Vet.Med.J.,Giza. Vol.50, No.4. (2002):1021-1038.

SEROPREVALENCE AND PRELIMINARY ISOLATION OF RESPIRATORY SYNCYTIAL VIRUS FROM INFECTED SHEEP IN EGYPT

H.A HUSSEIN*, S.A. EL-SOUALLY** and M.A SHALABY*

* Department of virology, Faculty of Veterinary Medicine, Cairo University, Giza-Egypt, 12515;

** Military research Center, Armed forces, Egypt

Received: 18 .8.2002.

Accepted: 17.10.2002

SUMMARY

In the current study, 380 serum samples and 47 nasal swabs were collected from sheep (Balady and Barki breed) of different ages showing respiratory manifestation in 3 different seasons distributed in 7 different Egyptian Governorates. 27 nasal swabs from contact goats were also collected. RSV antibodies were detected in sheep sera using SNT in a % of 36.8.The highest prevalence % was detected in Alexandria followed by El-Sharkia. RSV antibodies was detected in both breeds. The highest seropositive % was in lambs of 5 to 6 months (75%), in rams of 1-1.5 years (73%) and in ewes of 2-3years (48.1%). The prevalence was high in January (26.4%) followed by December (22.8%). Dot ELISA detected RSV in nasal swabs in 63.8% and 18.5% in sheep and goat, respectively. This study is the first to report the prevalence of RSV among sheep and goat population in Egypt and the preliminary isolation of the virus from diseased animals.

INTRODUCTION

Fatal pneumonia in sheep is an acute and infectious disease complex which have been associated with many of nematodes, bacterial, viral, Mycoplasma spp and chlamydial pathogens (AL-Darraji et al., 1982a; Bryson et al., 1983; Sprakker et al., 1984). Viral diseases constitute one of the important economic problems facing sheep, this viral agents causing respiratory tract diseases include: Infectious Bovine Rhinotracheities (IBR), Para influenza type-3 (PI3), Bovine Viral Diarrhea (BVD), Blue Tongue (BTV) and Bovine Herpes Virus-1 (BHV1) and recently Respiratory Syncytial Virus (RSV).

RSV is a member of genus pneumovirus subfamily pneumovirinae of the family Paramyxoviridae. The virus has been first isolated by Morris et al.,

(1956) from a chimpanzee showing "cold-like" signs and named the isolated agent "chimpanzee coryza agent". Chanock et al., (1957) isolated antigenically identical viruses from two infants, both with lower respiratory disease and their characteristic syncytial cytopathic effect were noted that RSV has been associated with mild upper respiratory tract infection in adults (Chanock and others 1961) and also has been associated with sever lower respiratory tract infection in children (Coates and Chanock, 1962).The subsequent detection of serum antibody to this virus in cattle was carried out by Doggett and others (1968). In addition, Lundgren et al .,(1969) detected RSV complement-fixing antibodies in the sera of different groups of dogs in similar proportion indicated their frequent exposure to this virus.

Inaba et al.,(1970) in Japan and Paccaud and Jacquier (1970) in Switzerland isolated the virus from clinical cases of respiratory diseases in cattle. The virus was then isolated in many countries including: USA in 1974 (Frey, 1983; Baker et al ., 1985); Hungary (Koves and Bartha.,1975); Belgium (Wellemans et al .,1970); Netherlands (Holzhauer and Neiuwstadt 1976); Northern Ireland (Bryson et al., 1978); UK (Pirie et al., 1981); Morocco (Mahin et al .,1982); and in Norway (Baker et al .,1985). BRSV was detected in cattle distributed in many Egyptian Governorates (Tawific 1992, Sahar, 1998) and the virus was isolated for the first time in Egypt in 1995 (Hanaa, 1995 and Saber et al., 1996). Recently, BRSV was also isolated from buffaloes (Salch, 2001 and Shalaby et al., 2002).

In the sheep population, serum antibody to respiratory syncytial virus is found to be widespread. Anti-RSV antibodies have been detected in many countries including: Europe, Canada, Peru and the United states at prevalence rates of 0 to 75% (Brako et al., 1984; El-Azhary et al., 1984 and Lehmkuhl et al., 1985). Respiratory syncytial virus induced a mild respiratory tract disease in colostrum-deprived lambs after experimental inoculation, and in combination with Pasteurella haemolytica. BRSV has an important role in the respiratory tract disease complex of sheep (Lehmkuhl et al., 1979) and the virus was isolated from sheep with mild rhinitis (Lea Master et al .,1983 ; Everman et al ., 1985 and Spraker et al ., 1985). Berthiaume and others , (1973) detected circulating antibodies to RSV in sheep and horses and they failed to detect antibodies in the sera of goats and pigs. The authors noted that mammals known so far to be RSV sensitive are domestic animals in close contact with humans or other primates. There is general agreement that the RSV is probably human in origin and mammals seem to be secondarily infected through contact with man. They proposed that, it would be important to investigate further which animal species of economic importance are susceptible to the RSV and to what extent the virus is pathogenic for these species. Lehmkuhl and Cutlip (1979) and Richardson et al., (1981) have detected antibodies to RSV

and the virus has been isolated from goats in the U.S.A.

There is no published data or study carried out to determine the prevalence of RSV infections in sheep and goats in Egypt. Moreover, the current situation of the virus and its contributions in the respiratory disease complex which commonly observed in sheep and goats is unknown. Therefore, the aim of the current study is to preliminary investigate the situation of RSV in sheep and goats in Egypt. To achieve such goal, our specific objectives planned as follows: (1) Determination of the prevalence of RSV antibodies in sera collected from sheep located in different Egyptian Governorates. (2) Determination of the role of season and age in relation to the incidence of the RSV infection in sheep under local condition. (3) Detection of RSV in nasal swabs collected from sheep and goats with respiratory manifestations. And finally, (4) Trials for isolation of the RSV from the detected positive samples.

MATERIALS AND METHODS

1. SAMPLES :

A total of 380 serum samples were collected from sheep (native breeds) of different sex and ages (ranging from 1 month ñ up to 3 years) on monthly bases from October 1998 to May 1999 representing seven Governorates in Egypt including : Port Said , El-Sharkia , Alexandria , El-Ismailia, El-Menia, Domiate and Cairo. These animals showed different clinical manifestation of respiratory tract including rhinitis , nasal discharge , cough and elevation of temperature. Nasal swabs were collected from 74 animals (47 sheep and 27 goats) showing sever Clinical manifestation of respiratory tract infection.

The detailed data of the colleted samples is shown in (Tables 1 and 2)

Table (1)

Detailed data of the collected sheep serum samples from different areas in seven Governorates

Governorate	Time of sample collection	Season	Breed	Number of serum sample
Port Said (first company)	October 1998	Autumn	Balady	55
Cairo (El-Marg)	November 1998	Autumn (Late)	Balady	25
El-Sharkia (Army farm)	December 1998	Winter	Balady	48
Alexandria (El-Amyria)	January 1999	Winter	Barki	50
El-Ismailia (Army farm)	February 1999	Winter	Balady	50
El-Menia	March 1999	Spring	Balady	50
Domiate	April 1999	Spring	Balady	63
Cairo (Basateen)	May 1999	Spring	Balady	39
Total				**380**

Table (2)

Detailed data of the collected sheep and goats nasal swabs from different areas in four Governorates

Animal species	Governorate	Time of sample collection	Season	Breed	Number of nasal swabs
Sheep	El-Sharkia	December 1998	Winter	Balady	15
	Alexandria	January 1999	Winter	Barki	13
	El-Ismalia	February 1999	Winter	Balady	9
	El-Menia	March 1999	Spring	Balady	10
Total					47
Goat	El-Sharkia	December 1998	Winter	Balady	8
	Alexandria	January 1999	Winter	Balady	11
	El-Ismalia	February 1999	Winter	Balady	8
Total					27

2. Preparation of polyclonal rabbit antisera against the standard BRSV strain

Two months old male rabbit was purchased and maintained in a clean secured and well ventilated place. Standard BRSV kindly supplied by Dr. Christopher Chase, South Dakota state University, USA and utilized in the SNT and in preparation of polyclonal rabbit antiserum against the standard BRSV strain. Three successive doses of the viral antigen were introduced to the rabbit before collection of the hyper immune serum.

3. Serum neutralization test

The neutralization test was carried out according to the standard technique of Rossiter et al., (1985) using Vero(African green monkey kidney) cell line.

4. Dot ELISA for the detection of BRSV in nasal swabs

This assay was applied according to Zheng et al., (1992) for detection of bovine respiratory syncytial virus in nasal discharges.

5. Trials for isolation of BRSV from nasal swabs

The dot ELISA positive nasal swab samples were diluted 1:10 and then filtered using 0.22 um pore diameter filter . A number of 25 ml tissue culture flasks of 80-90% confluent monolayers of Vero cells were prepared one day before the virus inoculation . The growth media (MEM +5-10%FCS) was decanted and 1ml of the diluted nasal swab samples were inoculated. The inoculated tissue culture flasks were incubated at 37°C for 90 minutes with gentle tilting every 10 minutes for adsorption of the virus. The media remaining over cells were decanted. 10ml of maintenance media (MEM +0.5-1%FCS) were added for each tissue culture flask. The tissue culture flasks were then incubated at 37°C for 3-7 days with daily observation and recording of the cytopathogenic effect (CPE) . After 7 days of incubation one cycle of freezing and thawing was applied for release of the virus from the culture cells in supernatant fluid. Five serial passages of each positive nasal swab by DOT ELISA were employed for isolation of BRSV .

6. Detection of the BRSV antigen in the suspected samples throughout the third passages using direct fluorescent antibody technique (FAT)

Direct immunofluorscent test (FAT) was applied for detection of the RSV antigen in the original nasal swab samples as well as in the supernatant fluid collected from different passages of each sample according to (Thomas and Sttot , 1981). Standard anti BRSV Fluorescence-conjugated antibodies was used.

RESULTS

1. Prevalence of BRSV among sheep in different Egyptian Governorates using serum neutralization test :

Of 380 sheep sera tested for BRSV by SNT, 36 . 3 % of the samples were positive . The majority of the samples that have 1 / 8 end point was located at Alexandria, followed by El- Sharkia. Neutralizing antibodies to BRSV were found in both Balady and Barki breed of sheep and the titers were in the same ranges in both breeds (Table 3) .

2. Percentage of BRSV infection in relation to age groups among lambs , rams and ewes :

The highest percentage of the prevalence of BRSV in lambs was in the age between 5 ñ 6 months. However, in rams, the highest percentage was detected in age ranging from 1- 1.5 year (30 . 2 %) and in ewes of 2- 3 years (15.6%) (Table 4).

3. Prevalence of BRSV infection in relation to seasons in sheep showing clinical respiratory manifestation :

The BRSV prevalence results in different seasons clearly showed that the infection is present in the three seasons tested in the study. The prevalence was high in winter and the highest percentage (26.4 %) was found in January followed by December (22.8 %) (Table 5).

4.4.Detection of RSV in nasal swabs collected from sheep showing respiratory manifestation:

BRSV was detected in 30 out of 47 nasal swabs in a percentage of (63.8%) . The majority of the detected positive samples was in December and January . The virus was almost detected in samples after 4 months of collection with varied percentage of detection ranging from 40 % to 77.7 %. It is clear that the winter season represented the highest season for virus detection or isolation (Table 6) .

5. Results of DOT ELISA for the detection of BRSV in nasal swabs collected from contact goats showing respiratory manifestation in sheep farms in 3 Egyptian Governorates:

Analysis of nasal swabs collected from goats showing respiratory manifestation and in contact with the diseased sheep revealed the presence of RSV in the samples in a percentage of 18.5 % . The goats tested from Alexandria showed high percentage of detection, 27.7 %, followed by El-Sharkia, 25 %, whereas samples collected from El-Ismailia were negative . Both male and female animals found to be positive.

Table (3)

Prevalence of BRSV antibodies in different Governorates using serum neutralization test

GOVERNORATE	DATE OF SAMPLE COLLECTION	SEASON	BREED	NO. OF SERUM SAMPLES	SCREENING OF SNT		SERUM NEUTRALIZING END POINT					
					+Ve	- Ve	1/2	1/4	1/8	1/16	1/32
Port Said (first company)	October 1998	Autumn	Balady	55	15	40	5	3	3	3	1
Cairo (EL-Marg)	November 1998	Autumn (Late)	Balady	25	12	13	-	3	-	7	2
El-Sharkia (Army farm)	December 1998	Winter	Balady	48	32	16	2	6	10	7	7
Alexandria (EL-Amyria)	January 1999	Winter	Barki	50	37	13	2	5	17	10	3
El-Ismalia (Army farm)	February 1999	Winter	Balady	50	21	29	4	5	5	7	-
EL-Menia	March1999	Spring	Balady	50	9	41	5	3	1	-	-
Domiat	April 1999	Spring	Balady	63	11	52	7	4	-	-	-
Cairo (Basateen)	May1999	Spring	Balady	39	3	36	1	2	-	-	-
Total (%)				380	140 (36.8%)	240 (63.1%)	26 (18.5%)	31 (22.1%)	36 (25.7%)	34 (24.2%)	13 (9.2%)

Table (4)

Percentage of BRSV infection in relation to age groups among Lambs, rams and ewes suing SNT

Age							
Lambs			Rams		Ewes		
1-2 months	3-4 months	5-6 months	1-1.5 years	2-3 years	above 3 years	1-1.5 years	2-3 years	Above3 years
No.of Serum Samples	28	7	47	140	28	47	27	32	24
No. of +ve	5	1	18	65	16	8	9	13	5
Overall +ve (%)	5 / 82 (6%)	1 / 82 (1.2%)	18 / 82 (21.9%)	65 / 215 (30.2%)	16 / 215 (7.4%)	8 / 215 (3.7%)	9 / 83 (10.8%)	13 / 83 (15.6%)	5 / 83 (6.02%)

Table (5): Screening for RBSV in different seasons in sheep showing clinical respiratory manifestation using SNT

Season	Autumn	Autumn (Late)	Winter			Spring			Total
Month	Oct..	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	
Total no. of samples	55	25	48	50	50	50	63	39	380
No. of +ve (%)	15 (27.2%)	12 (48%)	32 (66.6%)	37 (47%)	21 (42%)	9 (18%)	11 (17.4%)	3 (7.6%)	140 (36.8%)
% of +ve/ Total no. of +ve reactors (140)	10.7%	8.5%	22.8%	26.4%	15%	6.4%	7.8%	2.1%	

Table (6): Results of detection of RBSV in nasal swabs collected from sheep with clinical respiratory manifestation in different seasons using dot ELISA.

	Season				Total
	Winter		Spring		
Months (governorates)	December (El-Sharkia)	January (Alexandria)	February (Ismalia)	March (menia)	
Total Number of nasal swabs	15	13	9	10	47
Number of +ve (%)	11 (73.3%)	8 (61.5%)	7 (77.7%)	4 (40%)	30 (63.8%)
% of +ve / total reactors	11/30 (36.6%)	8/30 (26.6%)	7/30 (23.3%)	4/30 13.3%)	

6. Result of trial for BRSV isolation from nasal swabs collected in different governorates from diseased sheep and goat :

Trial for isolation of RSV from nasal swabs collected from both sheep and goats that showed clinical manifestations was carried out according to the published protocol for isolation of RSV . After propagation of the selected positive samples for RSV (by dot ELISA, 30 from sheep and 5 from goat samples) on MDBK cell line for 2 passages and applying the direct immunofluorscent assay on both the first and second passages, the results revealed that 9 out of 30 sheep and 2 out of 5 goat samples were positive in the first passage. However in the second passage , 10 out of such 30 sheep (6 of the positive nine samples in the first passage and 4 samples which gave negative FA in the first passage) and 1 goat sample (gave negative in the first passage), were positive by FAT. Inoculation of selected 7 sheep and 3 goat based on the FA results on Vero cells due to the discrepancy and changes of MDBK cells has been carried out. Three passages were applied on Vero cells and the results of FA on the first (consider third blind passage for the virus isolation) passage revealed positive for 4 out of the 6 sheep samples and 2 gave weak out of 3 goat samples. It should be noted that CPE behavior of the 10 isolates varied along the three Vero passages. Occasionally, CPE was observed and most of the three passages did not reveal a conclusive CPE .

DISCUSSION

In the present study RSV antibodies was detected in (36.8%) of the tested samples . This finding raised the possible role of RSV in the respiratory manifestation appeared in the examined animals. Such manifestation may be due to many factors as reported by others (Sharma and Woldehiwet , 1990 ; Lehmkuhl 1980 ; Al- Darraji ,1982c and Trigoet al., 1984) . Moreover the virus was isolated from sheep showing mild rhinitis (Everman, et al .,1985) . RSV was also shown to potentiate Pasteurella haemolytica in lung of lambs possibly by affecting lung vitality and thereby permitting secondary infection to become established producing a more sever disease (Al- Darraji et al .,1982 b). As well, RSV reported to cause illness associated by lung lesions in experimentally infected lambs and the virus was isolated from goat (Cutlip and Lehmkuhl ,1979).

Serum neutralizing antibodies (SN) to RSV have been detected in domestic sheep worldwide and the virus has been isolated from sheep with rhinitis (Evermann et al ., 1985). Likewise, RSV showed to induce acute fatal pneumonia in calf and sheep (Al- Darraji, et al ., 1982a; Bryson, et al ., 1983 and Pirie, et al ., 1981) . The crowdness

and bad hygiene measures play a role and predisposing factors in spread of infectious diseases in animal population (Healy, et al .,1993) . The obtained prevalence results provide a clear evidence on the existence and circulation of RSV in sheep and goats population in Egypt .

The distribution of SN titers in the SNT was somewhat high (8-16) unlike the recorded data in previous studies by Goyal, et al., (1988) who found that the majority of sheep had SN titer of 2 (61 %) and 29.5 % of 4 , 8.5 % of 8 , and only 1% of the samples was of 16. Discrepancy in SN titers may be due to many stress factors or may be represent the real local situation of RSV infection in the native breeds . Difference obtained in the prevalence of RSV titers with variability in the present study was absolutely expected (Lehmkuhl, et al .,1985) and is similar to many reports (Berithiaume ,et al., 1973 (81%) , Adair, et al .,1984 (55.5%) , El-Azhary, et al .,1984 (31%) , Tabba, et al., 1994 (67%) and Giangaspero, et al .,1997 (63.6 %) .

Many factors contributed in the differences in prevalence percentage in relation to age groups . In lambs, the low percentage of antibodies detected in the first 4 months may be antibodies due to maternal immunity whereas at the age between 5 ñ 6 months, may be due to active infection .These was clear in the rams which reach the maximum detection rate (30.2 %) . Rams of 2 years or more seemed to have low prevalence of infection. Interestingly, the majority of the samples collected from rams showed neutralizing titers ranging between 4-16 indicating evidence of RSV infection. It was reported in similar study in Minnesota that the majority of SN titer to BRSV ranged between 2-4 (Goyal, et al., 1988) . Indeed, the data obtained in the present study provide that rams with age of 1-1.5 year found to have the highest prevalence of RSV infection . In ewes with ages 2-3 years, the highest percentage of prevalence (15 .6 %) was similar to others (Goyal , et al ., 1988). Such high percentage could be due to the breeding age or may be due to active subclinical infection. It should be noted that all sera collected in the present study were from animals that show clinical manifestation. In fact, RSV infection reported in the present study in various age in groups. Such findings are concurrence with many researchers who detected BRSV antibodies in sheep in Europe , Canada , Peru and USA (Brako et al ., 1984; Elazhary et al ., 1984 and Lehmkuhl et al ., 1985). The existence of antibodies with various titers indicate an active infection could implicate RSV as a major cause in combination with other microbes specially Pasteurella haemolytica in the respiratory manifestation that seen in the sheep examined in this study. It is well documented that combination between BRSV and Pasteurella haemolytica play an important role in the

respiratory disease complex of sheep (Lehmkuhl et al ., 1979).

The distribution of RSV infection in relation to localities among Egyptian Governorates investigated in the present study, was greatly varied .The prevalence rate ranges between 17.4 -74% . The increase in the prevalence rate may be due to many reasons, firstly, the time of samples collection, which differed from Governorate to another. Secondly, the distribution of antibodies with different age groups within each Governorate was varied. Thirdly, such difference in the prevalence rate could be due to the animals are not equally distributed in the Governorate included in the study as sheep population in Egypt are living in a small numbers with uneven distribution (not more than 100 sheep in each group). Certainly, the consecutive testing of the same animals in 2 years period may be demonstrate answer to the question of the role of geographic distribution in relation to prevalence of RSV infection in sheep in Egypt .

Comparing Autumn ,Winter and Spring seasons in relation to the prevalence of RSV, it was clear that winter is the most convenient season to the RSV infection where the high prevalence (26.4%) found in January followed by December (22.8 %). Previous studies indicated that the majority of outbreaks occurred in early winter (Sharma and Woldwhiet,1991 ; and Baker et al .,1986). Yet, under our local season condition, the winter started later in comparison with the season distribution allover the world . Indeed, season may influence the occurrence of RSV infection in relation to the population size and geographic variation . Unfortunately no samples were collected in summer time at which some researchers reported the occurrence of some RSV epidemics (Sharma and Woldehiwet ,1991).

It is still difficult to demonstrate RSV throughout traditional isolation procedure and most of the research workers have directed their effort to develop a potential diagnostic test to detect and characterize RSV. Low rate for RSV isolation from field samples is common (Yamachyta., 1985). This may be due to the fact that the virus is fragil so rapidly loss its viability during the transportation to the laboratory and it is also thermo labile, hence sensitive to freezing and thawing (Baker et al.,1986 ; Anderson and Hierholzer1985 and Baker et al .,1997). Moreover, isolation and propagation of RSV needs at least 10-12 passages to obtain an isolate, these facts handicapped most of RSV researchers to isolate the field virus. Therefore, virus detection in random nasal discharges is considered the most reliable approach to screen the RSV positive animals . At the present study, Dot ELISA was employed to detect RSV in nasal discharge collected from animals that showing respiratory manifestation . ELISA proved to be sensitive , specific , rapid , diagnostic tool in comparison to other tests (Zheng et al ., 1992 ; Saleh

,2001). Employing this assay revealed the fast detection of RSV in a percentage of 63 .8 % of the nasal swabs collected from infected sheep. These results reflect the implication of RSV in such respiratory manifestation. The virus detection results were in accordance with the SNT results where the virus was detected in high percentage in December (36.6 %) and the antibodies were detected in high percentage in January. High detection rate of the virus in December confirms that the incidence of RSV infection occurs in early winter as reported by others (Jacobs and Edington, 1971; Koves and Bartha, 1975; Wellemans and Leunen, 1975 ; Bryson et al ., 1979; Pirie et al ., 1981; Baker et al ., 1986 and Ploegar et al ., 1986) .

RSV in lambs known to be detected after two days post infection and continued to be detected up to six days after infection (Al-Darraji ,1982c and Trigo et al., 1984). Therefore, the obtained detection results in the present study may suggest the onset of the virus infection occurred one week earlier to the samples collection or the development of clinical manifestation . Such fact may confirm the role of RSV in such manifestation and increase its major role in respiratory disease complex problem . Given all together, the season, age and existence of the virus besides the predisposing factors are the major causes of the respiratory disease complex in sheep. Rams seems to be the most affected animals by RSV in the present study. The reason of such higher percentage may be attributed to several stress factors including the accumulation, ventilation ,housing seasons and transportation . In Egypt, the housing season starting in the months of October, November and December, to be ready for slaughter in February and March. Therefore, the majority of the animals examined in the study may be housed at that time (November and December) or the large number of samples collected from rams was higher than lambs and ewes.

The implication of RSV in respiratory manifestation in goat was confirmed as goats examined in the present study showed clinical manifestation. The fact that RSV is transmitted by aerosol transmission route and goat were housed in the same area with sheep showing respiratory manifestation, raised the possible transmission of the disease may be the main reason of detecting the virus in such goats. Moreover , this rate was high in January than in December confirming the possible transmission of the virus as detected in higher percentage in sheep at that month. In conclusion ,the possible natural or acquired infection of goat by RSV could occur . In the present we report the occurrence of the RSV infection in goat in Egypt.

Trial to isolate the RSV from nasal swabs collected from diseased sheep and goats revealed prom-

ising results as we detect the viral antigens after three passages in cell culture using F.A. assay . Application of several passages of the propagated local isolates (7 sheep and 3 goat) and titration of such viruses will address the success of isolation as we did not able to increase the CPE of such viruses by limited passages applied in the present study. Indeed the present study confirms the existence of the RSV in sheep and goats in Egypt.

REFERENCES

Adair, B.M.; McFerran, J.B.; Mckillop, E.R.; McCullough, S.J. (1984): Survey for antibodies to respiratory viruses in two groups of sheep in Northern Ireland. Veterinary Record, 115, 403-406.

AL-Darraji, A.M.; Cutlip, R.C.; lehmkuhl, H.D.; Graham, D.L. (1982a): Experimental infection of lambs with bovine respiratory syncytial virus and Pasteurella heamolytica: pathologic studies. American Journal of Veterinary Research, 43,224-229.

Al-Darraji, A.M.; Cutlip, R.C.; Lehmkuhl, H.D.; Graham, D.L.; Kluge, J.P., Frank, G.H. (1982b) : Experimental infection of Lambs with bovine respiratory syncytial virus and Pasteurella haemolytica : clinical and microbiologic studies. American Journal of Veterinary Research, 43, 236-240.

AL-Darraji, A.M.; Cutlip, R.C.; Lehmkuhl, H.D. (1982c) :Experimental infection of lambs with bovine respiratory syncytial virus and Pasteurella haemolytica : immunofluorescent and electron microscopic studies. American Journal of Veterinary Research 43, 230-235.

Anderson LJ. and Hierholzer J.C (1985) : Antigenic characterization of Respiratory Syncytial Virus strains with monoclonal antibodies.J. Infect. Dis . 151: 626-633.

Baker, J.C.; Ames, T.R.; Markham, R.J.F. (1986) : Seroepizootiologic study of bovine respiratory synscytial virus in a dairy herd. American Journal of Veterinary Research, 47, 240-245.

Baker, J.C. ;Ellis, A. ;and Clark,E.G. (1997) : Bovine respiratory syncytial virus. Veterinary Clinics of North America: Food animal Practice, Vol. 13No. 3, 425- 454

Baker, J.C.; frey, M.L. (1985) : Bovine respiratory sysncytial virus. Veterinary Clinics of North America: Food Animal Practice, 1,259-275.

Berthiaume, L., Joncas, J.†;Boulay, G.†; Pavilanis, V. (1973) : Serological evidence of respiratory syncytial virus infection sheep. Veterinary Record, 93, 337-338.

Brako, E.E.; Fulton, R.W.; Nicholson, S.S.; Amborski, G.F. (1984) : Prevalence of bovine herpes virus-1, bovine viral diarrhea, parainfluenza- 3, goat respiratory syncytial, bovine leukemia and blue tongue viral antibodies in sheep.American Journal of Veterinary Research, 45, 813-816 .

Bryson,D.G.;Evremann,J.F.;liggitt,H.D.;forcyt,W.J.;Breeze, R.G.;(1988) : Studies on the pathogenesis and interspecies transmission of respiratory syncytial virus isolated from sheep. American Journal of Veterinary Research, 4, 1424-1430.

Bryson, D.G.; Mc Ferran, J.B.; Ball, H.J. (1978) : Observation on outbreaks of respiratory disease in housed calves : epidemiologic , clinical and microbiological findings

.Vet . Rec . 130: 485 - 489 .

Bryson, D.G.; Mc Ferran, J.B.; Ball, H.J.; Neill, S.D. (1979) : Observations on outbreaks of respiratory disease in calves associated with parainfluenza type 3 virus and respiratory syncytial virus infection. Veterinary Record, 104, 45-49.

Bryson, D.G. ;Mc Nulty,M.S.; Logan, E.F.;Cush,P (1983):Respiratory syncytial virus pneumonia in young calves: clinical and pathologic findings.American Journal of Veterinary Research , 44, 1648-1655 .

Chanock, R.M.; Kim ,H.W.;Vargosko, A.J. (1961) : Respiratory syncytial virus .I. Virus recovery and other observations during 1960 outbreak of bronchiolitis, pneumonia and minor respiratory diseases in children JAMA , 176:647-653.

Chanock RM, Roizman B, Myers R. (1957) : Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent: I. Isolation, properties and characterization. Am.J. Hyg; 66: 281-290.

Coates, H.V., Chanock, R.M. (1962) : Experimental infection with respiratory syncytial virus in several species of animals . Am.J. Hyg;76, 302 - 312.

Cutlip, R,C.; Lehmkuhl, H.D. (1979) : Lesions in Lambs experimentally infected with bovine respiratory syncytial virus. American Journal of Veterinary Research, 40, 1479-1482.

Doggett, J.E., Taylor-Robinson, D., Gallop, R.G.C. (1968): A study of an inhibitor in bovine serum active against respiratory syncytial virus. Archiv fur die gesamte VirusForsch, 23, 126-137.

Elazhary, M.A.S.Y.; Silim, A.; Dea, S.(1984) :Prevalence of antibodies to bovine respiratory syncytial virus, bovine viral.diarrhea virus, bovine herpesvirus ñ1, and bovine parainfluenza ñ3 virus in sheep and goats in Quebec. American Journal of Veterinary Research, 45, 1660-1662.

Everman, J.F.; Liggit, H.D.; Parish , S.M.; Ward, A.C.S.; Lea Master , B.R. (1985) : Properties of a respiratory syncytial virus isolated from a sheep with rhinitis. American Journal of Veterinary Research ,46 : 947 ñ 951.

Frey , M . L .(1983).Bovine respiratory syncytial virus and acute respiratory distress syndrome in cattle . Bovine Practic , 18 ,73 ñ78

Giangaspero M, Vanopdenbosch E, Nishikawa H, Tabbaa D (1997) : Prevalence of antibodies against respiratory viruses (Parainfluenza virus type 3, respiratory syncytial virus, reovirus and adenovirus) in relation to productivity in Syrian Awassi sheep. Trop anim Health Prod . May, 29 (2): 83-91

Goyal, S.M.; Khan, M.A.; McPherson, S.W.; Robinson, R.A.; Boylan, W.J. (1988) : Prevalence of antibodies to seven viruses in a flock of ewes in Minnesota. American Journal of Veterinary Research, 49, 464-467

Hanaa, A.A.G.(1995): Some virological studies on respiratory syncytial virus in cattle M.V.Sc. thesis in Vet. Sci., Virology Depart. Vet. Med. Cairo Univ.

Healy , AM ; Monaghan, ML; Bassett, HF; Gunn, HM; Markey, BK and Collins, JD. (1993) Morbidity and mortality in a large Irish : microbiological and serological findings in cattle with acute respiratory disease . British Veterinary Journal , 149: 6, 549 ñ 560 ; 24 ref .

Holzhauer, C; and Nieuwstadt, A.P.K.M.I. (1976) : Actiological role of bovine respiratory syncytial virus in bronchopneumonia of yearlings. Tijdschrift voor Diergeneeskunde, 101, 1023-1031.

Inaba, Y.; Tanaka, Y.; Omori, T.; Matumoto, M. (1970) : Isolation of bovine respiratory syncytial virus. Japanese Journal of Experimental Medicine, 40, 473-474

Jacobs, J.W.; Edington, N. (1971) : Isolation of respiratory syncytial virus from cattle in Britain.Veterinary Record, 88, 694.

Koves, B.; Bartha, A. (1975) : Isolation of respiratory syncytial virus from cattle diseased with respiratory symptoms. Acta Veterinaria Academie Scientiarum Hungaricae, 25, 357-362

LeaMaster, B.R.; Evermann, J.F.; Mueller, G.M.; Prieur, M.K.; Vander Schalie, J.(1983) : Serologic and virologic studies on naturally occurring RSV and Haemophilus somnus infection in sheep . Proceedings of the American Association of Veterinary Laboratory diagnosticians. P 265 .

Lehmkuhl , H.D., Cutlip, R.C. (1979) : Experimentally induced respiatory syncytial virus infection in lambs . AM. J. Vet. Res. 40, 512-514.

Lehmkuhl HD, Smith MH, Cutlip R.C. (1980) : Morphogenesis and structure of caprine respiratory syncytial virus . J Virol, 52, 364-369.

Lehmkuhl , H.D., Cutlip, R.C. ; Bolin, S.R.; Brogden,K. A. (1985) : Seroepidemiologic survey for antibodies to selected viruses in the respiratory tract of lambs. American Journal of Veterinary Research ,46,2601-260 .

Lundgren D . L . ,Magnuson ,M . G . and Clapper , W. E . (1969) : Lab . Anim .Care .19 , 352 .

Mahin , L. ; Wellemans , G. (1982:(Serological evidence for the intervention of bovine respiratory syncytial virus in a respiratory disease outbreak in Moroccan cattle . Zentralblatt fur Veterinarmedizin , B ,29, 76ñ79 .

Morris, J.A., Blount, R.E., Savage, R.E. (1956) : Recovery of cytopathic agent from chimpanzees with coryza .Proc. Soc. Exp. Biol. Med. 92, 544-549.

Paccaud, M.F., Jacquier, C. (1970): A respiratory syncytial virus of bovine origin.Archiv fur die Gesamte Virus-Forschung, 30, 327-342.

Pirie, H.M.; Petrie, L.; Pringle, C.R.; Allan, E.M.; Kennedy, G.J. (1981): Acute fatal pneumonia in calves due to respiratory syncytial virus. Veterinary Record, 108, 411-416.

Ploegar, H.W.; Boon, J.H.; klaassen, C.H.L.; Florent, G. Van (1986) : A sero-epidemiological survey of infections with the bovine respiratory syncytial virus in first-season grazing calves. Journal of Veterinary Medicine, B 33, 311-318.

Richardson - Wyatt, L.S.; Belshe, R.B.; London, W.T.; Sly, D.L., Camargo, E.; Chanock, R.M. (1981) : Respiratory syncytial virus antibodies in non ñ human primates and domestic animals.Laboratory Animal Science, 31, 413-415.

Rossiter , P.B.; Tessett, D.M. and Taylor,W.B. (1985). Microneutralization system for use with different strains of pest des petits ruminants virus and rinderpest virus . Tropical Animal Health Production .Vol . 17(no. 2): 75-81.

Saber, M.S; Hanaa, A.A; Salem, S. A. H; Mohamed, H.H.A; Hadia, A.A.H; Nawal, M.A and Fatheia, M.M

(1996). Isolation, identification and seroconversion studies on BRSV. Vet. Med. J. Giza: 44/4, 735-747.

Sahar M.M., (1998): Effect of respiratory syncytial virus vaccine on the immune response of animals . M.V.Sc. thesis infectious disease , Fac. Vet. Med. Cairo Univ.

Saleh A.A. , (2001): Detection and isolation of bovine respiratory syncytial virus in Buffaloes . Ph. D. Sci. thesis Virology, Fac. Vet. Med. Cairo Univ.

Shalaby, M.A; Saleh, A.A; Hussein, H.A; Baker, M.E.A and Samy, A.M (2002). Detection and isolation of bovine respiratory syncytial virus in buffaloes. Vet. Med. J. Giza 50/3: 389-404.

Sharma ,R. and woldrhiwet , Z. (1990) : Pathogenesis of bovine respiratory synctial virus in experimentally infected lambs . Vet. Microbiol., 23,267-272 .

Sharma, R.; Woldehiwet, Z. (1991): Class-specific antibody responses to bovine respiratory syncytial virus in experimentally infected lambs. Epidemiology and Infection.(in press) .

Spraker T R , Hibler C P , Schoonveld G G , (1984): Pathologic changes and microrganisms found in bighorn sheep during stress - related die ñ off . J . Wild Dis . ,20 : 319 - 327 .

Spraker,T.R.;Collins, J.k.; Adrian,W.J.;Olterman,J.H . (1985): Isolation and serologic evidence of a respiratory syncytial virus in bighorn sheep from colorado. Journal of wildlife Diseases, 22,416-418.

Tabba, D. (1994): Flock management system in Syrian Awassi sheep and its influence on the distribution of some viral respiratory diseases In proceedings of the 8th International congress on animal Hygiene, St. Paul,Minnesota,USA 12-16

Tawific, A.E.(1992): Pathological Studies on mortality of calves with special refernce to respiratory effections . Pathology Dept. Fac. Of Vet . med. Cairo Univ.

Thomas, L.H.; and sttot, E.J. (1981): Diagnosis of respiratory syncytial virus infection in Bovine respiratory tract by immunofluorescence. Veterinary Record, 432-435.

Trigo, F.J.; Breeze, R.G.; Evermann, J.F.; Gallina, A.M. (1984): Pathogenesis of experimental bovine respiratory syncytial virus infection in sheep. American Journal of Veterinary Reasearch, 45,1666- 1670 .

Wellemans, G.; Leunen, J. (1975) : Respiratory syncytial virus and respiratory disorders in cattle. Annales de Medecine VÈtÈrinaire, 119, 359-369.

Wellemans, G.; Leunen, J.; Luchsinger, E. (1970) : Isolation of a virus (220 / 69) serologically resembling human respiratory syncytial virus. Annales de MÈdecine Veterinaire, 114, 89-93.

Yamachyta, H.,(1985): Isolation and early fluorescent antigen detection of bovine respiratory syncytial virus in vero cell culture. J. Jap. Vet. Med. Ass. 38: 7, 436 - 442.

Zheng , S.J. ; Gan , M.H. (1992) . Studies of indirect DOT ELIZA for the detection of NDV. Acta Veterinara et Zootechnica Sinica . (23) (2) 187-192 .