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Salmonellosis in Botswana

I. Incidence in cattle

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SUMMARY

Clinical salmonellosis in cattle is infrequently recorded in Botswana, the majority of salmonella isolations being made from apparently healthy animals. A salmonella carrier rate of 6·7 % was found in testing faeces samples from 89 apparently healthy cattle. A total of 35 salmonella serotypes were isolated from specimens of bovine origin. *Salmonella dublin* was isolated infrequently, possibly in part because of prophylactic vaccination.

INTRODUCTION

The Republic of Botswana covers an area of 220,000 square miles (560,000 km^2) in central southern Africa. It has a subtropical climate, with mean summer temperatures of 25–38° C., and mean winter temperatures of 12–25° C. The average annual rainfall is in the region of 17 in. (43 mm.). The maximum rainfall occurs in the north of the country, which has an annual average of about 25 in. (65 mm.). The eastern part of Botswana enjoys from 15 to 25 in. (39–65 mm.) per year. Rainfall decreases towards the south-west of the country, which comprises part of the Kalahari desert. In all areas of the territory the annual rainfall shows marked variation.

The main industry in Botswana is livestock production. It was decided to investigate whether or not *Salmonella* is present in the country, and if so, to determine the incidence of infection, and also which serotypes are prevalent.

MATERIALS AND METHODS

Botswana's main abattoir, which is the only one dealing with export meat, is located in the south-east of the country. Animals from districts on the line of rail (which runs north–south along the eastern border) are railed direct to the abattoir. Animals from remote districts are walked to the line of rail and then railed. Animals from the west and south-west are either walked to the abattoir or transported by road.

Cattle are slaughtered between 12 and 48 hr. after arriving at the abattoir. The holding pens at the abattoir are provided with water.

There are four local abattoirs under constant supervision by the Veterinary Department, and numerous unsupervised slaughter houses. These deal with cattle within a radius of about 20 miles.

Samples

Pooled bovine bile samples

A total of 158 pooled bile samples were examined. All slaughtered cattle were examined before and after slaughter and no evidence of disease was found.

Of the total number of bile specimens examined, 60 samples were from local abattoirs. Each of these samples was from six to twelve cattle, representing about 33 % of the weekly kill.

The remaining 98 samples were from the export abattoir. In this abattoir the gall bladder from every animal is ruptured over a funnel. The bile passes along a pipe to a stainless-steel tank, where it is allowed to collect for 3–4 days. The bile is then drained through a tap at the base of the tank for concentration. After the tank has been emptied, the tank, pipe and funnel are sterilized by steam. Samples were collected from the tap at the base of the tank. The contents of the tank, when full, represent pooled bile from approximately 2000 animals. Samples of approximately 50 ml. were collected weekly, when the tank was full.

Meat and liver samples

A total of 752 meat samples from the neck, brisket, hump, the longissimus dorsi and the longus colli muscles were obtained from the country's export abattoir. Each sample consisted of about 150 g. From the same source, 117 liver samples were also collected. Carcasses and livers were random samples, and represented approximately 2 % of the daily kill. All samples were from apparently healthy animals.

Faeces samples

A total of 57 faeces samples from animals with gastro-intestinal symptoms were examined. In addition, faeces samples from 89 apparently healthy animals from the eastern part of the country were tested. Samples were collected over an 18-month period from March 1968 to September 1969.

Methods

Plates of deoxycholate citrate agar (Oxoid CM 227) and tubes of selenite broth (Oxoid CM 39) were inoculated with the samples. Meat samples were homogenized in a Waring blender before inoculation. After overnight incubation at 37° C. pale colonies were picked from the deoxycholate citrate plates into peptone water for further examination. A loopful of the selenite broth was subcultured onto a deoxycholate citrate agar plate after 18 hr. incubation, and again after 42 hr. incubation at 37 °C. Each plate was incubated overnight at 37° C. and pale colonies were picked as before.

Peptone water cultures of suspect organisms were tested for purity by plating on nutrient agar and MacConkey agar. When necessary, cultures were purified by subculture. Pure cultures were tested for urease production and lysine decarboxylase production. Cultures which gave a negative urease and a positive lysine

decarboxylase reaction were subjected to a range of biochemical tests (Cowan & Steel, 1966). Cultures giving the reactions of *Salmonella* were identified serologically, using firstly polyvalent antisera, and then single factor antisera. The subgenus of each salmonella isolated was determined according to Kauffmann (1965).

After 602 samples had been examined, strontium chloride enrichment medium (Iveson & Mackay-Scollay, 1969) was introduced, and this medium was used in parallel with the selenite broth in the remaining 571 samples. 'G.P.R.' grade strontium chloride (Hopkin and Williams Ltd.) and bacteriological peptone (Oxoid L37) were used in the preparation of this medium. A detailed comparison of the two enrichment media is in progress in this laboratory, to be published separately. The results of *Salmonella* isolations from the two media pertaining to the present study are detailed in Table 1.

Table 1. Salmonella *isolations from selenite broth and strontium chloride medium*

Samples examined ...	571
No. positive by	
Selenite alone	5
Selenite and strontium chloride	46
Strontium chloride alone	7

Table 2. Salmonella *serotypes from bovine bile from the export abattoir*

Serotype	No. of isolations	Serotype	No. of isolations
typhimurium	11	*newport*	2
anatum	11	*parow**	2
brancaster	7	*bovis morbificans*	1
tamale	7	*bradford*	1
essen	6	*derby*	1
saint-paul	6	*durbanville**	1
43:f,g,t:1,5*†	5	*heidelberg*	1
13,22:z_{39}:1,5,(7)*†	4	*newington*	1
jedburgh	3	*tinda*	1
dublin	2	*waycross*	1
hvittingfoss	2	1,4,12,27:z:1,5*†	1

* Subgenus II salmonellas.
† Hitherto undescribed serotypes.

RESULTS

Pooled bile samples

Salmonella spp. were isolated from two of the 60 samples from local abattoirs. The serotypes encountered were *Salm. typhimurium* and *Salm. fischerkietz.*

Thirty-nine of the 98 samples from the export abattoir yielded *Salmonella*, and more than one serotype was isolated from 24 of the samples. Twenty-two different serotypes were found, three of which were hitherto undescribed serotypes. The various types isolated, with the frequency of isolation, are given in Table 2.

Meat and liver samples

A total of 752 meat samples and 117 liver samples were received from the sites mentioned above. Isolations of Salmonellla from these are shown in Table 3.

Table 3. Salmonella *serotypes from meat and liver samples*

Site	No. of samples examined	Serotypes isolated
Brisket	237	—
Neck	128	windhoek* (2), offa (1)
Hump	119	—
Longissimus dorsi muscle	91	—
Longus colli muscle	177	—
Liver	117	braenderup (15), weltevreden (7), newington (3)

Figures in parentheses indicate the number of isolations.
* Subgenus II salmonella.

Faeces samples

Salmonellas were grown from 5 of the 57 faeces samples from sick animals (8·8 %) and from 6 of the 89 faeces samples from apparently healthy cattle (6·7 %). Serotypes found were *Salm. anatum*, *Salm. brancaster*, *Salm. donna*, *Salm. pomona* and *Salm. typhimurium* in the first group, and *Salm. colorado*, *Salm. enteritidis*, *Salm. goodwood*, *Salm. leopoldville*, *Salm.* 6,7:z:z_6 and one unidentified in the second group.

DISCUSSION

Overt salmonellosis does not appear to be common in Botswana cattle. It is probable that most infected animals contract subclinical infections and continue to harbour the organisms. Clarenburg, Vink & Schuurmans (1950) demonstrated that cattle may continue to harbour *Salm. dublin* for more than 100 days after infection, and Smith & Buxton (1951) found that 0·4 % of 750 apparently healthy cattle in England were *Salmonella* excreters.

From the isolations of *Salmonella* from pooled bile samples in the abattoirs, it appears that many serotypes are able to invade the gall bladder and to survive there. A large proportion of the isolations from meat and liver samples were from the liver, which is inevitably contaminated with bile if the gall bladder is accidentally ruptured. The rest of these isolations were from neck, which can easily be contaminated by intestinal contents during the handling of the carcass. It seems therefore that salmonellas may be confined to the biliary and intestinal tracts in apparently healthy cattle, and that invasion of the tissues by these organisms, if it occurs, is extremely rare.

Although a high salmonella carrier rate of about 7 % exists in Botswana cattle, it is encouraging that out of 752 samples of export meat tested, only three were found to contain *Salmonella*, a finding which compares favourably with many other countries. For example, a Public Health Laboratory Service working-party found

Salmonella in 1·9% of 1996 meat samples from abattoirs in England and Wales (Report, 1964).

It is interesting to note that three of the serotypes from sick animals, namely *Salm. anatum*, *Salm. brancaster* and *Salm. typhimurium*, were also isolated frequently from bile, whereas not one of the serotypes isolated from healthy cattle was found there.

It is possible that the appearance of *Salmonella* in the bile is related to the pathogenicity of the strain; on the other hand, it is also possible that serotypes such as *Salm. typhimurium* and *Salm. anatum* are able to survive longer in the gall bladder than are the more uncommon types.

A feature difficult to explain is the relative infrequency of isolation of *Salm. dublin* in Botswana. This serotype is the one most frequently observed in cattle in many countries, including Britain (Sojka & Field, 1970) and Botswana's neighbour, the Republic of South Africa (Henning, 1939). Vaccine against *Salm. dublin* is readily obtainable in Botswana, and its use is widespread. Vaccination may account in part for the paucity of isolations of this serotype.

The occurrence of a high proportion of subgenus II salmonellas, and also of higher somatic group salmonellas in Botswana cattle is of interest. Of the serotypes isolated, 43% fell into one or both of these categories. Iveson, Mackay-Scollay & Bamford (1969) suggested that in Australia salmonellas of higher somatic groups were more common in remote areas, whereas lower somatic group salmonellas were mainly confined to inhabited areas. Botswana is similar to parts of Australia both in climatic conditions and in sparseness of population, and it seems that a parallel condition exists between the two countries.

Subgenus II salmonellas are found mainly in reptiles (Taylor, 1969). A limited study (to be extended) has shown that there is a high carrier rate for *Salmonella* in reptiles in Botswana. *Salmonella* serotypes were isolated from 24 out of 30 reptiles examined, and many of these were higher somatic group salmonellas. Reptiles are abundant throughout Botswana, and it is probable that cross-infection occurs between cattle and reptiles in all parts of the country.

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