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CORRELATION BETWEEN BACTERIOLOGICAL AND SEROLOGICAL EXAMINATION FOR THE DETECTION OF SALMONELLA CARRIERS ON BUFFALO CALVES.

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SUMMARY: Ninety apparently healthy buffalo calves (one month old) were put under observation at El-Marg Farm for 9 months to study the correlation between cultural and serological examination of salmonella carriers. Salmonella strains were isolated from 25 out of 90 calves about one month old. The isolates belonged to 3 serotypes namely *S.tshiongwe* (group C), *S. anatum* (group E) and *S.worthington* (group G).

Tube agglutination test revealed the presence of salmonella antibodies in bacteriologically positive cases until they disappeared from the blood at the 8th month, while salmonella organisms were shed intermittently in the faeces till they disappeared at the 6th month. The repeated serological examination of the serum samples of the 90 buffalo calves revealed the presence of 'O' agglutinin titres that varied from 1:80 to 1:320 and 'H' agglutinin titres up to 1:640 in all bacteriologically positive calves.

INTRODUCTION

Bovine salmonellosis has attracted increasing attention during recent years. The problem of salmonellosis in calves is important not only from the economical point of view but also from the aspect of public health since the disease is transmissible to man.

Cattle and buffaloes of all ages are susceptible to infection with various salmonella organisms. In adults the disease is usually sporadic while in calves the infection spreads more rapidly (Lotfi and Kamel, 1964; Medanic et al .,1966 Robinson, 1966, El-Ghool et al., 1968, Markovic et al.,

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1973). Recovered cases may remain carriers and consequently constitute a potential disseminating factor of the infection.

In view of the role played by salmonella infection in calves on both economy and public health, the present study was designed to study the incidence of salmonella infection in buffalo calves and to follow up the positive salmonella carriers by cultural and serological methods until the organism and antibodies are no more detectable.

MATERIAL AND METHODS

The present study was performed on 90 buffalo calves immediately after their arrival at El-Marg Farm (Egyptian Company for Meat and Milk Production). The calves were isolated in a separate stable for 9 months, where they were given special attention and cleanliness measures. Blood and faecal samples (1080 each) were collected weekly during the first month, then monthly throughout the remaining 8 months.

Faecal samples from each of the examined calves were enriched into selenite F and tetrathionate broth for 18 h at 37 °C and subcultured onto MacConkey and S.S. agar media for 24 - 48 hours at 37 °C. Suspected non-lactose fermenting colonies were examined biochemically and typed serologically using salmonella polyvalent and monovalent diagnostic antisera "Wellcome" according to Edwards and Ewing (1972).

Heated O-antigensand formalized H-antigens were prepared from the three locally isolated faecal strains according to Kauffmann (1973) for agglutination test. The tube agglutination test was carried out on blood serum samples for 9 months to determine the end titre of the strain specific 'O' and 'H' salmonella agglutinins.

RESULTS

Bacteriological follow-up of salmonella carriers in a calf farm

From 1080 faecal samples collected from 90 calves during a period of 9 months, 118 salmonella isolates could be recovered giving an incidence of 10.9 %.

On the first week of the study, salmonella was isolated from 15 calves (16.6 %). The weekly repetition of isolation during the first month showed that the number of salmonella carriers has increased in the second week to 22 cases (24 %), in the third week to 25 cases (27.7 %), but in the fourth week only 15 cases (16.6 %) yielded positive salmonella cultures (Table 1).

Table 1: Bacteriological and serological follow-up of salmonella positiv cases for a period of 9 months.

Time of examination	Cultural examination		Serological examination	
	Number of positive cases	%	Number of positive cases	%
1st month				
1st week	15	16.6 %	25	27.7 %
2nd week	22	23.3 %	25	27.7 %
3rd week	25	27.7 %	24	26.6 %
4rd week	15	16.6 %	23	25.5 %
2nd month	15	16.6 %	22	24.4 %
3rd month	11	12.2 %	20	22.2 %
4th month	7	7.7 %	16	17.7 %
5th month	6	6.6 %	12	13.3 %
6th month	2	2.2 %	12	13.3 %
7th month	0	0	8	8.8 %
8th month	0	0	3	3.3 %
9th month	0	0	0	0
	118	10.9	190	17.6

Salmonella carriers in buffalo calves

The monthly repetition of bacteriological examination of the same calves for 9 months revealed the continuation of excretion of salmonella up to the 6th month, however the number of excretors decreased gradually so that only 2 calves voided salmonella in their faeces in the 6th month.

During the whole period of study, a total of 118 isolates recovered from 25 calves were identified serologically as *S. tshiongwe* (45.8 %), *S. anatum* (40.7 %) and *S. worthington* (13.5 %).

Serological follow-up of salmonella carrier in a calf farm

As shown in **Table 1**, all the 25 bacteriologically positive cases were serologically positive from the start of the study. On the other hand, the remaining (65) bacteriologically negative cases were all negative serologically. The titres of positive cases varied from 1:80 to 1:160 in case of O-agglutinins and 1:160 to 1:640 in case of H-agglutinins. The titre of both agglutinins decreased gradually and consequently. The serologically positive cases decreased so that only 3 positive cases could be confirmed on the 8th month. On the 9th month, all calves were negative.

The results of bacteriological and serological examinations of the 25 positive cases are presented in **Table 2**. The repeated examination of these cases led to the isolation of salmonella from the faeces in most of them, however, in two cases (No.14 and 17) salmonella could be isolated only once in the 2nd and 3rd months respectively and the faecal samples from these two cases remained negative during the whole period of study although agglutinins could be detected up to 7th month at a titre varying from 1:80 to 1:160. In case No.20 we failed to detect the organism in the faeces although agglutinins were found from the 1st week up to 8th month in a titre which reached up to 1:320.

Table 2:Bacteriological and serological follow up of the individual salmonella positive case for a period of nine months.

Positive calvesNo.	1st month	2nd month	3rd month	4th month	5th month	6th month	7th month	8th month	9th month	isolated salmonella serotypes
1	+ / +	- / +	- / +	+ / -	- / -	- / -	- / -	- / -	- / -	S. anatum
2	+ / +	- / +	+ / +	+ / -	- / -	- / -	- / -	- / -	- / -	S. anatum
3	+ / +	- / +	+ / +	+ / +	- / +	- / +	- / +	- / -	- / -	S.tshiongwe
4	+ / +	+ / -	- / -	- / -	+ / -	- / -	- / -	- / -	- / -	S.anatum
5	+ / +	- / +	+ / +	- / -	- / -	- / -	- / -	- / -	- / -	S.anatum
6	+ / +	+ / +	+ / +	+ / +	- / +	- / +	- / +	- / -	- / -	S.tshiongwe
7	+ / +	+ / +	+ / +	- / +	+ / +	- / +	- / +	- / -	- / -	S.worthington
8	+ / +	- / -	- / -	+ / -	- / -	- / -	- / -	- / -	- / -	S.tshiongwe
9	+ / +	+ / +	- / +	- / +	+ / -	- / -	- / -	- / -	- / -	S.tshiongwe
10	+ / +	+ / +	+ / +	- / +	+ / +	+ / +	- / +	- / +	- / -	S.anatum
11	+ / +	- / +	+ / -	- / -	- / -	- / -	- / -	- / -	- / -	S.tshiongwe
12	+ / +	+ / +	- / +	- / +	- / +	- / +	- / -	- / -	- / -	S.worthington
13	+ / +	+ / +	- / +	- / +	- / +	- / +	- / -	- / -	- / -	S.anatum
14	- / +	- / +	+ / +	- / +	- / +	- / +	- / +	- / -	- / -	S.anatum
15	+ / +	+ / +	- / +	+ / +	- / +	- / +	- / -	- / -	- / -	S.tshiongwe
16	+ / +	+ / +	+ / +	- / +	- / -	- / -	- / -	- / -	- / -	S.tshiongwe
17	- / +	+ / +	- / +	- / +	- / +	- / +	- / +	- / -	- / -	S.tshiongwe
18	- / +	+ / +	- / +	- / +	- / -	- / -	- / -	- / -	- / -	S.anatum
19	- / +	+ / +	- / +	- / +	- / -	- / -	- / -	- / -	- / -	S.anatum
20	- / +	- / +	- / +	- / +	+ / +	- / +	- / +	- / +	- / -	S.tshiongwe
21	- / +	+ / +	- / -	- / -	- / -	- / -	- / -	- / -	- / -	S.anatum
22	- / +	- / +	+ / +	- / +	- / +	- / +	- / -	- / -	- / -	S.tshiongwe
23	- / +	- / +	+ / +	+ / -	- / -	- / -	- / -	- / -	- / -	S.worthington
24	- / +	+ / -	- / -	- / -	- / -	- / -	- / -	- / -	- / -	S.tshiongwe
25	- / +	+ / +	- / +	- / +	+ / +	+ / +	- / +	- / +	- / -	S.tshiongwe
TOTAL	15/25	15/22	11/20	7/16	6/12	2/12	0/8	0/3	0/0	

++ Bacteriological /agglutination titre 1/80 or more.

x = Result of the first week of the first month.

From **Table 2** it is evident that salmonella was excreted intermittently in the faeces of most cases e.g. calves No. 1 and 4. In the first case, the faecal samples were negative in the 2nd and 3rd months then salmonella was excreted again in the 4th month then disappeared. In the second case, *S. anatum* could not be isolated in the 4th week of the 1st month but it was recovered again in the 2nd month then disappeared during the 3rd and 4th months and could be isolated on the 5th month.

DISCUSSION

The incidence of salmonella infection recovered in the present study among buffalo calves is significantly high when compared with the results of other authors who studied the salmonella carriers state in buffalo calves in Egypt. The rate of infection recorded by Kamel and Lotfi (1964) was 4.1 %. In the following year Lotfi and Kamel reported an incidence of 9.1 %. Zein El-Abden et al. (1966) recorded an incidence of 2 %. A salmonella carrier rate of 7.7 % was published by El-Amrousi et al. (1971), 9.3 % by Abdel-Galil et al. (1972), 10 % by Saad (1978) and 1.1 % by Abou Zeid (1979).

The high rate of carrier state among buffalo calves reported in this study demonstrates the importance of repeated examination particularly in view of the intermittent voiding of salmonella in faeces.

The isolation of 3 salmonella serotypes from the examined calves namely *S. tshiongwe*, *S. anatum* and *S. worthington* is certainly of epidemiological and public health significance since the presence of carriers with various salmonella serotypes in the farm constitutes a multiple risk of infection not only to other calves but also to human beings (Stewart and Decapito, 1953 and Hibbs and Foltz, 1964).

The intermittant excretion of salmonella in the faeces of carrier animals has been also described by Thornston (1957) who claimed that animals that recovered from

salmonella infection continued to excrete the organism in the faeces either continuously or intermittently for years if not for life. In the present study, the maximum time of excretion of salmonella in the faeces was however less than one year and in fact not more than 6 months.

The repeated serological examination of the 90 calves revealed the presence of O-agglutinins in titres which varied from 1:80 to 1:320 and H-agglutinins in titres up to 1:640 in all bacteriologically positive calves. On the other hand, the bacteriologically negative calves showed no detectable agglutinins i.e. the bacteriological and serological results were in agreement. However, in view of the intermittent nature of salmonella excretion in faeces, the serological examination of carrier calves is recommended for the follow up of such cases. Wray et al. (1977) mentioned that the results of their serological studies indicated that in the majority of cases diagnosis would have to be made on the basis of the somatic agglutinins. They considered somatic titres of 1:80 and above indicative of infection. The corresponding figures for the 'H' titre by Gibson (1965) were 1:40, 1:80, 1:160 and 1:320. This is quite in complete agreement with our results.

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