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DETECTION OF CORONAVIRUS ANTIGEN IN FAECAL SAMPLES OBTAINES FROM NEWBORN BUFFALOE CALVES

BY

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INTRODUCTION

Diarrhea remains one of the most important causes of calf mortality. An attempt has been made to gather current relevant clinical information relating to the aetiology of calf enteritis especially that of viral origin. The association between coronavirus infection and diarrhea in calves was studied by Mebus and Coworkers (1973 a and 1973 b).

Methods of diagnosing coronaviral infections of calves were studied clinically and pathologically (Stair et al., 1972; and Marin et al., 1974). Virological examination for coronavirus in calves was studied by Patel et al. (1982), where the virus was detected by indirect immunofluorescence and electron microscopy.

In this work, three different methods were used for detection of coronavirus antigen(s) in faecal samples of newborn Egyptian buffalo calves suffering from diarrhea. These were the Enzyme-Linked Immunosorbent Assay (ELISA), the Antigen Sopt Test (AST) and the Immunoperoxidase (IP) technique.

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MATERIALS AND METHODS

Collection of Samples:

86 faecal samples were collected from newborn buffalo calves (1-3 months old) located at a breeding governomental farm at kafr El-Sheikh governorate. 58 animals of these calves were suffering from diarrhea. Swabs were taken and moisted in sterile bottles, each containing 5 ml Minimal Essential Medium + 50 mg Gentamycin + 50 mg streptomycin and 100 I.U. penicilline per ml for each sample. Samples were then centrifuged at 300 r.p.m. for 30 minutes, the supernatent fluids were tested for bacterial contamination, and kept at -70°C untill used.

1. Detection of coronavirus antigen by Enzyme Linked Immunosorbent Assay (Elisa):

Materials:

- Virus: Nebraska reference virus strain was used as positive control antigen.
- Normal Minimum Essential Medium was used as nigative control antigen.
- Bovine corona mouse ascites clone I. were used as coronavirus-specific monoclonal antibodies.
- Bovine anticorona serum.

  These reagents were kindly supplied by Institute Fur Med. Mikrobiologie, Munchen, West Germany.
- Antibovine horseraddish peroxidase conjugate (Sigma).

Method:

A double antibody sandwich Elisa method was carried out according to Voller et al. (1979). Plates (Nunc) were coated with Bovine corona mouse ascites clone I monoclonal Abs. diluted 1:2000 (100 µl/well) and

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incubated overnight at + 4°C. Plates were washed 3 times with PBS pH 7.4 containing 0.05% Tween 20. Faecal samples were added (100 µl/well) and incubated at 37°C for 1 hour on a shaker,then kept at + 4°C overnight. Samples were discarded, plates were washed 3 times, then bovine anticorona serum diluted 1/50 was added (100 µl/well) and incubated for 2 hours at 37°C. Plates were then washed, antibovine peroxidase conjugate diluted 1:10000 was added and incubated for one hour. Substrate was then added (100 ml of citric acid buffer pH 5+40 mg 1.2 phenylenediamine dihydrochloride +20 µl 30% H_2O_2) for 15 minutes. Reaction was stopped by H_2SO_4 and plates were read on MCC titertek multiscan at 492 nm. Optical density values were detcted and the sample was considered positive if it is two times more than the negative control.

2. **The antigen spot test: (AST)**

The test was carried out according to Paul et al. (1982). A nitrocellulose filter was divided into pieces 4x4 mm squres (86 pieces for the samples). Each was placed in a microtitre well of tissue culture plates (24 wells per plate). The filters were washed with bidistilled water for 5 minutes, air dried, and 20 µl of each sample was blotted into each filter. The samples were left to dry thoroughly and kept at + 4°C overnight. Antigen-dotted filters were washed for 5 minutes in Tris buffer saline (50 mM Tris-HCl, 200 mM NaCl, pH 7.4, 0.05% Tween 20) then to each well 150 µl of PBS pH 7.4 plus, 10% normal horse serum were added and incubated for two hours, for blocking of nonspecific antigen binding sites on the filters. The blocking solution was aspirated and 100 µl of rabbit anticorona serum diluted 1:40 (diluted in the blocking solution) was added to each well and plates reincubated for one hour. The antibody solution was discarded and another blocking step was

repeated. Filters were washed, and 100 µl of anti-rabbit horseradish peroxidase conjugate (diluted 1:1000) were added for each of the samples and incubated at 37°C for one hour. The conjugated antibody solution was removed and filters were washed 3 times changes of TBS. The specific antigen-antibody reaction could be visually read by adding the membrane in freshly prepared enzyme substrate (4-chloro-naphthol, prepared as a 3 mg/ml stock solution in pure methanol). Just before use, 1.7 ml of the stock solution were mixed with 8.3 ml of TBS and 30 µl of H_2O_2 (3%). The enzyme reaction was stopped after 10-15 min. by washing the membrane with tap water. Specific positive reactions appeared as dark blue dots at the individual sites of the dotted antigens.

3. Immunoperoxidase technique (IP):

For performing the IP test (according to Pan et al., 1982), confluent monolayers of vero cells grown in 96 wells tissue culture plate (Nunc) were washed with 100 µl of media containing trypsin (25 µg/ml) for 10 minutes. Trypsin was discarded and samples were inoculated (100 µl/well). Control non-infected cells were included, then the plate was incubated at 37°C with 5% Co_2 incubator for 72 hours.

Staining of the plate: Media were discarded and cells were washed with PBS pH 7.6 then fixed in 20% acetone PBS, and left to dry at 70°C for 1 hour. Bovine corona mouse ascites clone I diluted 1:2000 was added to the fixed cells and incubated at 37°C for 1 h. Plate was then washed with PBS pH 7.4 plus 0.05%. Tween 20. Anti-mouse horradish peroxidase diluted 1 : 500 (100 µl/well) was added to the cells for 1 hour at 37°C. The plate was washed 3 times and the substrate was added (100 ml 0.05 mM. Tris buffer, 50 mg 3,3 Diaminobenzidine, and 10 µl 30% H_2O_2) for 15 minutes. Cells were examined under the microscope.

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RESULTS

1. Detection of corona virus antigen by Elisa:

Corona virus antigen could be detected in 65 samples and 21 samples were clearly negative. The optical density value (O.D.) of the negative control was 0.137 and the cut off value (2 times of the -ve control) was 0.274. The results of the test are presented in Table (1).

Table (1): Optical density values of coronavirus antigen in faecal samples.

A	0.765	0.649	0.572	0.457	0.658	0.444	0.916	0.198	0.370	0.154	0.135
B	0.333	0.508	0.639	0.563	0.595	0.467	0.362	0.362	0.413	0.358	0.342
C	0.665	0.644	0.620	0.387	0.573	0.803	0.747	0.271	0.204	0.270	0.216
D	0.718	0.583	0.795	****	0.887	0.433	0.266	0.222	0.166	0.145	0.180
E	1.691	0.568	0.560	1.790	0.473	0.342	0.349	0.340	0.233	0.256	0.356
F	1.946	0.470	0.410	1.710	0.366	0.358	0.262	0.355	0.228	0.221	0.212
G	0.979	0.603	0.578	1.701	0.414	0.368	0.368	0.259	0.243	0.282	+vec 1.652
H	1.107	0.782	0.452	0.712	0.424	0.731	0.441	0.389	0.209	0.272	-vec 0.137

- ve C = negative control + ve C = positive control

Figures in the table represents the optical density values (O.D.)

*** = O.D. more than 2.0

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2. The antigen spot test (AST):

Coronavirus antigen could be detectected in faecal samples of newborn buffalo calves by AST. The positive samples developed dark blue colour on the nitrocellulose filters while no colour developed for the negative samples (Photo. 1). Only 46 samples gave positive reaction by the antigen spot test.

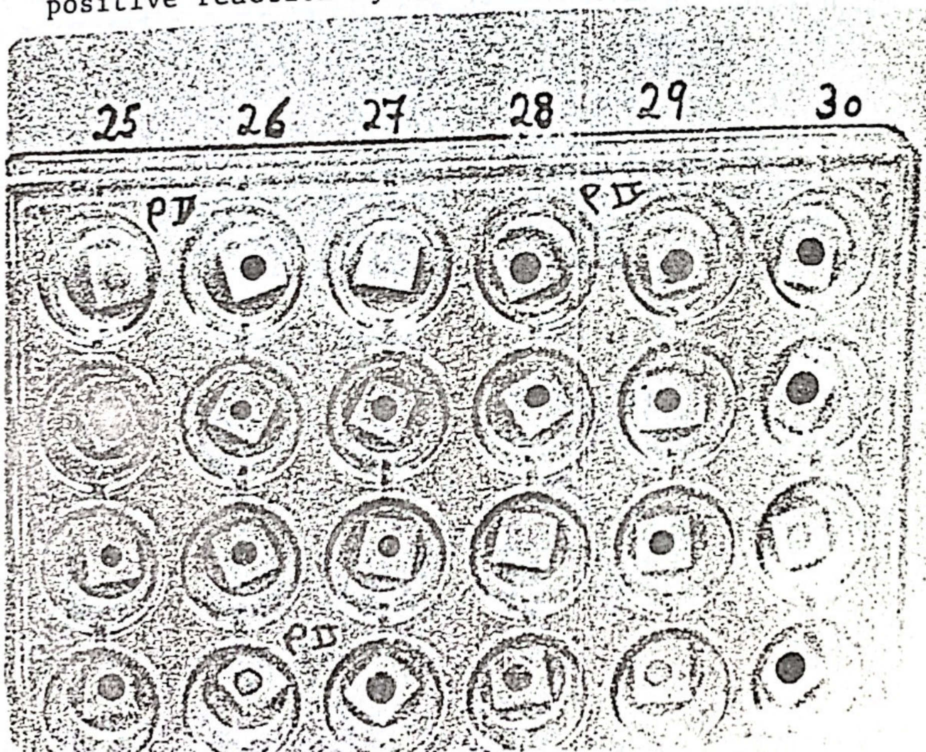

Fig. 1: Nitrocellulose filter discs showing blue coloured dots produced due to coronavirus antigen by the AST.

3. Immunoperoxidase test (IP):

Examination of the faecal samples by the IP revealed that the positive wells developed dark brown colour while the negative control vero cells showed no colour. Only 18 samples gave positive reaction by the IP technique for detection of corona virus antigen in vero cells.

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Comparison between the results obtained by the Elisa, AST and IP for detection of coronavirus antigen is shown in Table 2.

Table 2: Results obtained for detection of coronavirus antigen(s).

Test	Positive	Negative
ELISA	65	21
AST	46	40
IP	18	58

DISCUSSION

This study has been undertaken in order to detect coronavirus antigen(s) using different techniques as a mean of diagnosis of one of the causative agents of calf diarrhea. The use of the Enzyme-Linked Immunosorbent Assay (ELISA) was applied because it was a sensitive method for detection of virus antigen(s). The results showed that 65 faecal samples (75%) reacted positively for coronavirus **(Table, 1)**. In recent years, in addition to ELISA, various assays have been developed for immunological detection of antibodies and antigens, like the Antigen Spot Test (Paul et al., 1982). The AST could successfully detect coronavirus antigen in 46 faecal samples (53%) of the examined buffaloe calves. The AST is a valuable addition to the Elisa for detection of virus antigen. The results of the IP test showed that only 18 samples (21%) reacted positively for corona virus antigen. The lack of sensitivity of the IP for detection of coronavirus antigen directly from the faecal samples may be attributed to the fact that members of the coronavirus

group are known for their difficulty to adapt to culture cells (McIntosh, 1974). Briefly, the Elisa proved to be the most sensitive and specific test as a direct mean of detection for coronavirus antigen in diarrheic calves.

SUMMARY

Coronavirus antigen could be detected in faecal samples of newborn Egyptian buffaloe calves. Three different techniques were used, namely the immunosorbent assay (Elisa), the antigen spot test (AST) and immunoperoxidase technique (IP). Both the Elisa and AST proved to be sensitive and specific for diagnosis of coronavirus infection in diarrheic calves.

REFERENCES

1. Marin N., Lamothe P. and Gognon A. (1974): A case of viral neonatal calf diarrhea in a Quebec dairy herd. Can. J. Comp. Med. 38: 236-242.

2. McIntosh K. (1974): Coronavirus, a comparative review, cur. Top. Microbiol. Immunol 63: 85-129.

3. Mebus C.A, Stair E.L. and Rhodes M.B. (1973 a): Neonatal calf diarrhea: propagation, attenuation and characteristics of a coronavirus-like agent. Am. J. Vet. Res. 34: 145-150.

4. Mebus C.A., Stair E.L. and Rhodes M.B. (1973 b): Pathology of neonatal calf diarrhea induced by a coronavirus-like agent. Vet. Pathol. 10: 45-64.

5. Pan I.C., Huang T.S. and Hess W.R. (1982): New method of antibody detection by indirect immunoperoxidase plaque staining for serodiagnosis of african swine fever. J. Clin. Microbiol. 16: 650-655.

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6. Patel J.R., Davies H.A., Edington N., Laporte J. and Macnaughton M.R. (1982): Infection of a calf with the enteric coronavirus strain Paris. Archives of Virology 73: 319-327.

7. Paul H., Frans J., Van B and Sven O.W. (1982): The antigen spot test (AST): A highly sensitive assay for the detection of antibodies. J. of Immunological methods 48:293-298.

8. Stair E.L., Rhodes M.B. and White R.G. (1972): Neonatal calf diarrhea: purification and electron microscopy of a coronavirus-like agent. Am. J. Vet. Res. 33: 1147-1156.

9. Voller A. Bedwell D.E. and Bartlett, A. (1979): The enzyme linked immuosorbent assay (Elisa) A guide with abstracts of microplate applications Dynatech (Meda A/S).

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