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VIROLOGICAL STUDIES ON PARA-INFLUENZA VIRUS ISOLATED FROM KAFR EL-SHEIKH GOVERNORATE.

MERVAT, I.I. ABD EL-MONIEM and NELLY OMAR

Virology Dept. Animal Health Research Institute, Agricultural Research Center, Ministry of Agriculture, Dokki, Giza, Egypt.

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SUMMARY

In the present study, 250 swabs (174 nasal and 76 ocular) and 510 serum samples were collected from different farms at Kafr El-Sheikh Governorate from calves, feed lot and adult cattle of different ages for PI-3 isolation, identification and seroprevalence. Some of the animals were suffering from the disease.

The results indicated that 15 and 12 samples identified as PI3 positive by FAT and 12 VNT, respectively.

The serodiagnosis results of 510 examined serum samples by HI and SNT revealed that the PI3 antibodies were detected in diseased animals at percentages of 32% and 23.4%, respectively. While in apparently healthy animals in percentages of 31% and 13.1%, respectively.

INTRODUCTION

Bovine respiratory diseases of animals are of paramount importance because of high morbidity and mortality.

In Egypt, and El-Dobeigy (1975) found that parainfluenza-3 virus is one of the important respiratory viruses. They added that it affected all ages especially young ones and caused high annual losses.

Parainfluenza-3 (PI-3) virus was isolated from pneumoentritis in buffalo calves (Singh and Baz, 1966). Also PI-3 virus was incriminated in many respiratory disorders in calves and other animal species (Singh and El-Cicy, 1967; El-Dobeigy,

respiratory disorders in calves and other animal species (Singh and El-Cicy, 1967; El-Dobeigy, 1975; Kite et al., 1994 and Steinhagen and Hubent, 1995). Clinical features of PI-3 disease is particularly important in recently weaned calves and young cattle, especially when they are maintained in closely confined conditions. Infection is characterized by mild to severe respiratory involvement with fever and nasal discharge (Marshal and Frank, 1975; Kahrs, 1981 and Kite, 1994).

The aim of this study is to isolate the principal pathogen PI-3 as well as its identification in addition to sero-prevalence.

MATERIAL AND METHOS

I. Material:

*** Samples:**

Serum samples: A total of 510 serum samples were collected from diseased and apparently healthy Friesian cattle from intensive beef and milk production farm at Kafr El-Sheikh Governorate, Egypt with different age (one day old up to 4 years) (Table 1). The sera were separated and kept at - 20°C until examined.

*** Swabs:**

250 swabs (174 nasal and 76 ocular) were collected from diseased animals on transport media (Kahrs, 1981) and stored at - 20°C until used for virological investigation.

*** Cells:**

MDBK cell line was obtained from Veterinary Serum and Vaccine Production and Research Institute Abbasia, Cairo, Egypt.

*** Media:**

Eagle's minimal essential media (MEM) (GIBCO).

*** Reference Virus:**

Parainfluanza-3 virus (PI-3, strain 45, isolated and identified by Singh and Baz (1966) was used.

*** Positive hyperimmune serum:**

Positive hyperimmune serum of PI-3 was supplied from Serum and Vaccines Production and Research Institute, Abbassia, Cairo, Egypt.

*** Conjugate:**

Antibovine fluorescin isothiocyanate conjugated was supplied by Sigma immunochemicals.

*** Laboratory animals:**

Guinea pig erythrocytes for HA and HI tests for PI-3 virus was prepared in the Department of

Virology, Animal Health Research Institute, Dokki, Giza, Egypt.

II. Methods:

1. Isolation:

Adaptation and propagation of reference virus and examination of prepared collected swabs on monolayer of MDBK cell line were done according to Kahrs (1981).

2. Identification of viral isolates by :

a. Virus Neutralization Test (VNT) according to Singh and Baz (1966)

b. Immuno-fluorescent Antibody Technique (IFAT) according to Baczynisski et al. (1974).

3. Serodiagnosis:

a. Serum Neutralization Test: SNT was carried out according to Carbery and Lee (1966).

b. Haemagglutination Inhibition Test: This test was done according to Maglione et al. (1992).

RESULTS

Isolation from nasal and ocular swabs was conducted on MDBK cells. There was rounding of the cells with progressive synthytial formation. The characteristic cytopathic effects (CPE) were obvious 5-7 days post inoculation. It is clear from table (2) that 20 viruses could be isolated from collected swabs.

Identification of isolated virus by FAT and VNT revealed that 15 virus isolates out of 20 cytopathic agents by FAT and 12 virus isolates by VNT (Table 3 and photo 1).

Prevalence of PI-3 antibodies were detected by HI and SNT in diseased animals as shown in table (4). The result indicated that 112 (32%) serum samples out of 350 were positive by HI, while 82 (23.4%) were positive by SNT.

Table (5) demonstrated seroprevalence of PI-3 antibodies in sera of healthy animals detected by HI and SNT. The result indicated that 31(19.4%) serum samples out of 160 were positive by HI, while 21 (13.1%) were positive by SNT.

The incidence of positive reactors were inversely proportional to the age of the animal against PI-3 by both HI and SNT. The highest levels of antibody was in calves (43.4% by both HI and 32.4% by SNT in diseased calves while 25.7% by HI and 18.6% by SNT in healthy calves.

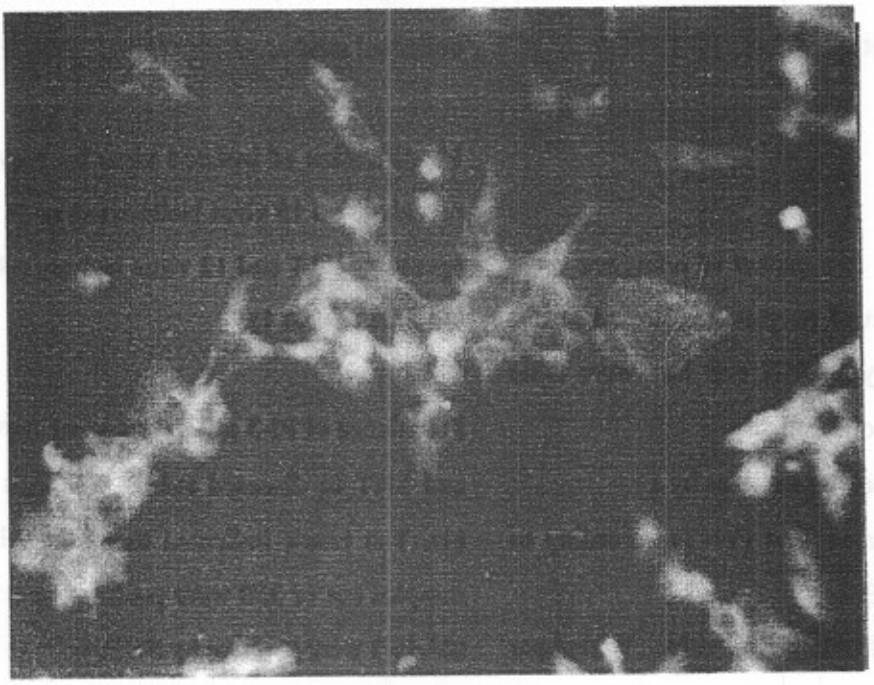

Photo 1: MDBK cells infected with PI-3 virus showing intracytoplasmic perinuclear fluorescence.

Table (1): Distribution of collected serum samples.

Animal	Total No.	Calves	Feed Lot	Adults
Disease	350	145	110	95
Health	160	70	55	35
Total	510	215	165	130

Calves: < 7-12 months.
Feed Lot 13-24 months.
Adult: Over 2 years.

Table (2): No. of isolates from swabs.

Animal	Total No. of serum samples	No.of N.S.	No.of CPS Positive	No.of O.S	No.of CPS Positive
Calves	120	83	11	37	3
Feed Lot	85	55	4	30	1
Adults	45	36	1	9	-
Total	250	174	16	76	4
%			9.2%		5.3%

N.S: Nasal swab.
O.S: Ocular swab.

Table (3): Identification of isolates by FA and VNT.

Animal	No.of isolate from N.S	Identified isolate		Isolates O.C.	Identified isolate	
		FA	VNT		FA	VNT
Calves	11	9	8	3	2	1
Feed Lot	4	2	2	1	1	1
Adults	1	1	-	-	-	-
Total	16	12	10	4	3	2

Table (4): Sero-prevalence of PI-3 by HI and SNT in serum of diseased animals.

Animal	Total No. of serum samples	HI		SNT	
		No.of +ve	%	No.of +ve	%
Calves	145	63	43.4	47	32.4
Feed Lot	110	34	31.0	26	23.6
Adults	95	15	15.8	9	9.5
Total	350	112	32%	82	23.4%

Table (5): Sero-prevalence of PI-3 by HI and SNT in serum of healthy animals.

Animal	Total No. of serum samples	HI		SNT	
		No.of +ve	%	No.of +ve	%
Calves	70	18	25.7	13	18.6
Feed Lot	55	9	16.4	6	11
Adults	35	4	11.4	2	5.7
Total	160	31	19.4	21	13.1

DISCUSSION

PI-3 is one of the respiratory viral infections which causes great annual losses for animal breeder in most if not all parts of the world.

Several outbreaks of respiratory distress occurred in the farm in Kafr El-Sheikh Governorate. The clinical signs were nasal discharge, conjunctivitis with signs of pneumonia. Severe clinical signs may be due to adverse environmental conditions stress due to over crowding and transportation (Selim et al. 1996). The signs agree with those described by Frank and Marshall (1971) and Kite et al. (1994). Regarding the virus isolation 20 out of 250 gave the typical CPE in our study, this result agreed by Frank and Marshall (1971) and Woods et al. (1964).

Regarding the identification of isolates 3 more identified isolates by using FAT than VNT. This result means that the FAT is more sensitive than VNT. The same conclusion was stated by Baczynisski et al. (1974) who stated that FAT is more rapid and sensitive.

PI-3 antibodies were detected by HI and SNT. The gained results by HI were 112 (32%) in diseased animals while 31 (19.4) in healthy animals. On the other hand, the results of SNT were 82 (23.4%) in diseased animals while 21 (13.1%) in healthy animals. The same results were obtained by Virakul et al. (1985) who stated that HI more sensitive than SNT for detection of PI-3 antibodies. The previous results were in agreement with that found by others, Frank and Marshall (1971) who stated that although SNT is rather difficult and laborious yet it

although SNT is rather difficult and laborious yet it provides more dependable results and hence it is more sensitive for studding the antibody level.

In this study high level of antibodies were detected in calves. This may be due to higher susceptibility (Selim et al., 1996).

Concerning the serological status against PI-3 in healthy animals examined by HI and SNT (Table 5) clarify that 31 (19.4%) and 21 (13.1%) were positive for PI-3 by HI and SNT respectively. This results clarify the subclinical infections or previous infections was very high and should be looked for with seriousness for the unmeasured losses in body weight, still births, abortions etc. The history of these farms indicated poor weight gains. These result agree with Sweat (1966) who comparing the growth of weaning calves with and without PI-3 infection, indicated that the calves fail to gain weight for 50-60 days in infected calves although food consumption was normal. This adds to the losses of PI-3 infections.
There are many records of the benefit of vaccine against PI-3 (Hamdy et al., 1965; Soulebot et al., 1981 and Sweat, 1966).

From this study, it can be realized that vaccination with PI-3 vaccine 30 days before weaning and at the time of weaning is probably a sounder procedure altogether with vaccination of dams before parturition to raise their antibody level to be transferred through colostrums.

It is a high time to control PI-3 infections in newly born cattle and buffalo-calves in Egypt being incorporated in a high percentage in pneumoenteritis syndromes to help in raising more healthy calves.

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دراسات فيرولوجية على بارا انفلونزا -٣ معزولة من محافظة كفر الشيخ

مرقت إبراهيم إبراهيم عبدالمنعم و نيللى عمر

قسم الفيرولوجيا - معهد بحوث صحة الحيوان

مركز البحوث الزراعية وزارة الزراعة - الدقى -

الجيزة - مصر

فى هذه الدراسة تم جمع ٢٥٠ مسحة (١٧٤ أنفية و ٧٦ عينية) و ٥١٠ عينةة سيرم من أبقار مريضة وسليمة ظاهرياً من مزرعة لإنتاج الألبان بمحافظة كفر الشيخ.

وقد تم التعرف على ڤيروس بارا أنفلونزا - ٣ فى عدد ١٥ و ١٢ عينة بإستخدام الإختبار الفلورسينتى وإختبار الڤبروس المتعادل على الترتيب. وقد أظهرت نتائج الفحص اليرولوچى لعدد ٥١٠ عينة سيرم وجود الأجسام المناعية للڤيروس بنسبة ٣٢٪ و ٤ . ٢٣٪ بإستخدام إختبارى تلزن الدم والسيرم المتعادل على الترتيب . بينما كانت النسبة فى الحيوانات السليمة ظاهرياً ٣١٪ و ١ . ١٣٪ على الترتيب.