Outbreaks of *Salmonella* Dublin Infection among Calves on a Dairy Farm Applying *Salmonella* Bacterins in Zambia

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ABSTRACT. In Zambia, a dairy farm keeping about 600 cows and self-contained calves had applied *S.* Dublin and *S.* Typhimurium bacterins to pregnant cows and calves in combination with all-in all-out pen system for rearing calves. Only relatively small scale outbreaks of *S.* Dublin infection occurred repeatedly in these years from 1989 to 1991 among fattening calves on the farm. The results obtained from the epizootiological study suggest that the preventive measures including the vaccination with *Salmonella* bacterins gave insufficient protection against *S.* Dublin infection to the calves, but they might have prevented large scale outbreak of the disease. This is the first report of the epizootiological study on outbreak of bovine *S.* Dublin infection on farm in Zambia.—KEY WORDS : calf, *Salmonella* Dublin, Zambia.

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Bovine salmonellosis including infection of *Salmonella choleraesuis* subspecies *choleraesuis* serovar dublin (*Salmonella* Dublin) is an economically important disease and its wide occurrence has been reported [1, 5, 10]. In Zambia, being located southern area of Africa, salmonellosis is one of the most important diseases in cattle. Since the first isolation from cattle in 1950's (R. N. Sharma, unpublished data), frequent isolations of *S.* Dublin from bovine specimens submitted for laboratory examinations have been reported [3, 4]. However, there has been no report of epizootiological study of outbreak of *S.* Dublin infection on farms. We encountered outbreaks of *S.* Dublin infection among Holstein-Friesian calves on a dairy farm in 1989–1991 in Zambia and carried out an epizootiological study. This report will be useful to better understanding of epizootiological feature of bovine salmonellosis in Zambia.

The farm involved is in Lusaka and keeps about six hundred Holstein-Friesian cattle and only self-contained calves are reared for dairy and for fattening. Groups of these calves are kept separately in a total of 6 pens on pasture (Fig. 1). There is no shelter on the farm. All-in all-out policy had been applied for each pen with some intervals between groups of calves. On the farm, a vaccination program had been applied to all pregnant cows and calves to prevent some bacterial diseases in calves. The vaccine (Bovivac Plus — Hoechst) contained formalinized and aluminium-absorbed cells of selected *Escherichia coli* serotypes of bovine origin, strains of *S.* Dublin, *S.* Typhimurium and Robert's types 1, 2, 3 and 4 of *Pasteurella multocida*. For pregnant cows, 2 subcutaneous injections of 5 m*l* had been made at approximately 6 and 3 weeks prior to calving to reinforce the specific antibodies transferred to the suckling calf via the colostrum. For calves, 2 subcutaneous injections of 2 m*l* at approximately 10 and 24 days of age had been practiced. However, newborn calves in No. 3 pen in 1990 had been vaccinated with the bacterins at 3 and 18 days of age, one week earlier than usual, resulting in possible reduction of immunity.

In December, 1989, the first isolation of *S.* Dublin from diseased fattening calves in No. 3 pen was recorded. Histopathological examination of 2 dead calves revealed acute phase showing centrolobular necrosis in the liver, fibrinous pneumonia and few glanulomatous lesions systemically. During the outbreak, 5 out of 30 calves in the pen died of salmonellosis. Further epizootiological investigations on the outbreak were not carried out on the farm.

In the beginning of October, 1990, 18 of a group of 40 about 3 month old calves kept in No. 3 pen showed severe watery diarrhea, dysentery, loss of appetite and general weakness (Fig. 2). At that time, the farm kept 380 dairy cows and a total of 211 calves (128 heifers and 83 steers of 8 weeks to 6 months of age). The calves had been separated into 6 pens of Nos. 1–6 as indicated in Fig. 1. Except No. 6 pen where 40 heifers were reared for dairy, all 5 pens kept fattening calves. Two of the affected calves died on the 5th and 6th of October and the other one male calf with severe symptoms was submitted for diagnosis to the University of Zambia, School of Veterinary Medicine in Lusaka on the 11th of October.

The autopsied calf was 3 month old male and killed by bleeding at terminal stage. Macroscopically, pinpoint-sized whitish spots were scattered diffusely in the liver, kidney and spleen. General lymphoid tissues were enlarged, especially in mesenteric lymph nodes (Fig. 3) and tonsil. Peritonitis, serofibrinosa and edematous thickening

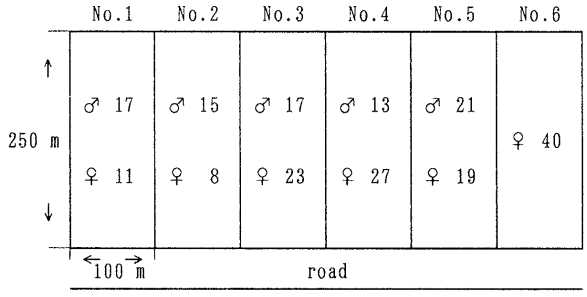

Fig. 1. Number of calves in the pens in October, 1990, and the infection seen in No. 3 pen.

of the intestinal mucosa were found. For histopathological examination, main organs of the autopsied calf were fixed in 10 % formalin and their paraffin sections were prepared and stained with hematoxylin and eosin (HE). The autopsied case revealed more chronic phase showing scattered necrotic granulomatous lesions in the liver, spleen (Fig. 4), kidney and lung. Embolic thromboses were observed generally (Fig. 5).

Pieces of heart, liver and spleen and jejunal and ileal contents of the autopsied calf were cultured on plates of blood agar, MacConkey agar, desoxycholate hydrogen sulphide lactose (DHL) agar and heart-infusion agar and into selenite broth, and incubated at 37°C for 24 hours. The plates were then examined for the growths. Subcultures from the incubated selenite broth cultures were made on MacConkey and DHL agar plates, incubated and subsequently examined. Four fecal samples obtained from the affected calves were also examined in a similar way. The postmortem materials and the 4 fecal samples from affected calves gave *Salmonella* suspect colonies. These isolates were examined biochemically for *Salmonella* and serotyped using Diagnostic *Salmonella* Antisera for O and H (Denka) and *Salmonella* H sera for Phase Induction (Denka) according to the manufacturer's instructions. Finally these isolates were identified as *S.* Dublin. These *S.* Dublin isolates from the postmortem materials were examined for antibiotic sensitivity with 6 Monodisks (Showa) using Oxoid Sensitivity Test agar. They were strongly sensitive to tetracycline, oxytetracycline, kanamycin, ampicillin and gentamicin, and moderately sensitive to streptomycin.

Intestinal contents of the postmortem materials and the 4 fecal samples from affected calves were examined parasitologically by the floatation methods. No parasitic ova or coccidial oocysts were detected from any samples examined.

Results obtained from the pathological, bacteriological and parasitological examinations described above indicated that *S.* Dublin infection broke out on the farm. Then the following measures for treatment and control of salmonellosis were taken on the farm.

All affected calves were soon transferred from No. 3 pen into an isolation pen. Moreover, medication for affected calves with chemotherapeutics started. One bolus of Cotrox (Interchem, Zambia) was given to the calves twice daily for 6 days. Each bolus contained 1.0 g sulfadiazine and 0.2 g trimethoprim which were supposed to have potentiation against *Salmonella* (M. Ngoma, unpublished data). All affected calves medicated recovered within 3 to 4 days after treatment. On the 15th of October, all calves in the pens Nos. 1–6 received one booster vaccination with the bacterins mentioned above. Other control measures such as cleaning and disinfection of pen environment except mangers, and detection of excretors of *S.* Dublin from calves and cows to prevent prolongation of the outbreak of salmonellosis [10] were not practiced. Since this outbreak in 1990, no further outbreak of *S.* Dublin infection had been recorded on the farm until June in 1991 when a small scale outbreak was confirmed among calves (D. S. Misra, unpublished data). Unfortunately no detailed data of the outbreak were available.

No attempt to search the source of *S.* Dublin infection among calves was made at the time of the outbreak in 1990. However, as this farm kept only self-contained calves, possibility of introduction of *S.* Dublin from outside source seemed to have been very low or have been negligible. On the other hand, there seemed to have been 2 possible sources of infection of *S.* Dublin after the outbreak in 1989. One of the possible sources seemed to be environmental contaminations with *S.* Dublin such as soil, water and others in the No.3 pen where affected

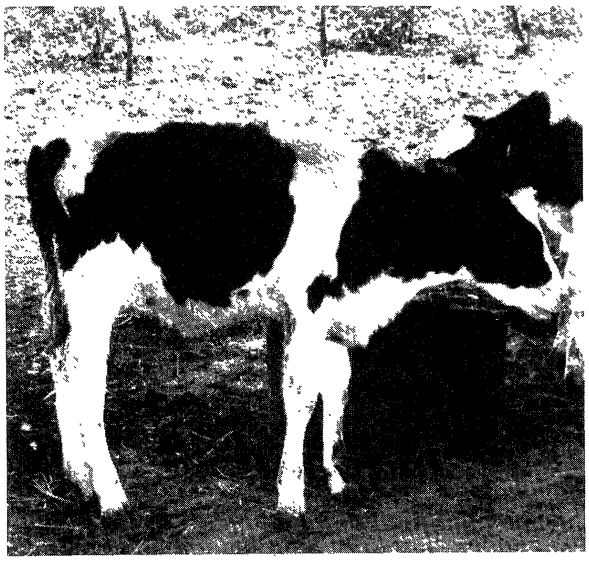

Fig. 2. An affected calf in the isolation paddock showing diarrhea, weakness and emaciation.

Fig. 3. Conspicuous enlargement of mesenteric lymph nodes.

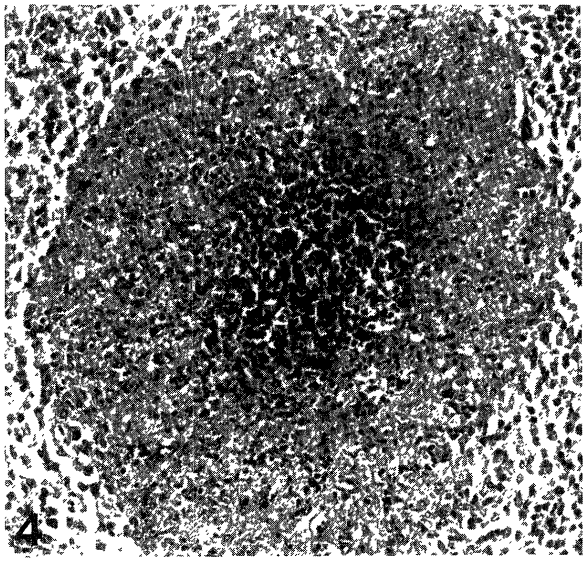

Fig. 4. Granuloma seen in the spleen. HE stain. × 155.

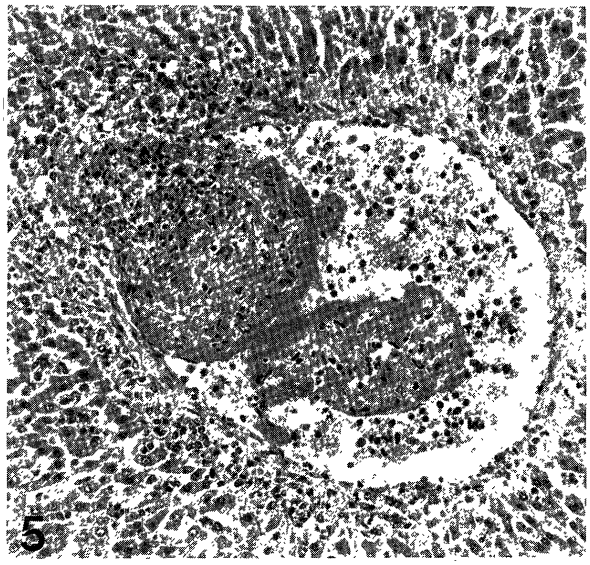

Fig. 5. Thrombosis seen in the central vein in the liver. HE stain. × 155.

calves had been kept during the outbreak in 1989. Although *S.* Dublin survived for at least 1,069 days in artificially contaminated and dried feces and probably died out within 6 months in infected feces out of doors while in feces splashed on walls it might survive up to 10 months [10], no information has been given about the survival of *S.* Dublin in pen environment under weather conditions in Zambia with about 3 months' hot dry season (September to November) with strong sun light and with about 5 months' hot rainy season (December to April) with heavy rainfall [7]. Since *S.* Dublin has higher ability to produce active carrier in cattle than other *Salmonella* serovars [10], another one of the possible infection sources of *S.* Dublin seemed to have been incidence of carrier cattle.

It has been reported that, although vaccines derived from killed organisms do result in increased resistance, it appears that a more solid immunity results from either natural infection or vaccination with living *Salmonella* [8, 10]. Vaccination of the calf and the dam with subsequent colostral passage of antibody to the calf have been studied, using formalin-killed aluminium hydrooxide-precipitated vaccines [2, 6]. *S.* Dublin bacterins were found to protect calves against homologous exposure [2], but Smith *et al.* [9] pointed out the results lacked statistical validity, due to the small number of calves used. Henning [6] in South Africa reported that, when the colostral immunity was challenged with virulent *S.* Dublin cultures given orally the calves exhibited a fair degree of resistance. Thus, he believed that this immunity is sufficient to protect young calves against natural exposure to paratyphoid, and he stated that the immunization of pregnant cows as a means of protecting new-born calves against paratyphoid is recommended an additional method of combating the diseases.

As stated above, only relatively small scale outbreaks of *S.* Dublin infection occurred repeatedly in these years from 1989 to 1991 on the dairy farm conducting the preventive measures such as the vaccination with *Salmonella* bacterins in combination with the all-in all-out pen system. The results obtained from our epizootiological study suggest that the preventive measures including the vaccination with *Salmonella* bacterins gave insufficient protection against *S.* Dublin infection to the calves on the farm, but they might have prevented large scale outbreak of the disease.

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