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ISOLATION, IDENTIFICATION AND SERO CONVERSION STUDIES ON BRSV IN EGYPT

By

SABER*, M.S., HANA** ABDEL-AZIZ, SALEM**, S.A.H., MOHAMED **, H.H. AHMED, HADIA**, A.A. MOSSA, NAWAL**, M. ALI, and FATHEIA MOHAMED M**.

* Faculty of Vet. Med. Cairo Univ.
** Animal Health Res. Inst..

Brsv: Bovine respiratory syncytial virus.

SUMMARY

The present work describes for the first time in Egypt the serodiagnosis isolation, and identification of respiratory syncytial virus from affected calves as well as the seroprevalence of infection among cattle.

In the present survey 250 nasal swabs and 400 sera sample were collected from calves, feed lot and diary cattle with different ages, seasons and different degree of respiratory signs, for BRsv isolation, identification and sero diagnosis. In addition to 150 calves apparantly normal contact animals were examined.

The collected samples represented six farms distributed throughout 5 Egyptian governorates. The obtained results proved that 5 out of 7 virus isolates were from calves have 1-6 months age and another 2 isolatates from unweaned calves that 2-3 weeks old, 6 isolates were detected in spring, season while only one during winter season.

Only 3 out of 7 titrated viral isolates were identified as BRSV by SNT and one more by direct FAT. The isolated strains were designated as RBSV Salhai 1994 and RBsv Alexanderia 1994.

The serodiagnosis results of 400 examined sera sample by SNT and ELISA revealed that 65 (16.25%) and 110 (27.5%) sera sample were positive respectively. the antibodies titer ranged from ($^1/_8$ to $^1/_{64}$). Maternal immunity through seroconversion study showed a fadding of passive immunity completely at 5th month age.

INTRODUCTION

Bovine respiratory syncytial virus disease (RBSV) seems to be an "emerging disease" the cause of more and more pneumonia, interstitial pulmonary oedema, and emphysema, especially in recently weaned calves and young cattle. Sheep are also

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susceptible to bovine RBSV. The virus was first detected in Japan, Belgium and Switzerland in 1970, and it was isolated a little later in England and United States in 1974. It probably occurs worldwide (Baker et al., 1985) and (Frank et al., 1993). Clinical features of RBV, disease is particularly important in recently weaned calves and young cattle, especially when they are maintained in closely confined conditions. Infection is characterized by sudden onset of fever, hyperpnea, lethargy, rhinitis, and cough, Bronchiolitis and multifocal and interstitial edema and emphysema, and cases progressing to severe bronchopneumonia may end in death. In general, in outreak situations morbidity is high but mortality is low. The fatality rate is variable ranging up to 20% and usually due to bacterial pneumonia (Baker et al., 1987 and Fenner et al., 1993). Bovine and human RSV and antigenically related also share similarity between their clinical, epidemiologic and atholgoical features (Fenner et al., 1981).

In Egypt the BRSV was delt with from the pathological aspects by (Twafik 1992). The aim of present article was illustarted to describe this problem through a survey carried out on different localities in Egypt using diagnostic techniques designed primarily to detect and/or isolate the principal pathogen (BRSV) as well as its indentification, in addition to seroprevalence and seroconversion of BRSV antibodies in acute and convalescent neonatal calves.

MATERIAL AND METHODS

1- Samples

a) Blood samples and nasal swabs:

400 blood samples and 250 nasal swabs were collected from Fresian cattle of different age (one day old upto 4 years) and different localities (6 farms distributed throughout 5 governorates) including Alexandria, giza, Kafer el-Sheikh el-Menofia and Ismailia) for serological investigation and viral isolation respectively.

N.B.: The preparation and transportation of samples according to (Pierre and Michel 1993).

b) Twenty five off spring of pregnant dams were suffered from mild respiratory signs in Salihia dairy farm were submitted for seroconversion examination.

2- **Laboratory animals:** Dual routes of inoculation (S/C and I/V) in 2 male rabbits were used for preparation of hyper immune serum against standared BRSV strain 375 L. the viral antigen and local antisera were perpared as the method described by (Grist et al., 1979).

3- **Standared virus, and antibovine conjugated FITC.**

a) Referance BRSV 3751 strain used for preparation of viral antigens and serodiagnosis.

b) Antibovine FITC conjugated RBSV antiserum used for direct FAT detection of unknown isolates

Both "a" and "b" supplied by Maryland University Microbiology Dept. USA.

4- Viral antigen

Positive and negative viral antigens were locally prepared from infected and non infected

MDBK cell culture with reference virus, according to (Robert et al., 1979).

The viral antigen was used for ELISA serodiagnosis.

5- Enzyme conjugate:

Peroxidase labelled, affinity purified antibodies to bovine IgG (H-L.) goat was supplied by Institute of Research and Development, Birmingham Research Park, Vincent Drive, Birmingham, B152, Sq, England. It used for ELISA.

6- Cell culture

MDBK and VERO cell line were supported by the National Vet. Disease Lab. (NVDL) USA. They were used for virus isolation, titration, identification, viral antigens preparation and serodiagnosis.

Methodology:

1- **Isolation,** adaptation and propagation of viral isolates on monolayer culture of MDBK cell line from prepared nasal swabs according to Pierre and Michel, (1993).

2- **Infectivity titration:** Of viral isolates according to Pierrre and Michel, (1993) serial dilution (ten fold) of each isolate were inoculated onto confluent MDBK cell. The virus titer was calculated according to (Reed and Muench 1938).

3- Preparation of hyper immune serum:

a) Preparation of BRSV antigen
b) Procedure for hyper immune sera preparation according to (Grist et al., 1979).

4- Identification of viral isolates by:

a) SNT according to Kruger and Smiths (1986).
b) Direct FAT according to Thomas and Stott., (1981).

5- Serodiagnosis:

a- Screening and quantitative estimation of neutralizing antibodies against BRSV in examined sera sample according to Gillette and Smith, (1985) and Baker et al. (1985) respectively.

b) ELISA tech. according to Robert et al. (1979). Calculation and determination of cut off value according to Peterfy et al. (1983).

RESULTS

Isolation from nasal swabs was conducted on MDBK cells, the characteristic cytopathic effects (CPE) were obvious 5-7 days post inoculation. It is clear from Table (1) that 7 viruses could be isolated from samples collected from Alexanderia and Ismailia governorates only, while the other localities were negative for virus isolation. Severe virus isolates were obtained from calves at an age ranging between (2-3 weeks to 1-6 month). Most of virus isolates were recovered in spring while only one was recovered in winter season. It is clear form the table that a maximum virus titer measuring $10^{5.8}$ $TCID_{50}$/ml was obtained for an isolate collected from Alexanderia governorate while the lowest titer was 10^{2}-$TCID_{50}$/ml from Ismailia.

Potancy assay of locally prepared hyperimmune

Table (1): Some epizootiological data for BRSV isolation and identification

Date of samples collection	Gover.	Farm	No. of nasal swabs	No. of isolates	Age*			Fres. breed	cod us of isolates	Titer of isolates	Identified isolate	
					D	M	Y				SNT	FA
Feb. 1993	Alex.	Dina	9	1		3-6		9	Dalla 9	$10^{5.8}$ $.10^{4}$	+ -	+ -
May 1993		Dalla	13	4		1-2		13	10	$10^{3.2}$	+	+
Dec. 1993	Giza	Agri. exp. station	10 8 20			8	2 1-3 2-4	10 8 20	11 12 Dina 20	$10^{3.6}$ $10^{3.7}$	- - -	- - +
Jan. 1994												
Feb.1994	Kafr El-	Saka	17					17	Salihia	$10^{3.2}$	+	+
Feb. 1994	Sheikh		80		1-7		1-3	80	40 42	10^{2}	-	-
Mar.1994		Toukh	40	2	14-21			40				
Nov.1994	Menofia	Tanbisha	13			3-6		13				
Dec. 1994		Dairy farm	15			8-12	3-4	15				
Dec. 1994	Ismailia	Salihia	25					25				
Total	5	6	250	7				240			3	4
%			——	2.8		------		2.91				

* D = day - M = month - Y = year

Table (2): Sero-prevalence of BRSV and serum neutralization titer in different governorates

Date of samples collection	Gover.	Farm	No. of sera sample	Age*			Screening		Titer			
				D	M	Y	-ve	+ve	1:8	1:16	1:32	1:64
Feb. 1993	Alex.	Dina	20		3-6		17	3	3	-	-	-
May 1993		Dalla	18		1-2		16	2	2	-	-	-
Dec. 1993	Giza	Agri.	28		8	2	23	5	3	2	-	-
		exper.	38			1-3	33	5	3	2	-	-
		station	30		2-4		23	7	3	2	2	-
Jand. 1994	Kafr El-Sheikh	Sakha	38			1-3	32	6	4	2	-	-
Feb.1994	Menofia		80	1-7			67	13	6	4	2	1
Feb. 1994	Ismailia	Toukh	70	14-21			61	9	5	2	1	1
Mar.1994		Tanbisha	20		3-6		17	3	2	1	-	-
Nov.1994 Dec. 1994		Dairy farm	28		8-12	3-4	22	6	6	-	-	-
Dec. 1994		Salihia	30				24	6	3	3	-	-
Total	5	6	400				335	65	40	18	5	2
%							83.75	16.25	61.5	27.6	7.6	3

* D = day - M = month - Y = year

Table (3): Sero conversion of calves in acute and convalescent stage against BRSV by using SNT

Stage	Ab-titer							
	< 4	4	8	16	32	64	128	> 256
Acute	72	52	39	17	5	2	1	-
No-of sera sample								
Total 188	39.546	28.5	21.5	9.3	2.7	1.09	0.5	-
%		31.5						
Convalescent								
No. of sera samples	46	35	16	9	17	10	8	5
Total 146	31.5	23.9	10.9	6.16	11.6	6.8	5.4	3.4
%		55.2						

Table (4): Estimation of maternal antibodies against BRSV in young calves using SNT & ELISA Salihia farm (Ismailia)

Age of animals	No. of ser. samples	ELISA		SNT	
		No. of positive	%	No. of positive	%
1 day	25	11	44.00.	8	33.76
1 week	25	11	44.00	8	33.76
2 week	25	11	44.00	8	33.76
3 week	25	8	33.76	5	20.00
1 month	25	7	28.00	5	20.00
2 month	19	7	28.00	3	15.78
3 month	18	4	22.20	2	11.20
4 month	16	2	12.50	-	-
5 month	16	-	-	-	-

Fig. (4) : Histogram of ELISA % results : for detection of antibody level in tested sera samples.

225 (56.25 %) Sera samples : less than 60 % are negative samples
65 (16.25 %) Sera samples : reading between 60 - 90 % are doubtful
110 (27.50 %) Sera samples : more than 90 % are considered positive

serum of inoculated rabbits with standard BRSV antigen by using SNT and ELISA, the neutralizing titer were 1:16 and 1:32 respectively.

Identification of isolated virus by SNT and direct FAT revealed that 3 virus isolates (9, 11 and 40) out of 7 cytopathic agents and one more isolate by FAT (9, 11, 40 and 20). The results showed complete neutralization for the three isolates by SNT while a faint green yellowish granulated cytoplasmic fluorescence by direct FAT.

Prevalence of BRSV antibodies were detected by SNT and ELISA, as showen in Table (2) the results were 65 (16.25%) positive out of 400 sera samples while 335 (83.75%) were negative by SNT, on other hand ELISA recorded that 110 (27.5%) sera samples were positive and 290 (72.5%) were negative.

Table (3) demonstrated the BRSV sera conversion of diseased animals. The serum samples were obtained from 188 calves in the acute stage, they were initially treated for respiratory dieases. Convalescent serum samples were obtained 3 weeks after initial treatment and any rise in BRSV antibody titer were determined. At the time of initial treatment 68% of animals were negative for BRSV neutralizing antibody and the vast majorty of those with detectable antibody had low titers. At three weeks post-treatment, only 55.2% of the animals were negative and those animals that did respond produced high titers to BRSV.

Concerning the age susceptability the results showed the immune response against BRSV in different ages of examined cattle (calves, feed lot and dairy cattle by using ELISA and SNT. The highest incidence age was found in calves (54.5% by ELISA and 61.5% by SNT). The moderate precentage was found in feed lot (27.27% by ELISA and 27.69% by SNT). The lowest incidence was found in dairy cattle (18.18% by ELISA and 10.76% by SNT). Table (4).

The incidence of positive reactors were inversely proportional to the age of the animal against BRSV by both ELISA and SNT. The highest level of antibody was in one day old animals (55% by ELISA and 45% by SNT), and the lowest level of antibody at 3 months age (14% by ELISA and 8.3 by SNT).

DISCUSSION

Bovine respiratory syncytial virus (BRVS) is one of the respiratory viral infection in human, cattle and sheep causing respiratory disease specially in young age in many countries. The disease is responsible for economic losses, that may be directly related to death due to severe interstitial pneumonia or indirectly releated to unthriftness, delayed marketing, and treatment costs (Evermann et al., 1985)

The role of respiratory syncytial virus in endemic pneumonia (Shipping fever) in cattle and sheep is not clearly understood, the virus has been isolated from the respiratory tract of sick calves and lambs after arival in the feed lot, and antibody prevalance studies have indicated that infection at this time is wide spread; and virus is probably endemic in this environment, along with other viral respiratory tract pathogens. The unresolved question is whether or how often infection lead to fibrinous pneumonia caused by Pasteurella haemolytica, the true end event in shipping fever (Frank et al., 1993).

BRSV is an enveloped RNA-virus releated to genus Pneumo virus of family Paramyxoviridae

(Baker 1987). The present article was planned to illustrate the prevalance of BRSV infection among cattle as emphasized by BRSV isolation and identification as well as some of its epizootiological aspects associated with respiratory symptoms.

The epizootiological data obtained from 5 governorate are shown in Table (1). The main clinical respiratory signs agree with those described by Pirie et al., 1981; Trigo et al., 1984 and Verohoeff et al., 1988), such clinical manifestation may be attributed to several microbial factors including BRSV infection (Baker, 1987).

Regarding the virus isolation seven out of 250 virus isolates from prepared nasal swabs gave the typical CPE in the form of syncytial appearance at the 5th day post inoculation and aggregation around the long axis at the 7 the PI on MDBK cells. These finding agreed with Gershwin et al., 1989.

The number of isolates is limited may be due to the BRSV is labile during the freezing and thawing cycles and transportation (Baker, 1987). Most of virus isolates obtained from animals suffered from clear respiratory manifestation. The isolation was achieved only in 2 farms in Alexanderia the isolated strain was designated Alexanderia 1994 and in Ismailia governorate from Friesian calves designated BRSV Salhia 1994 these results supported by (Gershwin et al., 1989). Table (1).

The crowdness, transportation and bad hygenic measures may play an important role as predsiposing factors in wide spread of infection and lowering the immunity of the herd (Baker, 1987). Regarding the seological identification of the isolates, the employed methods were the virus neutralization test (VNT) and direct fluorescent antibody technique (Fat). Three out of 7 viruses isoaltes could be completely neutralized by the VNT using local BRSV positive serum, the neutralizing titer of viral isolates were different with the same local BRSV positive serum, these may be due to the presence of minor antigenic difference between the isolated strains (Buxton and Fraser, 1977, and Gillette and Smith 1985).

Parallel to the previous results one more identified isolate by using direct FAT, by this confirmatory findings for the presence of BRSV antigen, simillar to that described by (Adair and McFerram 1987). The two serological techniques confirmed the identification and the presence of BRSV antigen according to (Thomas and Stott, 1981) and Wellemans (1977) who stated that direct FA is more rapid and simple over both virus isolation and serology in the diagnosis of BRSV infection. Serosurveillance study for BRSV infection specially in acute phase of respiratory diease, was carreid on 400 sera samples examined by SNT and ELISA. The gained results by SNT were 65 samples (16.25%) reacted specifically with BRSV standard virus, the neutralizing titers ranging between 1:8 to 1:64, this finding agreed with those of Gillette and Smith, (185) and Baker et al. (1985) who stated that the neutralizing titer considered to be positive when 1:8 or more. On the other hand the results of ELISA were 110 (27.5%) out of 400 sera samples positive, in contrast to 290 (72.51%) sera samples were negative. Table (2) and Fig. (1) denote the positive and negative results as determined by standard ELISA curve. The positive antigen and

antisera used for examination of 400 sera sample by ELISA, are locally prepared according to (Robert et al., 1979). The presence of neutralizing antibodies to the disease may be the result of natural exposure to BRSV infection as there is no vacciantion policy adopted in Egypt (Kimman et al., 1986).

It is suggested that the antibody detected by ELISA in serum samples and negative in the neutralization test mostly IgG (Probably residual antibodies from a previous infection), while most of the neutralizing antibodies were IGM (Westenbrink and Kimman, 1987).

Concerning the seroconversion of calves in acute and convalescent stage (Table 3) showed that 64 out of 188 (34%) cases in acute stage were positive by SNT (antibody titer ranged from 1:8 to 1:128) while in convalescent period 65 calves out of 146 cases were positive (45%). (antibody titer ranged from 1:8 to 1:256). The highest titer is noticed in convalescent than in acute stage. These findings are in coincidence with (Baker et al., 1986), (Vannieuwstat and Verhoeff., 1983) and (Adiar 1986).

Concerning the serological status against BRSV in different ages of examined cattle by ELISA and SNT table (4) clearify that the highest percent of antibody level was present among calves less than 6 months and the lowest antibody level in dairy cattle more than 2 years in both ELISA & SNT. This results are supported by (Baker et al., 1985). The serological state in neonatal calves in Salhia dairy farm against BRSV by using ELISA and SNT, revealed in the present work the reversible relationship between ages of animals and passive maternal immunity. The highest precent (44%) was detected by ELISA and (33.76%) by SNT at one day old calves. This percent is gradually decreased with animal aging till reaching to zero at 4th to 5th month by SNT and ELISA correspondingly. Our results coincided with Westenbrink et al. (1985) who stated that the maternal antibodies to BRSV were detectable in neonates from one day old until five month of age. The ELISA and virus neutralizaiton test appeared to be more sensitive for detection the low level of maternal antibodies.

Also these findings are supported by many authors Vannieuwstadt and Verhoeff., (1983), Smith et al., (1975), Baker et al. (1986), and Baker et al. (1985). In conclusion the BRSV disease is endemic in Egypt among imported Friesian cattle specially in calves, also it affected feed lot and dairy cattle. Further investigation should discuss the size of this problem later on, in comparison with our gained results. Although the minor percentage of identified viral isolation (1.6%) the BRSV is considered as one of the complex respiratory disease among cattle.

For control of the disease it should be kept in mind that clinical disease of the respiratory tract is often caused by several factors acting together therefore, careful diagnosis is needed before control measures are applied and serological examination of nonvaccinated imported animals before introduced in Egypt.

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