NEONATAL CALF PROBLEMS DUE TO SOME VIRAL INFECTIONS

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Abstract

Seroprevalence study has been done using Enzyme Immuno Assay (ELISA) for the detection of specific antibodies to some viruses known to be recorded in Egypt. A total of 451 serum samples were collected from calves aging 2 weeks to 8 months from different governorates of Egypt and were tested for antibodies to RP (56 %), FMD (36 %) , BVD (35 %), IBR (33 %) and RVF (0%)

The Prevalence rates werre 56 % , 36 % , 35% , 33 % and 0 % to the previously mentioned viruses respectively, suggesting a passive transfer of colostrum antibodies to the calves, or active exposure to the viruses specially those where vaccines are not available (BVD and IBR viruses).

Trials have been done for isolation and identification of viral agent from nasal and rectal swabs collected from diseased or contact calves in tested governorates of Egypt.

Out of 121 swabs collected, only 9 viral agents have been isolated and identified serologically by IFA and EIA techniques using reference sera available. Four out of nine isolates were + ve to enteroviruses, three were positive to IBR virus and one was PI-3 positive, while the remaining one was negative to all reference sera used.

The pathogenicity of these isolates was confirmed by experimental inoculation of susceptible animals.

All isolates were recovered from nasal and /or rectal swabs 5 days after experimental infection in susceptible calves.

INTRODUCTION

Because of the great economic losses due to the calf mortalities and morbidity, many researches were carried out to detect the main causative agent involved, and to control the problem. It was found that the major causes were enteritis and respiratory affections. The prevalence of viral infection antibodies of adenovirus(Lehmkuhl *et al.* 1979), bovine respiratory syncytical (BRS) virus (Martin and Bohac 1986), bovine virus diarrhoea (BVD) (Onken 1986) , coronavirus (Koromyslov et al . 1984) infectious bovine rhinotracheitis (IBR) virus (Hojbjerg 1982), parainfluenza-3 (PI-3) virus (Moreno-Lopez 1979) and rotavirus (Koromyslov *et al.* 1984) were detected in serum samples , while the virus isolation from the natural infection revealed that adenovirus (Dyakov 1982), BRS virus (Harrison and Pursell 1985), BVD virus (Amber et al . 1988), coronavirus (Mostl and Burki 1988) , enteroviruses (Wilhelm 1980), IBR virus (Martin and Bohac 1986), PI-3 virus (Haralambiev et al . 1987), rotavirus (Castrucci 1988), rift valley fever (RVF) virus (Nafady et al . 1987) and rinderpest (RP) virus (Wafula and Kariuki 1987) could be isolated from the calves with enteric and respiratory manifestations. The experimental studies on different infectious agents also were recorded (Potgieter *et al.,* 1984; Saif 1987; Bryson *et al.,* 1979; Frias *et al.,* 1986) . Different techniques were used for antibody and antigen detection; the more sensitive and specific techniques were serum neutralization test and enzyme immuno assay (ELISA) (Libeau and Calvez 1988).

The immunization of calves against the different infectious agents showed many problems, e.g. active immunization (Syurim et al . 1983) and passive immunization (Mechor *et al* . 1987 and Mostl and Burki 1988).

The aim of this work is to detect specific antibodies against the main viral diseases recorded in Egypt , viz. BVD, IBR , RP, RVF and FMD in calves from 2 weeks to 8 months. Also , attempts to isolate the causative viral agent from diseased and contact animals are carried out . Lastly, experimental infection with the identified viruses to study their pathogenicity and excretions will be undertaken.

MATERIALS AND METHODS

Sampling : Swabs: Fecal, lacrimal and nasal swabs were collected form calves aged one week to 7 months and showing diarrhoea, lacrimation and nasal se-

cretion respectively.

Serum samples were collected form the same above mentioned calves as well as from apparently healthy contact calves. Sera were stored at - 20°C till used. A total of 121 swabs and 541 serum samples were collected.

Hyperimmune sera:

1) Rabbit hyperimmune serum to rotavirus was obtained from Microbiology Dept. Fac. Vet. Med. Cairo University .
2) Bovine anti-BVD and IBR sera and guinea pig anti-FMD type O serum were obtained from States Bakt. Laboratories, Stockholm-Sweden, through Virology Dept., Animal Health Research Institute, Dokki, Cairo , Egypt.
3) Rabbit anti-RP serum, bovine anti - BRS virus and bovine anti-parainfluenza were obtained from States Bakt. Laboratories, Stockholm, Sweden.
4) Rabbit anti-adenovirus serum : SBL - substrat, USA.
5) Horse anti-enterovirus polyvalent serum was obtained from NIH research reference reagent, Bethesda, Maryland, USA.
6) Guinea pig and rabbit anti-PI-3 virus sera were obtained from National Bacteriological Laboratories Stockholm, Sweden.
7) Rabbit anti-IBR virus serum was obtained from Virology Dept., Animal Health Research Institute, Dokki, Cairo, Egypt.
8) Bovine anti-RVF serum was obtained from Plum Island, USA through Virology Division, Naval American Medical Research Unit , NO. 3, Cairo, Egypt.

Cell cultures for virus isolation

1) Bovine kidney cell culture was prepared according to Plowright and Ferries (1959).
2) Vero Cell line Africa and green monkey kidney cell was prepared according to Fields *et al.* (1985).

Antigens used in the study

1) Tissue culture antigens prepared on vero cell lines on BK for BVD , IBR, FMD type O, and rinderpest viruses were obtained from Virology Dept., Animal Health Re-

search Institute, Dokki , Cairo , Egypt.

Experimental animals:

Clinically normal Friezian calves, aged 6 to 8 months, were used for experimental infection with the isolated viruses (only the serologically negative calves were used).

Animals were kept under observation, and body temperatures werre recorded for 10 days before starting the experimental inoculation with the isolates obtained from nasal and rectal swabs. Serum samples from these calves were tested for viral antigen or antibodies.

Procedure:

1. Processing of specimen for virus isolation : The collected swabs on Hank's balanced salt solution with 10 % antibiotic were kept overnight at 4 ^{o}C, then, the swabs were discarded and the solutions were centrifuged at 2000 rpm for 20 minutes in a cooling centrifuge. The supernatant was collected and stored at 70^{o}C
2. Inoculation of the cell culture: Three prescription tissue culture flasks of confluent vero and BK cells were inoculated for each sample with 0.5 ml of the supernatant , incubated at 37 ^{o}C for one hour adsorption, then, the maintaining medeium was added , incubated at 37 ^{o}C and examined daily for any cytopathic effect (CPE). After development of CPE , the flasks were re-inoculated several times till a consistent CPE was obtained .
3. Identification of the isolated viral agents:

a) The indirect fluorescent antibody technique (IFA) : The test was carried out after Fields *et al.* (1985).

b) Indirect enzyme immunoassay (EIA) : The test was performed for either identification of the isolated viral agents or antibodies according to Voller *et al* . (1976) and Payment *et al.* (1979).

4. Experimental infeciom of calves with the isolated viral agents: The isolated viruses were titrated for infective dose ($TCID_{50}$) before use.

- IBR and enteroviruses were inoculated intramuscularly according to York (1968).
- The PI-3 virus was inoculated intranasally according to Bryson *et al.* (1979).
- Daily observation for 3 weeks from both inoculated and control was performed.
-Temperatures were recorded for 7 days after infection , nasal and rectal swabs

were collected for 5 days post-infection .
-Serum samples were obtained 1,2 and 3 weeks post-inoculation.

5 - Antigen detection by the antigen capture EIA was carried out according to Scott and Olson (1986).

RESULTS

Incidence of specific antibodies to tested viral agents using EIA:

A total of 451 serum samples collected from either apparently healthy or diseased calves were tested for specific antibodies against many viruses using EIA as shown in Table 1.

Table 1. Results of tested serum samples against different viruses.

Virus	Total serum samples	Positive	Percentage
BVD	451	157	35
IBR	451	151	33
RP	451	253	56
FMD	451	161	36
RVF	451	0	0

Trial for isolation of viral agents from clinically and apparently healthy calves:

Nasal and rectal swabs were collected from 100 calves showing either respiratory or intestinal symptoms or both, with or without thermal reactions. Samples were also collected from 21 apparently healthy contact calves. Each sample

Table 2. Type of viral isolates from diseased calves .

Sample No.	Locality	Type of swab	Age	Symptoms	Type of isolates
1-7	Noubaria	Nasal & rectal	4 days 5 month	Rhinitis fever	1-4 (entero) 5-7 (IBR)
8-9	Fayoum	Nasal	1-3 m	Cough, diarrhoea	8 (PI-3)

was inoculated into bovine kidney cell culture and vero cell line, and subjected daily to microscopic examination.

The results revealed that out of 121 samples , 35 showed cytopathic changes (CPE) within the first passage. On the subsequent 5 successive passages, only 9 samples produced CPE (Table 2).

Identification of the isolated viral agents

1. CPE changes : Provisional microscopical examination of the isolates was carried out by detection of the characteristic cytopathic changes (CPE) in the infected cell culture. The pronounced CPE observed in isloates 1,2,3 and 4 was mainly cell lysis , while isolae 5,6 and 7 were ballooning with degenation and polykaryocytosis. Isolate No. 8 demonstrated cyncytia and intranuclaear inclusions. Isolate No. 9 revealed threading vacuolation of the infected cells.

2. The indirect fluorescent antibody (IFA) technique : Virus infected cell cultures were examined by IFA technique using the hyperimmune sera against BVD , IBR RP, RVF , FMD , PI-3 adeno-3 , BRS , entero and rotaviruses.

3. The indirect enzyme immunoassay (EIA) : Using above mentioned reference sera , parallel results for identification of the isolated viral agents were obtained by using the indirect EIA compared with those obtained by the IFA technique.

Table3. Clinical manifestations of infection of viral agents in susceptible ealves.

Calf No.	Type of identified isolate	Clinical symptoms
1	IBR	Fever, rhinitis.
2	Enterovirus	Apparently healthy
3	PI-3	Apparently healthy
4	IBR+PI-3	Fever, rhinitis.
5	IBR + enterovirus	Rhinitis only.
6	PI-3 + enterovirus	Fever.
7	IBR + enterovirus + PI-3	Apparently healthy .

Experimental infection of the isolated viral agents in susceptible calves:

Firstly, the clinical manifestations were recorded as shown in Table 3.

The virus or viral antigen was successfully recovered by the antigen capture enzyme immunoassay from most of the nasal or rectal swabs collected 5 days after infection except for the PI-3 virus. When inoclated simultaneously with IBR virus, neither the nasal nor the rectal swabs demonstrated the virus.

The immune response was generally poor in calves inoculated with PI-3 virus alone or together with IBR and /or enteroviruses.

On the other hand, IBR infected calves developed good antibody response to the virus alone or together with PI-3 virus, while, the calf inoculated with IBR together with enteroviruses demonstrated weak immune response . Calves experimentally infected with enterovirus alone demonstrated specific antibodies in serum samples 1, 2 and 3 weeks post-infection, while, the enterovirus inoculated with PI-3 virus or IBR virus or with both of them, induced a moderate to weak immune response.

In general, experimental infection with the isolated enteroviruses and PI-3 produced no clinical signs, while IBR viruses inoculated separately produced respiratory syndrome. Meanwhile, mixed experimetal infection with two of the identified

viruses produced rhinitis and / or fever, while , the 3 identified isolated viruses used together for experimental infection resulted in no clinical signs during the experimental period (3 weeks).

DISCUSSION

In Egypt, many reports documented the wide spread nature of viral infections among calves; the effects were predominantly accompanied with enteric and or respiratory syndrome specially in those calves aging 2 weeks to 6 months (Awad 1963).

Experiment 1 aimed to evaluate the rate of infection or immune status of calves by detection of specific antibodies to the previous recorded viruses in Egypt. EIA was used as a specific sensitive reproducible method to a variety of viruses (Mohanty and Dutta 1981 and Hsiung 1982).

Incidence of BVD antibody was 35 % in tested calves. This may denote active infection or previous infection of their dams , a result which coincided with that of Howard *et al.* (1986) , while that IBR virus was 33 % might be due to exposure to the virus , agreeding with Nakashly (1981). High positive calf serum antibodies to RP virus 56 % reflected a good response of their dams to the continuous vaccination programmes applied in Egypt. These findings support the records of Singh et al. (1965). Low prevalence rates of antibodes to FMD virus type O (36 %) were recorded in the tested calf sera. This may indicate a poor response of pregnant cows to FMD vaccine. On the other hand, no antibodies to RVF virus were detected in all serum samples collected from calves from different governorates , which might indicate the absence of circulating virus in Egypt .

According to the results obtained in experiment 2, all isolated viruses demonstrated a clear cytopathic effect within 2-4 days post-inociulation of BK and /or vero cells.

Out of 121 swabs obtained from diseased calves, as well as apparently healthy contact calves, viral agents were successfully isolated from 9 swabs only (4 nasal and 5 rectal) and they were all from diseased calves. On the other hand, trials for virus isolation from swabs collected from apparently healthy contacts were negative; this result agreed with Ross *et al.* (1983) on cell culrures for virus isola-

tion. In experiment 3 and from Table 2 four, out of nine isolates were positi for enteroviruses , three were IBR , one was PI-3 and one was unidentified. Enteroviruses were previously isolated in Egypt from diseased and apparenly healthy calves as reported by Ayad (1982). The 4 isolates of enterviruses were correlated with diarrahoea and rhinitis in newly-born, this agreed with Wilhelm *et al.* (980).

The three viral isolates idenified as IBR virus, were obtained from calves showing cough, rhinitis with or without thermal reactions as reported by Corkish and Richards (1983) .

The nine isolates shown in Table 2 were experimentally infected in susceptible calves as shown in Table 3 and confirmed the previous results of serological and identification techniques.

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بعض مشاكل العجول حديثة الولادة بسبب بعض الفيروسات

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– تم عمل مسح سيرولوجى عن الأجسام المناعية لبعض الفيروسات المعروف تواجدها فى مصر وذلك بتجميع عينات من مختلف محافظات مصر .

– قد يرجع السبب فى وجود هذه الأمراض لإنتقالها سلبياً من خلال السرسوب او بسبب التعرض المباشر لهذه الفيروسات

– تم عزل تسعة فيروسات من المسحات التى اخذت بعد ان مررت فى خلايا الزرع النسيجى عدة مرات .

– لإختباو قدرة الفيروسات المعزولة على إحداث اعراض مرضية ، تم حقن عجول التجارب فى عمر ٦ شهور لهذه الفيروسات المعزولة .

– اثبتت النتائج ان الفيروس موجود بنسبة عاليه فى مسحات الشرج عنها فى المسحات الأنفيه.