from mangers at Farms A and B were also negative.

Table 1. *Isolation of* S. mbandaka *from cattle*

		February		March										April	
Farm	Animals	18	25	4	5	9	11	12	13	16	17	18	19	31	2	14
A	Milking cows	0/105	NT	9/105	NT	16/106	NT	NT	NT	NT	NT	2/106	NT	NT	NT	0/106
	Calves	NT	0/44	NT	NT	NT	0/44	NT	NT	NT	NT	NT	NT	0/44	NT	NT
B	Milking cows	0	0/95	NT	12/95	NT	NT	NT	NT	NT	NT	3/95	NT	NT	0/95	NT
	Calves	0/23	NT	0/20	NT	NT	NT	NT	NT	NT	NT	0/20	NT	NT	NT	NT
C	Milking cows	NT	NT	NT	NT	NT	NT	NT	2/106	NT	NT	NT	1/107	NT	0/107	NT
	Calves	NT	NT	NT	NT	NT	NT	NT	NT	0/40	NT	NT	NT	NT	NT	NT
	Dry cows / Steers / Heifers	NT	NT	NT	NT	NT	NT	3/33	NT	NT	0/65	0/48	0/89	NT	NT	NT

NT = not tested.

Table 2. *Isolation of salmonellas from milk filters*

Date (1981)	Farm A	Farm B	Farm C
February 20	—	—	—
February 24	NT	NT	–
February 26	**	**	–
March 4	NT	NT	–
March 10	**	+	–
March 12	NT	NT	–
March 17	NT	NT	+
March 19	–	–	+
March 25	**	**	–
March 27	NT	NT	–
April 2	**	+	NT
April 140	–	**	–

NT = not tested; + = *S. mbandaka* isolated; ** = *S. saint-paul* isolated.

Re-examination of the ingredients of 'complete mix' on 10 March 1981 revealed the presence of *S. mbandaka* in an unopened bag of vegetable fat. Subsequently a further 19 bags of vegetable fat were examined (13 unopened bags, 6 previously opened bags) and *S. mbandaka* was isolated from 17. These represented ten different one-ton batches of vegetable fat all of which contained the organism. The 'Most Probable Number' of organisms present in two batches examined was 240/100 g. The batches were delivered on 10 March 1981 and 20 March 1981. A previous delivery made on 9 February 1981 was used as an ingredient in the 'complete mix' from which *S. mbandaka* was isolated on 9 March 1981. This batch had been fully used by this latter date and was thus not available for examination.

Virulence of Salmonella mbandaka. *S. mbandaka* was lethal in only two of the 30 mice inoculated. Both received $2{\cdot}0 \times 10^8$ organisms.

In rats the administration of *S. mbandaka* and *S. dublin* resulted in a similar level of mortality. At a dose of 10^9 organisms *S. mbandaka* was lethal in 10 of 12 rats compared to 11 of 12 for *S. dublin*, and at 10^8 it was not lethal in any of 10 rats compared to one death in 10 caused by *S. dublin*.

The effect of *S. mbandaka* in calves is summarized in Table 3. Apart from a transient pyrexia none of the animals exhibited signs of salmonellosis. The organism was not isolated from blood and was recovered on one occasion only from a calf which received $1{\cdot}1 \times 10^8$ organisms. The maximum number of salmonellas excreted in the faeces of calves given $1{\cdot}9 \times 10^9$ *S. mbandaka* was $4{\cdot}7 \times 10^4$ and $2{\cdot}6 \times 10^4$ organisms/g respectively. Excretion at levels detectable by surface spread counts continued for only two days in both animals. Salmonellas were only detectable in the faeces of calves given $1{\cdot}1 \times 10^8$ organisms by enrichment. At necropsy salmonellas were isolated from only one calf at each dose level.

Table 3. *Effect of oral challenge with* Salmonella mbandaka *in calves*

Animal	Inoculum	Mean maximum daily temperature	Clinical observations	No. of isolations from faeces/no. possible	Tissues containing *S. mbandaka* at necropsy
1	$1{\cdot}1 \times 10^8$	39·3 (day 4)	No illness	1/22	None
2	$1{\cdot}1 \times 10^8$	39·2 (day 4 and 7)	No illness	10/22	Retropharyngeal lymph node Omasum contents Colon contents Caecal node
3	$1{\cdot}9 \times 10^9$	40·1 (day 2)	No illness	7/16	
4	$1{\cdot}9 \times 10^9$	39·5 (day 3)	No illness	7/16	None

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DISCUSSION

Detection of *S. mbandaka* might not have occurred had cattle at Farms A and B not been sampled as part of an investigation of a disease outbreak associated with *S. saint-paul*; the former did not appear to be pathogenic for cattle. Salmonellas from feed and other sources may often be present in cattle without their presence being suspected. The organism was recoverable in the herd for less than a month and it was excreted by relatively few animals. This may have been a result of resistance acquired as a result of the presence of *S. saint-paul*, but since this organism was never present on Farm C this is unlikely. A more reasonable explanation is the apparent lack of virulence of the organism for cattle. It produced little observable effect in mice or calves at dose levels which would normally be lethal if a virulent strain of *S. dublin* were used. The latter is usually lethal in mice at doses of 10^4 or greater and in calves at 10^8 or greater (P. W. Jones and M. M. Aitken, unpublished observations). The comparable virulence in rats of *S. dublin* and *S. mbandaka* contrasts with the non-pathogenicity of the latter in mice and calves.

That the vegetable fat supplement was the source of *S. mbandaka* was confirmed by isolation of the organism from 13 unopened bags. The organism was not isolated from workers in contact with the animals or from wildlife, thus excluding these as alternative sources, and there had been no recent purchase of cattle. The time at which the organism first infected the cattle can also be accurately assessed, since two of the herds were being continually monitored and at the third milk filters were being examined. These were negative prior to the isolation of the organism from cattle. Similarly with the exception of three steers at Farm C the organism was only isolated from animals which had consumed the vegetable fat in their diet and there was evidence that steers may have come into contact with the infected feed. A concentration of 240 salmonellas/100 g of feed is higher than can be explained by chance contamination and greater than that normally found in animal feeds (Report, 1959).

It is surprising that the organism was not isolated from pig slurry which was examined in preference to swabbing individual animals, since the effectiveness of this method of demonstrating the presence of salmonellas in a pig herd had been shown previously (Jones & Hall, 1975).

It is cause for concern that a feed containing only constituents of vegetable origin could be incriminated as a source of infection for cattle. Three separate deliveries made between 9 February 1981 and 20 March 1981 were contaminated, indicating that a large quantity of raw material used by the manufacturer was contaminated. This could have led to the widespread dissemination of an organism of greater virulence than the strain of *S. mbandaka* involved in the present study. It is also important to note that *S. mbandaka* may be a risk to public health.

S. mbandaka was first reported under the Zoonoses Order in 1975 when it was isolated from one incident in a calf and two in poultry. It has since been isolated sporadically from cattle (7 adults, 6 calves), but is more common in poultry, from which a peak 88 isolations were made in 1978. The isolations from cattle appear

to have been incidental, perhaps confirming the relative lack of virulence of this organism for bovines (Duncan, 1981, personal communication).

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