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COMPARISON OF THREE METHODS FOR THE DETECTION OF ROTAVIRUS ANTIGEN IN THE FAECES OF NEW BORN CALVES

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SUMMARY

Immunofluorescence on incoulated cell cultures, latex agglutination test and enzyme-linked immunosorbant assay (ELISA) were compared for the detection of rotavirus antigen in faecal sample from new born calves.

Rotavirus were detected in 20 faecal sample by immunfluorescence test, latex agglutination test demonstrated rotavirus in 27 faecal sample, and rotavirus were detected in 29 out of 82 faecal sample by ELISA.

INTRODUCTION

Rotavirus has been implicated as one of the major causative agents of diarrhoea in new born calves (1,2).

Because many stains of the virus do not grow well in cell culture (2), the diagnosis is based on direct detection of the virus in the faeces.

In this study we compared a number of previously described techniques with respect to their sensitivity, simplicity and applicability for large-scale testing. Immunofluorescence test (IFT) on inoculated cell culture (10), latex agglutination test (LAT) (3) and

enzyme-linked Immunosorbont assay (ELISA) (4,5), were the techniques used.

MATERIALS AND METHODS

Faecal Specimens:

Eighty two of faecal sample were collected from the rectum of 2 to 10 days old calves with severe watery diarrhea at Al- Naser dairy farm station, faecal samples were diluted with Hank's solution media to make a 30% solution. Samples were clarified by centrifugation at a 3000 r.p.m. for 20 min. The supernatant was treated with 500 µg streptomycin, 500 i.u. of penicillin and used as inoculum in cell culture of calf kidney and also as a sample for ELISA and LAT.

Cell Culture:

Primary cell culture of calf kidney was prepared as described(6) and grown in Hank's balanced salt solution supplemented with 0.5 percent lactalbumin hydrolysate and 10 percent fetal calf serum with 100 i.µ penicillin and 100 µg streptomycin per ml.

Immunofluorescence Test (IFT):

Secondary bovine kidney monolayers cells grown on coverslips in Leighton tubes using Hank's solution containing 10% calfserum monolayars were inculated with 0.2 ml faecal samples. After 1 hour incubation at 37°C the cells were washed and maintenance media containing 5 µg Trypsin were added(7). After 48 - 72 hours post in inoculation the culture was washed with PBS. fixed with aceton and stained by using indirect method of Immunofluorescente (8) using reference anti-rotavirus serum (National Animal Disease center, Ames. Iowa..) to detect the rota-virus antigen in the infected CK cells.

Latex Agglutination Test (LAT):

The test prepared by using commercial ready kit: virotect-rota, Omega Diagnostics limited, using latex particles with rabbit (U.K) antibodies against human rotavirus (3).

One drop of the supernatant was transferred to each well of the test slide. A drop of test the latex suspension added to the first well and another drop of control latex reagent to the second well, mixing the contents of each well and examing the agglutination after 2 minutes.

Enzyme-Linked Immunosorbent Assay (ELISA):

Using commercial ready kit prepared by (Rotavirus ELISA) kit, Dakopatts, Denemark).

The test was performed by using the lg-fraction or rabbit antibodies with specificity to human rotavirus as catching antibodies and the same antibodies conjugated with horseradish peroxides as detecting antibodies(5).

The polystyrene microtest plates were coated conjugated antibodies (Anti-human) rotavirus rabbit lgG, while other wells were coated with normal rabbit lgG.

The plate were washed and faecal samples were added, after incubation for 30 min. the plate were washed with citric acid phosphate buffer. The peroxides conjugate anti-human rotavirus were added to all wells. After incubation period. 1M H_2SO_4 was pipetted to all wells.

The result can be read visually by comparing the colour of test wells with colour of the corresponding negative control wells.

RESULTS

Out of 28 faecal samples rotavirus antigens were detected in 20 samples by IFT and samples using LAT, whereas the ELISA technique demonstrated the presence of the antigen in 29 samples.

No. rotavirus antigens were detected in 53 samples

(Table No. 1).

Table 1: Detection of Rotavirus in faecal sample of calves by IFT , LAT,ELSIA

Test used	Positive / total sample
LFT	20/82
LAT	27/82
ELISA	29/82

DISCUSSION

The using of the IFT technique for the diagnosis of rotavirus is useful, and number of cell showing fluorescence was greatly enhanced by using trypsin in the maintenance media. The technique is not rapid and is difficult to use when large number of samples need to be examined, our results agree with other workers (6,8).

The Latex agglutination test is a sample and sensitive technique and can used for the examination of large number. Also could be done in the field, however, small amount of antigen may not be detected. The same conclusion was reach by other study (3).

The possibility of using antibodies with specificity to human rotavirus in ELISA for diagnostic detection of calf rotavirus exists because of common antigen of rotavirus (5). It is better to use of an ELISA test with specific antibodies against calf rotavirus in studies done on calves.

The sensitivity of ELISA test seemed to be that the other methods used in this study. These finding agree with results obtained by other worker(3,9) and it could be used as screening test to check large herds with greet ease and simplicity.

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مقارنة لثلاث طرق للتحري عن مستضدات فايروس الروتا في براز العجول حديثة الولادة

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الخلاصة

تم مقارنة الأختبارات التالية : التآلف المناعي في خلايا الزرع النسيجي مع أختبار التلازن بأستخدام حبيبات اللاتكس مع أختبار المقايسة بالأنزيمات المناعية (الأليزا) للتحري عن وجود مستضد فايروس الروتا في نماذج مأخوذة من عجول حديثة الولادة مصابة بالأسهال . أمكن التحري عن فايروس الروتا في (20 نموذج) بأستخدام أختبار التآلف المناعي و (27 نموذج) بأستخدام أختبار التلازن بوجود حبيبات اللاتكس وفي (29 نموذج) براز من مجموع (82 نموذج) بأستخدام أختبار الأليزا .