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ANTIGEN CAPTURE ELISA TECHNIQUE FOR RAPID DETECTION OF *SALMONELLA TYPHIMURIUM* IN FAECAL SAMPLES OF DIARRHOEIC COW CALVES.

(With 2 Tables)

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اختبار الأليزا بالقبض الأنتيجينى فى سرعة الكشف عن السالمونيلا تيفيميوريم فى براز العجول البقرى المصابة بالأسهال.

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تم فحص ٦٦ عينة براز من عجول بقرى مصابة بالإسهال بالطرق التقليدية للزرع وطريقة الأنتيجين القابض للأليزا. تم عزل ١٢ عترة من السالمونيلا تيفيميوريم بنسبة ١٨,٢ % بالطرق التقليدية بينما تم عزل ١٦ عترة من السالمونيلا تيفيميوريم بنسبة ٢٤,٢ % بطريقة الأنتيجين القابض للأليزا. بمقارنة طريقتين للتحضين قبل أستخدام الأليزا وجد ١٣ عينة إيجابية باستخدام شوربة التتراثيونات فقط قبل طريقة الأليزا بنسبة ١٩,٧ % بينما وجد ١٦ عينة إيجابية باستخدام شوربة التتراثيونات ثم شوربة الإم بنسبة ٢٤,٢% قبل طريقة الأليزا. وجد ان طريقة الأنتيجين القابض للأليزا سرعة وحساسة فى الكشف عن السالمونيلا فى الحيوانات المصابة.

SUMMARY

A total of 66 faecal samples was collected from cow calves suffering from profuse watery diarrhoea and examined by conventional cultural method and antigen capture ELISA. Twelve strains of *Salmonella typhimurium* were isolated in a percentage of 18.2 % by culture method while 16 samples were detected as positive for *Salmonella* infection with a percentage of 24.2 % by antigen capture ELISA. Comparison of two

pre-enrichment procedures by ELISA test revealed that 13 samples were positive with tetrathionate broth and subjected directly to ELISA test with a percentage of 19.7 %, while 16 samples were positive using M-broth after tetrathionate enrichment with a percentage of 24.2 %. Antigen capture ELISA was rapid and sensitive for identification of *Salmonella* infected animals.

Key Words: *Elisa, Salmonella Typhimurium, Diarrhoea, Calves*

INTRODUCTION

Bovine salmonellosis is an economically important disease as a public health problem (Corrier et al., 1990). Although bovine salmonellosis affects cattle of all ages, calves are more susceptible to infection than adults (Wray and Sojka, 1981). It is also known that newlyborn calves could be infected with *Salmonella* at time of parturition or sooner after birth (Jones et al., 1983 and Peel et al., 1990). *Salmonella typhimurium* plays an important role in calf diarrhoea (McLarn and Wray, 1991 and Lance et al., 1992) and was isolated in an endemic area in a high incidence.

The faecal excretion of *Salmonella* by infected calves may be intermittent and multiple faecal samples collected over several days may be required to detect the infection (Palmer et al., 1985). Subsequently, it is impossible to determine exactly the rate of *Salmonella* infection in a herd depending on one day faecal sample (Wray, 1985).

The detection of *Salmonellae* in faeces and clinical samples involves a series of enrichment steps because these pathogens, when present in the samples are somehow found in low numbers and sublethally injured (Minnich et al., 1982). Therefore, detection methodologies for *Salmonellae* must be sensitive and allow for resuscitation and growth initiation of injured cells.

Faecal culture may under estimate the herd prevalence rate of *Salmonella*. The time consuming and the insensitivity of faecal culture for diagnosis of *Salmonella* infections were the important factors affecting this diagnosis (Lance et al., 1992). So, the use of ELISA assay is very important to overcome the disadvantages of the conventional method and to detect the presence of *Salmonella* microorganisms in faecal samples of diarrhoeic calves in a more rapid and sensitive manner which would help

in the rapid treatment of diseased cases and control the *Salmonella* infections in farm animals.

In this study, the usefulness of the antigen capture ELISA, following two enrichment methods, in detecting *Salmonella typhimurium* in faecal samples of diarrhoeic calves was determined and compared with culture procedures.

MATERIAL and METHODS

Faecal samples:

A total of 66 faecal samples were collected from calves of 2-3 months old suffering from profuse watery diarrhoea . Each sample was divided into 2 portions, the first one was subjected to cultural method and the second portion was prepared and tested by enzyme immunoassay for the detection of *Salmonella typhimurium*.

Cultural method:

Approximately 1 gram of faeces sample was placed into 8 ml of tetrathionate broth (Oxoid) for enrichment and incubated at 43°C for 24 hours. Broth cultures were then streaked on Hekton enteric agar (Oxoid) plates and Salmonella-Shigella (Oxoid) plates and incubated at 37°C for 24 hours (Emswiler et al., 1984 and Pelton et al., 1994).

Suspected growing colonies were identified by colony characteristics and biochemical reactions according to Krieg and Holt (1984). All tests were done using media from Difco Laboratories, Detroit, Mitchigan, USA.

Antigenic characterization was performed according to Edwards and Ewing (1972) and Kauffmann (1973) using a slide agglutination test with monovalent "O" and "H" antisera against *Salmonella typhimurium* obtained from Wellcome Diagnostics, Dartford, England.

Enzyme immunoassay:

- **Faecal preparation:**

Each faecal sample was suspended in 9 volumes of tetrathionate broth (Emswiler et al., 1984) at 42°C for 24 hours. Five milliliters of tetrathionate broth culture were mixed with 5 ml of Rhozyme 41 (a commercial protease) solution (1 % in 0.01 M phosphate buffer [pH 8.0] with 0.7 % Tween 20), incubated at 37°C for 1 hour (Zierdt, 1982 and Rigby, 1984) to eliminate non specific reactions. One ml of the incubated broth was then transferred to tube containing 10 ml of M-broth (Difco)

with 10 µg of novobiocin to enhance flagellar production by bacteria (Desmidt et al., 1994) and was incubated at 42°C for 6 hours. The M-broth culture was centrifuged at 1000x g for 20 minute. The cell pellets were resuspended in 2 ml phosphate buffered saline (PBS, pH 7.4) and heated for 1 hour in boiling water bath then stored at 4°C until used for ELISA test. Optimum dilution of test sample was 1: 100 in bovine serum albumin (Kirke Gard and Perry Laboratories, Inc. USA).

• **Preparation of flagellar hyperimmune sera:**

The clinically normal Boskat rabbits of about 1.5 - 2 kg proved to be free of *Salmonella* were used for preparation of flagellar hyperimmune serum aginst *Salmonella typhimurium* (local strain, obtained from Serology Unit, Animal Health Research Institute) according to the methods of Hartman and Minnich (1981). Repeated serum samples were collected from the injected rabbits regularly at 15 days intervals to obtain the highest antibody titre. The antibody level was measured by tube agglutination test according to the method of Cheebrough (1985). The obtained antisera undergoes purification and fractionation to obtain IgG fraction used in an ELISA test by using gel filteration technique (Fey, 1979). Purification was done to overcome the false positive reaction in ELISA due to presence of cross-reacting (Swaminathan et al., 1985). Optimum dilution of used antisera was 1:50 in phosphate buffer saline.

• **ELISA procedure:**

Two pre-enrichment procedures were tested, the first procedure using tetrathionate broth and the second using M-broth after tetrathionate enrichment. The antigen capture ELISA was performed according to the method described by Rigby (1984) and Desmidt et al. (1994). The microtitre plate wells were coated (100 µl per well) by the flagellar hyperimmune sera (anti-H IgG) against *Salmonella typhimurium* in a dilution of 1:50. The prepared samples were added as 100 µl per well to allow capture of *Salmonella* antigen, incubate the plates at 37°C for 1 hour at moist atmosphere. Control positive and control negative samples were included. The wells were washed 6 times and 100 µl of goat anti-rabbit IgG was diluted as 1:2000 in bovine serum albumin, was added to each well, which would bind to the captured *Salmonella* antigen. The plates were incubated at 37°C for 1 hour, then washed 6 times to remove the unbound conjugate. ABTS (2,2`, Azino-di-3-ethyl-benzthiazoline sulfonate) substrate was pipetted (100 µl) into each well and allowed to react for 15 minute at room temperature. The stop solution was added

and the plates were read within 5-10 minutes on microELISA reader at 405 nm. The sample was considered positive if the absorption value was greater than or equal to average absorption of the negative control plus 0.25.

RESULTS

Results of conventional culture method:

The bacteriological examination of 66 faecal samples collected from calves suffering from profuse watery diarrhoea was applied and revealed that 12 strains of *Salmonella typhimurium* were isolated in a percentage of 18.2 %.

These isolates were identified as *Salmonella typhimurium* according to their cultural characterstics, biochemical, and finally their serological behaviour.

Results of ELISA immunoassay:

The using of ELISA test for detection of *Salmonella typhimurium* from faecal samples of diarrhoeic calves revealed that 16 samples were detected as positive for *Salmonella* infection out of 66 tested samples with a percentage of 24.2 % as shown in Table (1). Only twelve of these positive samples were also positive by the culture method.

Table 1. Results of ELISA test in comparison with culture method for detection of *Salmonella typhimurium* in faecal samples.

Type and No. of Samples	Positive by both methods	Negative by both methods	EIA positive, culture negative	EIA negative, culture positive
66 faecal samples	12	50	4	0

Comparison of the two pre-enrichment procedures by ELISA test revealed that 13 samples were positive with tetrathionate broth and subjected directly to ELISA test with a percentage of 19.7 %, while 16 samples were positive using M-broth after tetrathionate enrichment with a percentage of 24.2 % (Table 2).

Table (2): Comparison of the ELISA test after tetrathionate broth and tetrathionate with M-broth.

Type and No. of Samples	ELISA method	Positive	
		No.	%
66 faecal samples	Tetrathionate broth	13	19.7
	Tetrathionate with M-broth	16	24.2

DISCUSSION

Salmonellosis in calves is still an important problem throughout the world which has both zoonotic and economic importance (Corrier et al., 1990). Calves are susceptible to *Salmonella* infection and several outbreaks were recorded causing severe economic loss. On the other hand, infected calves disseminate the organisms in their faeces and hence spread the infection to other animals (Losinger et al., 1995).

In this study, 66 faecal samples from diarrhoeic calves were examined using conventional culture method and ELISA assay. By the culture method, 12 strains of *Salmonella typhimurium* were isolated and identified biochemically and serologically with a percentage of isolation reaching 18.2 %. The high incidence rate of *Salmonella typhimurium* among calves suffering from diarrhoea was reported in Egypt by Khalil (1988) who isolated *Salmonella typhimurium* in 20.6 % among calves. Also, McLarn and Wray (1991); Lance et al. (1992) and Shah and Hala (1992) proved that, *Salmonella typhimurium* was the most commonest of salmonellosis in diarrhoeic calves of 4 months-1 year old and the rate of isolation ranged from 15 %-21 %. The same observation was recorded by Battisti et al. (1994) and Hemmatzadeh and Salemi (1994) who recovered *Salmonella typhimurium* from diarrhoeic calves in an incidence of 15.5 %.

Positive results by culture methods require two conditions. First, sufficient numbers of *Salmonellae* must be present in the inoculated selective enrichment medium to assure one or more *Salmonella* cells in aliquots streaked onto plates. Second, the relative proportion of *Salmonellae* to other organisms capable of growth on the selective/differential isolation media must be such that at least one isolated colony of *Salmonella* can be obtained (Flowers et al., 1995).

In the present study, the ELISA assay was used for detection of *Salmonella typhimurium* in faecal samples of diarrhoeic calves, the results which were obtained within 2 days detected 16 cases of *Salmonella typhimurium* (24.2 %) compared with (18.2 %) of positive samples by conventional culture method. All positive samples by culture method were positive also ELISA test. In this respect, Emswiler et al. (1984) proved that no positive samples by culture method were negative by ELISA test. The same observation was reported by Alexio et al. (1984) who detected *Salmonella typhimurium* by ELISA test in a

percentage of 42.5 % while by using the culture method the percentage was 32.5 %.

In this study, antigen capture ELISA technique was rapid (2 days) and sensitive when compared with the culture method. Several authors applied different techniques of ELISA including antigen capture method for detection of *Salmonellae* in different clinical and environmental samples with a highly sensitive results which agreed with antigen capture ELISA Rigby (1984); Minga (1988); Drew and Wilcock (1990) and Desmidt et al. (1994). Also, Smith et al. (1994) used successfuly the antigen capture ELISA for detection of *Salmonella typhimurium* in faecal samples of calves and cows.

In this study, 13 samples were positive with pre-enrichment tetrathionate broth and subjected directly to ELISA test, while 16 samples were positive using post-enriched M-broth after tetrathionate pre-enrichment. This is because the number of *Salmonella* in the preenriched broth of these samples was not within the range of sensitivity of the assay and the presence of some contaminant which grew in the pre-enrichment broth would interfere with the binding of *Salmonella* antigen to immoblized capture antibody. This finding agrees with that of Swaminathan et al. (1985) who reported that 75 % of total positive samples tested after post-enrichment broth were positive after pre-enrichment broth only.

Blackburn and Patel (1990) and Desmidt et al. (1994) reported that ELISA assay was easy to use and not labour intensive and results were obtained 1 - 2 days earlier than the conventional culture methods for detection of *Salmonella* and can detect any small number of microorganisms in faecal samples even 5×10^4 C.F.U / ml. From the present study, it can be concluded that the antigen capture ELISA was rapid and sensitive for the identification of *Salmonella* infected animals.

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