EPIDEMIOLOGICAL STUDIES ON INFECTIOUS BOVINE RHINOTRACHEITIS (IBR), BOVINE VIRAL DIARRHOEA (BVD), PARAINFLUENZA-3 (PI-3) AND BOVINE ADENOVIRUS TYPE-3 IN CALVES OF SOME GOVERNORATES IN EGYPT

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ABSTRACT

Epidemiological studies on some viral diseases of calves (316 buffalo and cow) calves in Kafr El-Sheikh, Kaliobia, Dakahlia, Domyat, Beni-Suef and New Valley governorates in Egypt was carried out.

The results showed that bovine respiratory virus infections exists in the country where it reached in buffalo calves 34.78%, 30.43%, 73.47% and 26.08% in Kafr El-Sheikh and 30%, 42.5%, 45%, 20% in Kaliobia, while, in cow calves 43.75%, 45%, 51.25% and 30% in Dakahlia; 33.84%, 43.07%, 49.23% and 27.69% in Domyat and 40%, 35%, 60% and 30% in Bani-Suef against for BVD, IBR, PI-3 and BAV3 viruses, respectively, while New Valley governorate was free from those viral infections.

The results obtained by SN was compared by ELISA to detect the sensitivity and specificity of an enzyme linked immunosorbant assay for detection of BVD, IBR, PI-3 and BAV3 antibodies in cattle sera.

125 clinical specimens in the form of ocular, nasal swabs, buffy coats and sera of suspected diseased bovine calves suffering from pneumonia and pneumo-enteritis obtained from the different localities at Kaliobia, Dakahlia and Domyat in Egypt was used for isolation of the viruses, on Madin darby bovine kidney cell culture, our results revealed one isolate of adenovirus type 3, four isolates of IBR virus, five isolates of PI-3, the isolates were identified using virus against reference hyperimmune serum against each virus by virus neutralization (VN) and haemagglutination inhibition (HI) and Immunofluo-rescence (IF). Due to the presence of non-cytopathic strain of BVD (ncpBVDV) all negative specimens and suspected samples for BVD virus were subjected to Polymerase Chain Reactions (PCR) and indirect immunofluo-rescence (IF) for detection and biotyping the BVD virus. The result indicated that 14 isolates of BVD virus identified cytopathic strains of BVD and 5 non cytopathic strains of BVD virus. The IF technique detected 10/14 of viral isolates, the single step reverse transcription polymerase chain reaction (RT-PCR) utilizing the 5 untranslated region (5 utR) of BVD genome detected 14/14 of viral isolates. The second round PCR utilizing another pair of PCR primers from the 5 untranslated region allowed rapid genotyping of BVD virus.

INTRODUCTION

Respiratory diseases in neonates are a major problem for the people who raise neonates especially with the development of intensive system for production.

Bronchopneumonia causes a great economic losses in neonatal calves especially in intensive farm production and large flock (Barrett, 1998).

Environmental risk factors includes extremes of temperature, poor ventilation, dust, ammonia and over-crowding. High pressure sprays used for cleaning produce aerosols that may effectively disperse pathogens in the environment and predispose neonates to respiratory infection (Bickert and Herdt, 1985).

The sub-clinical infection should be looked for with seriousness for the unmeasured losses in body weight, still births and abortions etc. The history of these farms indicated poor weight gains. The work of Sweat (1967) by comparing the growth of weaning calf with and without respiratory infection indicated that the calves fail to gain weight for 5-60 days in infected calves although food consumption was normal, in addition to the losses from viral infection. Sub-clinical and clinical affection should not looked for with non-consideration for the unmeasured high losses in animal industry-beside the presence of an infective agent spreading among susceptible population.

Immunity of immune system increases the susceptibility of neonates to contagious and opportunistic infections at birth foals, calves and lambs are hypoglobulinemic or a gammaglobulinemic (Selim et al. 1995). Infectious Bovine Rhinotracheitis virus, Bovine Viral Diarrhoea and Bovine Adenovirus type-3 all may produce respiratory disease in calves. Viral infections increase the risk of opportunistic bacterial infection by their immune suppressive effects and damage to the respiratory epithelium and pulmonary clearance mechanisms (Potgieler, 1997).

Control measures for BVD, IBR, PI-3 and BAV-3 viruses depend mainly on through screening of animals for infection periodically and prior to entering a herd as well as vaccination.

Two biotypes of BVDV exists cytopathic BVDV (cpBVDV) and non-cytopathic BVD (ncpBVDV); the cpBVDV induces cytoplasmic vacuolation and cell death in susceptible cell cultures where as the ncpBVDV has little effect in cell culture. Either separately or in combination, both viral biotypes induce diseases that range from clinically mild to the fatal syndrome (mucosal disease).

Cell cultures established from infected foeti may remain indefinitely infected. As the ncpBVDV does not induce a cytopathic effect infection of cell culture may remain undetectable unless immunochemical or immuno-fluorescence assays are conducted. Nevertheless, BVDV is found as a frequent contaminant of commercial fetal calf serum, a commonly used component in tissue culture systems, this leads to contamination of cell culture, viral stocks and biological products such as vaccines (Lohr et al., 1983).

Recently since (1994), BVDV was classified according to molecular differences in the 5 untranslated region of the viral genome to genotypes 1 and genotype 2 (Ridpath et al., 1994).

Discrimination between genotypes of BVDV by classical virological and serological means is cumbersome due to the antigenic relatedness of the viruses. The primer directed nucleic acid amplification using the polymerase chain reaction provides a rapid and sensitive tool for detection and typing of BVDV (Ridpath et al., 1994 and El-Kholy et al., 1998).

The aim of this study is to affirm the presence of bovine respiratory viral disease infection (BVD, IBR, PI-3 and BAV-3) in the country, (1) Incidence of positive reactors in serum of farm animal (cow and buffalo) in Kafr El-Sheikh, Kaliobia, Dakahlia, Domyat, Bani-Suef and New Valley governorates and to determine its distribution in the

investigated governorates. (2) Trials for virus isolation and identification from the clinical bovine specimens of bovine calves suffering from pneumonia and pneumo-enteritis and the use of a PCR assay for detection and biotyping of BVDV isolates.

MATERIAL AND METHODS

1. Viruses and cells :

BVD : Local Egyptian Iman strain (a cytopathic strain).
IBR : Local Egyptian strain (Abou Hammad strain).
PI-3 : Local Egyptian strain (strain 45).
BAV3 : Egyptian strain (Type 3).

All viral strains were propagated and titrated on Madin Darby bovine kidney MDBK cell culture. Both MDBK cells and foetal calf serum were tested to be sure free from adventitious BVDV by indirect immunofluorescence (El-Kholy et al., 1998).

2. Serum samples:

A total of 316 serum samples were obtained from calves (buffalo and cow) aged from 2-6 months from different governorates represent lower Egypt (Kafr El-Sheikh "Mahalet Mousa Farm", Kaliobia "23 July Farm", Domyat "El-Serw and El-Basateen Farms", Dakahlia "El-Mansoura" and desert area New Valley) governorates and upper Egypt (Beni-Suef "Sedes Farm" governorate). These farms not used any vaccine program for controlling these respiratory viral diseases. The collected sera were kept at -20°C after inactivated at 56°C for 30 minutes.

3. History and clinical findings :

Samples were collected from suspected diseased calves at least showing one or more of the following signs; fever, respiratory distress and eye affection, nasal and ocular discharge, anorexia, depression, rapid respiration, cough, salivation, dyspnea, conjunctivitis and occasionally corneal opacity, hyperemia, oedema and haemorrhagic focal necrosis of the nasal mucosa and diarrhoea.

Some animals showed clinical signs with serious complication and emergency slaughter or death occur.

4. Clinical specimens :

A hundred and twenty five bovine clinical specimens, each represented one suspected animal with signs of pneumo-enteritis, were obtained from different localities in different provinces (Kaliobia, Dakahlia, Domyat) of 50% specimens from buffaloes (23 July Farm) of Kaliobia and the reminder from the other bovine farms as 20 specimens from (El-Serw farm), 25 specimens from (El-Basateen farm) and 30 specimens from (Mansoura farm) in Egypt to be for trials of virus isolation for BVD, IBR, PI-3 and BAV-3 viruses. The specimens include uninactivated sera, buffy coat, ocular and nasal swabs. All specimens were processed for virus isolation on MDBK cells then the clarified supernatant harvested from inoculated tissue culture were examined by FA staining.

Serological studies :

1. Serum neutralization test :

It was performed according to the method of Carbrey (1971).

2. Enzyme Linked Immuno Sorbent Assay (ELISA) :

It was described by Voller et al. (1976). The serological status of each test sample was determined from the P/N ratio (positive/Negative ratio) which is the mean optical density (OD) of the duplicate sample divided by the mean OD of the reference negative serum. The tested sera were assigned a status of positive (P/N ratio) > 2.00, suspicious (P/N ratio > 1.50 < 2.00 or negative (P/N ratio < 1.50).

The performance of the ELISA was considered in terms of the following parameters as defined by Vecchio (1966) :

$$\text{Specificity (\%)} : \frac{\text{No. of non infected cattle with a negative test}}{\text{All non infected cattle tested against each virus}} \times 100$$

$$\text{Sensitivity (\%)} : \frac{\text{No. of infected cattle with a positive test}}{\text{Non infected cattle tested against each virus}} \times 100$$

Virological studies :

1. Trials of virus isolation :

All specimens were processed for virus isolation. The used method was described by El-Kholy et al. (1998).

2. Identification of isolated agents :

a. Virus neutralization test : It was utilized as described by Carbrey (1971) for the identification of the reisolated viral agents.

b. Haemadsorption and Haemagglutination : The haemadsorption test was performed according to Vogel and Shelokov (1957) and plate haemagglutination test was carried out according to the method described by Gale and King (1961).

c. Haemagglutination inhibition test (HI) : HI test was used to identify viral isolates that were positive for haemadsorption and haemagglutination tests according to Carbrey (1971).

3. Indirect immunofluorescence (FA) : The FA technique was carried out in tissue culture microtitration according to El-Kholy et al. (1998).

4. Polymerase chain reaction :

For detection and biotyping bovine viral diarrhoea viruses isolated from clinical specimens.

- Reverse transcription polymerase chain reaction (RT-PCR) assay using two sets of oligonucleotide primers spanning the 5 utR of the BVD virus genome was performed on RNA isolated from cell cultures inoculated with processed clinical specimens (El-Kholy et al., 1998).
- First round polymerase chain reaction (one-step reverse transcription-polymerase chain reaction, to detect RNA of BVDV in samples as El-Kholy et al. (1998).
- Second round polymerase chain reaction, it was used to identify genotype 2 of BVD virus as El-Kholy et al. (1998).

RESULTS

The results of serum neutralization test as shown in Table (1) indicates that bovine respiratory virus infection is wide spread among cattle throughout Egypt the incidence of positive reactors 1:4 or higher antibody titre in buffalo calves reached 34.78%, 30.43%, 43.47% and 26.08% in Kafr El-Sheikh 30%, 42.5%, 45% and 20% in Kaliobia, while in cow calves; 43.75%, 45%, 51.25% and 30% in Dakahlia 33.84%, 43.07%, 49.23% and

27.69% in Domyat 40%, 35%, 60% and 30% in Bani-Suef for BVD, IBR, PI-3 and BAV-3 viruses, respectively, while New Valley governorate was free from those viral infections.

The results of SN compared by ELISA and the sensitivity and specificity of an enzyme linked immunosorbent assay for the detection of BVD, IBR, PI-3 and BAV-3 antibodies in cattle sera as shown in Table (2) indicated that relative sensitivity 91.4%, 95.1%, 90.24% and 92.7% of positive reactions; 5.37%, 2.94%, 6.5% and 5.88% of suspicious reactions and relative specificity 3.28%, 1.96%, 3.25% and 1.47% of negative reactions against BVD, IBR, PI-3 and BAV-3, respectively.

1. Isolation of IBR, PI-3 and BAV-3 viruses :

IBR, PI-3 and BAV-3 viruses have been isolated from sera, nasal, ocular swabs and buffy coat of clinically sick animals characteristic cytopathic effect (CPE) against each virus, haemadsorption in MDBK cells and haemagglutinating activity (HA) were studied. The isolates were also identified using reference hyperimmune serum against each virus by virus neutralization test (VNT) and haemagglutination inhibition (HI). One isolate of adenovirus type 3, four isolates of IBR virus and five isolates of PI-3 virus. The results were confirmed by IF test (Table 3).

2. Isolation of BVD virus :

All negative specimens were subjected to indirect immunofluorescence (IF) which detected 10 of the BVD virus isolates 8 of the cp strain of BVDV isolates and 2 of the ncp strain of BVD virus isolates (Table 4) and polymerase chain reaction (PCR) for detection of biotyping the BVD virus. The results indicated that out of 14 isolates of BVD virus identified nine isolates of cytopathic strains of BVD and five isolates of non cytopathic strain of BVD virus were identified. The single step RT-PCR using primers derived from 5 utR of BVD genome detected 14 of the virus isolates (which detect any genotype and/or 2 of BVD virus) (Table 4 and Fig. 1a, b).

Second PCR assay using primer was selected from 5 utR of a genotype 2 of BVD virus detected 11/14 of the viral isolates (which detect only genotype 2 BVD virus strain) (Table 4 and Fig. 2).

DISCUSSION

The results obtained in this investigation very interesting. So, many facts and situation on respiratory infection in newborn cattle and buffalo calves were represented. The high incidence of SN antibodies indicated that BVD, IBR, PI-3 and BAV-3 infection are widely spread in these farms (Table 1) where it posses a serious threat to the calf industry in Egypt. The number of reactors of serum dilutions 1:4 or higher was higher in all farms due to the susceptibility of the species, ineffective sanitary control measures, effect of the season of heavy rainfall and floods, poor ventilation, dust, ammonia and over-crowding that reflect on lowering the immune status of animal and spread of respiratory infection especially in wet and dirty farm while the New Valley governorate reported no respiratory virus infection which might be due to the nature of this area of dry air and desert land. The previous mentioned interpretation came in agreement with El-Dobeigy et al. (1983), Carriollo et al., 1986 and Atia et al. (1999). Comparison evaluation of enzyme linked immunosorbent assay (ELISA) and serum neutralization tests for detection of BVD, IBR, PI-3 and BAV-3 virus antibodies in cattle and buffalo sera (Table 2).

Increase ELISA titre of serum samples than SN titre since the ELISA probably detects, not only neutralizing antibody but also other antibodies reacting with antigenic determinants not involved in serum neutralization test (Anderson et al., 1983). The low level of ELISA positive (relative sensitivity against BVD, IBR, PI-3 and BAV-3 (Table 2). In spite of relatively higher percentage of SN-antibodies could be explained on the basis that we are dealing mostly with (IgM) antibodies and might have been from animals at an early stage of infection.

Based on the results obtained the ELISA method appears to be an efficient serological test in terms of excellent specificity and sensitivity it agrees closely with the SN test in detecting both seronegative and seropositive animals. The test is also more practical and faster than SN test for detection both seronegative and seropositive animals of BVD, IBR, PI-3 and BAV-3 because of its many advantages over the SN test as Hyun et al. (1991).

Isolation of respiratory viruses from clinical specimens IBR, PI-3 and BAV-3 and cp strain of BVD viruses were isolated by cultivation on MDBK cell cultures and identified by the utilizing of hyperimmune serum against each viruses using VN and HI tests and the results were reconfirmed by immunodetection using IF method (Table 3).

Due to the presence of non cytopathic strain of BVD virus, all negative samples were subjected by and using polymerase chain reaction to detect and genotyping of BVD virus of clinical specimens. Out of 125 clinical specimens tested, 101 specimens gave negative results for virus isolation in all tests used in this study.

One isolate of Adeno virus type 3, 4 isolates of IBR virus, 5 isolates of PI-3 as Baz et al. (1986) and 9 of the viral isolates were cpBVDV while 5 isolates were ncp BVD as Howard et al. (1987) (Table 4). Immunofluorescence detect 10 of the BVD viral isolates 8 of cp BVDV and 2 isolates of ncp BVDV (due to poor growth of virus). This could be as the latent BVD virus or presence of toxic elements in the sample (Horner et al., 1995) (Tables 3 & 4). The one step RT-PCR which used the 5 utR primers detected BVD from 14/14 of clinical specimens tested in the first round (genotype 1 and/or genotype 2) (Table 4 and Fig. 1a, b).

The second round PCR detected 11/14 of the viral isolates as BVD genotype 2 (Table 4 and Fig. 2). These findings support the claim that the 5 utR for both molecular detection and genotyping of BVD strain from isolates (Ridpath et al., 1994) and the PCR detects both viable and non infectious viral particles (dead or immature) since it identifies specific nucleotide sequences as Lannette (1992). From the previous results indicated that the BVD genotype 2 spread allover Egypt as the local Iman strain identified as genotype 2.

The apparent wide distribution of respiratory viral affection due to their enoumerous causative agents and enoumerous host range, no routine vaccination against these diseases.

From these results, it can be realized that vaccination with combined inactivated respiratory virus vaccine (pneumo-3) at 30 days before weaning and at the time of weaning is probably a sounder procedure all together with vaccination of dams before parturition to rainse their antibody level.

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Table (1) : Distribution of bovine respiratory viral antibodies (BVD, IBR, PI-3 and BAV type 3) in collected serum samples in different localities in Egypt as screened by SNT

Governorates	Species	No. of bovine	No. of positive serum*							
			BVD		IBR		PI-3		BAV-3	
		serum samples	Positive	%	Positive	%	Positive	%	Positive	%
1. Kafr El-Sheikh Mahalet Mousa Farm	Buffalo calves	46	16	34.78	14	30.43	20	43.47	12	26.08
2. Kaluobia 23 July Station Farm		40	12	30	17	42.5	18	45	8	20
3. Dakahlia El-Mansoura	Cow calves	80	35	43.75	36	45	41	51.25	24	30
4. Domyate El-Serw and El-Basateen farms		65	22	33.84	28	43.07	32	49.23	18	27.69
5. Bani-Suef Sedes Farm		20	8	40	7	35	12	60	6	30
6. New Valley		65	-		-		-		-	

* Positive samples with 1/4 or higher titre

Table (2) : Comparative detection of BVD, IBR, PI-3 and BAV-3 virus antibodies in bovine sera by ELISA and SNT

SN titre	ELISA result															
	BVD				IBR				PI-3				BAV-3			
	No.	N	S	P	No.	N	S	P	No.	N	S	P	No	N	S	P
1:4 - 1:8	12	3	4	65	80	2	3	75	82	3	6	73	50	1	2	47
1:16 - 1:32	12	0	1	11	16	0	0	16	20	1	2	17	12	0	2	10
1:64 - 1:128	5	0	0	5	4	0	0	4	17	0	0	17	4	0	0	4
≥ 1:128	4	0	0	4	2	0	0	2	4	0	0	4	2	0	0	2
No.	93	3	5	85	102	2	3	97	123	4	8	111	86	1	4	81
Sensitivity & specificity %		3.28	5.37	91.4		1.96	2.94	95.1		3.25	6.5	[illegible]		1.17	5.88	92.7

Specificity N : Negative (P/N ratio < 1.5)
S : Suspecious (P/N ratio 1.5 – 2.00)
Sensitivity P : Positive (P/N ratio > 2.00)

Table (3) : The results of IBR, PI-3, BAV-3 and cytopathic strains of BVD virus from bovine clinical specimens virus serum neutralization (VN) and confirmed by haemagglutination inhibition (HI) and immunofluorescent (IF) test.

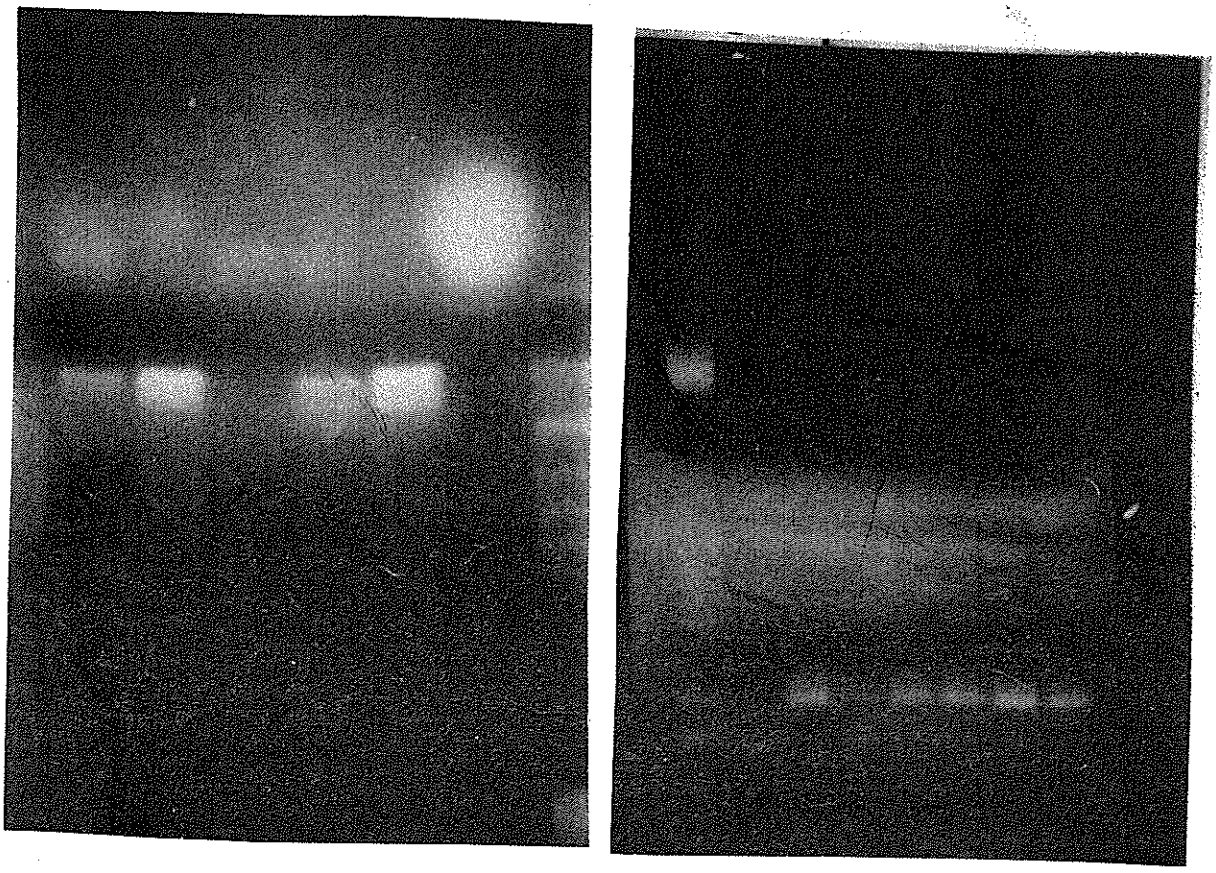
Clinical specimens	Number	Negative and suspected nep BVD virus	IBR	PI-3	BAV-3	CP strain of BVD
			Using virus serum neutralization (VN)			
Uninactivated serum	26	25	- ve	- ve	- ve	1
Buffy coat	49	39	2	2	1	5
Nasal swab	30	25	1	2	- ve	2
Ocular swab	20	17	1	1	- ve	1
Total	125	120	4	5	1	9
HI		0	0	-	-	0
IF			4.4	5.5	1.1	8.9

-ve : No virus isolation.
- : Presence of haemagglutinating activity.
. : No haemagglutinating activity

Table (4) : The use of single step reverse transcription polymerase chain reaction (RT-PCR) and 2nd PCR for detection and genotyping of bovine viral diarrhoea virus isolated from clinical specimens.

Clinical specimens	Number	Negative for PCR test against BVDV	BVD biotype using RT-PCR		IF		RT-PCR 1	2nd PCR 2
			CP	NCP	CP	NCP		
Uninactivated serum	26	25	1	-	1	-	1	1
Buffy coat	44	37	5	2	4	1	7	6
Nasal swabs	27	23	2	2	2	0	4	3
Ocular swabs	18	16	1	1	1	1	2	1
	115	101	9	5	8/9	2/5	14/14	11/13

NCP : Non cytopathogenic isolates
CP : Cytopathogenic isolate.
BVDV: Bovine Viral Diarrhoea Virus.
(1) Detected any geotype 1 and 2 of BVD virus.
(2) Detected only genotype 2 of BVD isolates



Lanes :

1 : 100 bp DNA ladder.

2-8 : Viral isolates from clinical specimens.

The arrow refers to a unique size of the amplified cDNA fragments of approximately 198 base pairs (bp).

Fig. (1a, b) :

Agarose gel electrophoresis of the first-round polymerase chain reaction (PCR) amplicons derived from 5 untranslated region of bovine viral diarrhoea viral isolates from clinical specimens.

Agarose gel (1.5%) stained with ethidium bromide.

Lanes :
1 : 100 bp DNA ladder.
2-8 : Viral isolates from clinical specimens.
The arrow refers to the size of the amplicons of approximately 105 base pairs (bp).

Fig. (2) :
Agarose gel electrophoresis of the second-round polymerase chain reaction (PCR) amplicons derived from 5 untranslated region of bovine viral diarrhoea viral isolates from clinical specimens.
Agarose gel (1.5%) stained with ethidium bromide.

الملخص العربى

دراسة وبائية لاستكشاف مدى الإصابة بالتهاب القصبة الهوائية والميكوزا والبارإنفلونزا-٣ والأدينو فى الماشية فى مصر

مجدى محمد على الصباغ* و أحمد عبده على الصوالحى**و سميرة سعيد طه*و حسين متولى غالى*

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تم تجميع ٣١٦ عينة سيرم من محافظات مصر المختلفة ممثلة فى كفر الشيخ والقليوبية من العجول الجاموسى والدقهلية ودمياط وبنى سويف والوادى الجديد من العجول البقرى وذلك لإجراء اختبار التعادل فى السيرم. وأوضحت الدراسات مدى انتشار العدوى بالأمراض المعدية التنفسية فى المحافظات موضع الدراسة فى مصر ولم تكن هناك أى نسبة للإصابة فى محافظة الوادى الجديد. وتم مقارنة النتائج باستخدام اختبار الأليزا على العينات الإيجابية باختبار التعادل فى السيرم وذلك لاختبار مدى حساسية اختبار الأليزا. كذلك تم أخذ عينات دم وسيرم ومسحات من العين والأنف لعزل الفيروسات المسببة لهذه الأعراض من محافظة القليوبية والدقهلية ودمياط حيث تم عزل واحد من فيروس الأدينو-٣ وأربع معزولات من التهاب القصبة الهوائية المعدى وخمس معزولات من البارأنفلونزا-٣ بعد مطابقته لاختبار الفلورسنت المشع ونتيجة لوجود فيروس الميكوزا الذى لا يحدث تأثيراً مرضياً بخلايا النسيج الزرعى وعدم قدرة اختبار الفلورسنت المشع على كشفه بنسبة ١٠٠% كذلك تم استخدام تفاعل التبلمر المتسلسل PCR فى الكشف عن فيروس الميكوزا حيث تم عزل ٩ معزولات قادرة على إحداث التأثير المرضى فى خلايا الزرع النسيجى وكذلك عدد خمسة معزولات غير قادرة على إحداث التأثير المرضى فى الخلايا وكذلك تصنيفه طبقاً للتركيب الجينى.

ودلت كل النتائج مجتمعة على وجود وانتشار العدوى بالأمراض التنفسية فى مصر حيث انه لا يستخدم أى تحصين فى تلك المزارع من قبل مما يؤدى لوجود العدوى. لذلك ينصح فريق العمل بتكثيف استخدام اللقاحات للوقاية من هذه الأمراض خاصة المنتجة محلياً مثل لقاح الأمراض التنفسية الفيروسى الميت.