# STUDIES ON RESPIRATORY SYNCYTIAL VIRUS IN CATTLE AT SHARKIA GOVERNORATE

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## 1-SUMMARY

Bovine respiratory syncytial virus (BRSV) is considered an economic important respiratory tract pathogen, causing serious losses in calves and adult cattle. Therefore, the present study was conducted to isolate and identify the BRSV in cattle and buffalo showing respiratory manifestations. One hundred and eighty samples, 129 nasal swab and 51 lung tissue were collected from different localities of Sharkia Governorate for this study. Eighty eight out of 180 samples were screened by sandwich ELISA commercial kits for detecting BRSV antigen. Only one lung tissue sample was positive with a percentage of 1.1 %. All collected samples were used in isolation attempts on MDBK cells and antigen detection by fluorescent antibody test. The virus was isolated from 35 samples out of 180 tested samples with a percentage of 19.4 %. Viral antigen was detected in 39 samples out of 180 tested samples with percentage of 21.6% by fluorescent antibody test.

## 2-INTRODUCTION

Bovine respiratory syncytial virus is one of the most important causes of respiratory tract disease in cattle; especially in young calves in which RSV is associated with sever pneumonia, interstitial edema and emphysema. Infection with BRSV occurs in many cattle in rearing areas of the world **(Gillette and Smith, 1985)**. In outbreak situations, morbidity is high but the fatality rate is variable, reach up to 20% and usually due to bacterial pneumonia **(Murphy et al., 1999)**. BRSV infection is characterized by sudden onset of fever, rhinitis, coughing, respiratory distress, increased bronchial sounds, abdominal breathing and reduced appetite. Pathological lesions in cattle with sever disease is characterized by bronchiolitis, multifocal and interstitial edema, emphysema and some cases progressing to sever bronchopneumonia may end with death **(Ellis et al., 2001)**.

BRSV belongs to the genus Pneumovirus, in the family Paramyxoviridae. The virus genome is a 15.2 kb long single stranded, negative –sense and non-segmented

RNA. It is associated with viral proteins to form a nucleocapsid core. The nucleocapsid is packaged within a lipid envelope, which is derived from the host cell plasma membrane and contains virus-encoded glycoproteins responsible for viral attachment and entry into host cells **(Mink et al., 1991).**

Diagnosis of BRSV infections by virus isolation is difficult to be carried out and often requires blind passages of suspect material in cell culture before viral cytopathic effects (CPE) are evident. BRSV can be isolated on Madin-Darby bovine kidney (MDBK) cells and show CPE after 5-7 days of inoculation after 3-5 passages. The virus develops numerous syncytial cells and intracytoplasmic eosinophilic inclusion bodies **(Rosenquist, 1974 and Murphy, 1999)**. Fluorescent antibody test is more rapid and simple over virus isolation in diagnosis of BRSV infection. Serological investigation such as SNT, CFT, IP and ELISA is considerably more fruitful in viral diagnosis **(Gillette, 1983; and Masot et al., 1993)**. BRSV infection can be accurately diagnosed by using reverse transcriptase polymerase chain reaction (**Boxus et al., 2005)**.

Although many researches were done on BRSV in Egypt **(Tawifik, 1992, Hanaa, 1995, Saber, 1996 and Ashraf, 2001),** it still one of the greatest problems that threaten livestock and causes heavy losses among susceptible animals. The present work was planned to detect BRSV antigen in the collected nasal swab and lung tissue samples using ELISA, isolation and propagation of BRSV on cell culture and identification of viral isolates by direct fluorescent antibody test.

## 3-MATERIALS AND METHODS

**Samples: 1-Nasal swabs:** One hundred twenty nine (129) nasal swabs were collected from clinically affected cattle and buffaloes of different ages. These animals were representative of farms distributed through out Sharkia Governorate. Nasal swabs were squeezed then centrifuged at 3000 rpm for 10 minutes. The supernatant was aspirated by using an automatic pipette and divided into aliquots into small cryovials, labeled and kept at -70$^{o}$c until used for both antigen detection and virus isolation.

**2-Lung tissues:** Fifty one (51) lung tissue samples, showing some pathological lesions were collected from diseased cattle and buffaloes slaughtered in Sharkia Governorate abattoirs and transported in sterile bags under cooled conditions. The lung tissue was homogenized in 2 ml of the dilution buffer and centrifuged at 3000

rpm/15 minutes to obtain the supernatant. The supernatant was aspirated into small cryovials, labeled and kept at -70$^{o}$c until used.

**Reference BRSV and BRSV antiserum conjugated with fluorescein isothiocyanate (FITC):** Reference BRSV and BRSV antiserum conjugated with FITC were originally obtained from (National Veterinary Services laboratory -Ames, Iowa, USA), and were kindly supplied by **Dr. Ahmed Abdel-Samie,** Virology Department, Faculty of Veterinary Medicine, Zagazig University.

**Cell culture:** A continuous cell line of MDBK cells were supplied by Rinder-Pest like diseases Department, Veterinary Serum and Vaccine Research institute, Abassia, Cairo. The cell culture was grown and propagated using modified Eagle's minimum essential medium (EMEM). The MDBK cells were used for virus isolation and fluorescence antibody technique.

**Detection of BRSV-specific antigens using ELISA kit:** ELISA kit for bovine respiratory syncytial virus detection (Bio-X Diagnostics-Site du Complexe des Postes-22, rue J. Wauters-5580 Jemelle-Belgiue) was used for detection of BRSV antigen. A total of 88 nasal swabs and lung tissue samples from clinically diseased cattle and buffaloes were assayed for the presence of BRSV-specific antigens. The test procedure was carried out as described by manufacture.

**Isolation of BRSV in cell culture:** The virus was isolated accord to the protocol of **(Smith et al., 1975).** Isolation attempts were done using 180 samples in trial to isolate BRSV on MDBK cells. MDBK cells were distributed in plastic 96 wells tissue culture plate for 70% confluence, the growth media were discarded and 50 ul from each sample was inoculated into triplicate wells. For each plate, cell control and virus control were included .The plates were incubated at 37$^{o}$C for one hour for adsorption, the virus inoculum was discharged and the plates were washed using media and 200 ul of EMEM were added to each well, then incubated at 37$^{o}$C for 5 days with daily examination for recording the development of cytopathic effect. After 5 days, the virus was harvested and used for subsequent passages, after the 3$^{rd}$ passage, the cells were harvested and kept at -70 $^{o}$C for virus isolates identification.

**Identification of BRSV isolates by direct fluorescent antibody technique:** The technique was applied on BRSV isolates according to **Gillette and Smith, 1985.** Two ml of MDBK cells were seeded and grown in each tube with growth medium till the

monolayer confluent sheet was formed. Growth medium was removed from the Leighton's tubes; 200 ul from each isolate was used as inoculum to infect the MDBK cells. The tubes were incubated at 37 $^{o}$C with 5% $CO_2$ for 24 hours. By using wormed maintenance medium, the lieghton tubes were filled, inverted on its concave surface and the cover slides was picked up by clean sterile forcipes and washed by PBS then dried for 10 minutes. Fixation was done by immersing the cover slips into Petri dish containing acetone 100 % for 10 minutes then dried at room temp and fixed on glass slides. The cover slips were placed into a humified chamber and stained with bovine anti BRSV antiserum conjugated with FITC. After 60 minutes of incubation at 37 $^{o}$C, the cover slips were rinsed and washed with PBS 3 times each for 5 minutes at room temperature. The slides were then drained, dried and mounted with 50% buffered glycerol and examined for specific fluorescence. Uninfected control cells, reference positive virus control were included in each assay.

## 4. RESULTS

**Detection of BRSV specific antigens using ELISA:** ELISA was used for detection of BRSV antigens in 88 tested cattle and buffaloes samples (51 lung tissues and 37 nasal swabs). As shown in table (1), specific BRSV antigens were detected in one out of the 88 tested cattle and buffaloes samples with a percentage of 1.1% and there were also 3 suspected samples. The only positive sample (lung tissue) was obtained from a buffalo in Belbis slaughtering house.

**Isolation of BRSV on MDBK cells:** As shown in table (2), a virus was isolated from 35 samples out of 180 tested samples with a percentage of 19.4 %. There was a great variation between the samples that collected from imported and native cattle. A virus was isolated from 27 samples (2 from lung tissue and 25 from nasal swabs) out of 134 tested imported cattle samples, with a percentage of 20%, but not isolated from samples collected from native cattle. In addition, a virus was isolated from 8 (5 from lung tissue and 3 from nasal swab) samples out of 34 tested buffalo samples with a percentage of 23.5%. The positive samples showed the characteristic syncytial cells, which firstly occur in the form of progressive degeneration, then fusion of the cells forming a multinucleated giant cells. CPE was obvious 5 –7 day post-inoculation and gradually increased until 70% of the cell sheet is completely detached in some samples (figure 2). Figure 1 shows the negative control MDBK cells

**Identification of BRSV isolates by direct fluorescent antibody technique:** As shown in table (3), BRSV antigens were detected in 39 samples out of the 180 tested samples with a percentage of 21.6%. Two samples out of 12 tested native cattle samples with a percentage of 16.7%, 29 samples out of 134 tested imported cattle samples with a percentage of 21.6 and 8 samples out of 34 tested buffalo samples with a percentage of 23.5% were positive. The infected cells showed bright-greenish yellow intra-cytoplasmic fluorescence after 24 hours post inoculation figure (3).

## 5. Discussion

Bovine respiratory syncytial virus is one of the most important viruses affecting respiratory tract in cattle, especially in young calves. In this study, a total of 180 samples (nasal swabs and lung tissues) were collected from diseased cattle and buffaloes, suffering from respiratory manifestations in different localities at Sharkia Governorate. These samples proved to be the best one for the process of the virus isolation. This fact was explained by **(Viuff et al., 1996)** who mentioned that BRSV replication was demonstrated in epithelial cells of trachea, bronchi and bronchioles and in alveolar cells.

The first approach to deal with the problem was testing the prepared samples by ELISA. Out of the 180 collected samples, only 88 samples were tested by ELISA for detection of BRSV antigen due to the level of supplied materials. As shown in Table (1), specific BRSV antigen was detected in only one lung tissue sample out of 88 tested samples with a percentage of 1.1% and there were also 3 suspected samples. These results in agreement with the findings of **(Percivalle et al., 1989)** who mentioned that ELISA lacks sensitivity in detection of BRSV antigen. Our results were different from these obtained by **(Masot et al., 1993)** who considered ELISA as diagnostic tool and most suitable technique for identifying BRSV antigen in the lung. The difference between our results and others may be returned to time of sample collection that may be not at the time of acute infection or due to the presence of low levels of antigens in samples.

The second approach of our work was to isolate the virus from the collected nasal swabs and lung tissues. The collected samples were subjected to three successive passages, the percentage of samples that showed CPE increased with the number of passages. It was found that some samples were not isolated by the first

passage, but it needs 3-5 passages to be isolated on MDBK cells. As shown in table (2), the virus was isolated from 35 samples out of 180 tested samples on MDBK cells with a percentage of 19.4 %. The percentage of identified viral isolation (19.4%) was low because BRSV is considered as one of the complex respiratory disease among cattle. Tissue samples containing high concentration of BRSV antigens frequently do not reproduce the virus in cell cultures. Several factors are involved, mainly high virus liability **(Mohanty et al., 1976 and Baker et al., 1986**). Virus isolation was achieved from samples collected from imported cattle which may be explained by higher sensitivity of imported cattle to BRSV due to stress factors. These results were in agreement with the findings of **(Thomas and Stott, 1981)** who isolated 21 samples out of 107 nasopharyngeal specimens with a percentage of (19.6%). Our results disagree with these obtained by (**Gillette and Smith, 1985 and Arns et al., 2003)** who isolated BRSV from only 2.2% and 3% of samples respectively. The differences could be attributed to the type of cell culture or the severity of infection and subsequently the virus titer in the samples.

Finally, all collected samples were subjected to direct fluorescent antibody technique for identification of virus isolates in the collected samples. As shown in table (3), the virus isolate was detected in 39 samples out of 180 tested samples with percentage of 21.6%. The results agreed with **(Thomas and Stott, 1981)** who recorded that 24 out of 107 nasopharyngeal specimens with a percentage of (22.4%) were positive by fluorescent antibody technique. However, the result is less than obtained by **Kimman et al., (1986)** who mentioned that fluorescent antibody technique could detected the antigen with a percentage 65.6%.

Although, it is hard for us to compare the results of the used tests due to difference in the number of tested samples, but it seems that fluorescent antibody test and virus isolation are more sensitive for antigen detection than ELISA. These results agreed with the findings of **(Percivalle et al., 1989 and Lokensgard et al., 1992)** who stated the direct FAT is more rapid and simple over both virus isolation and serology in the diagnosis of BRSV infection. In conclusion, the results showed the presence of BRSV infection in Sharkia Governorate among imported cattle and buffalo. For control of the BRSV disease serological examination should be done to non-vaccinated imported animals before introduced in Egypt.

**Fig 1: Normal MDBK cells.**

**Fig 2: MDBK cells inoculated with field samples, showing CPE in the form of syncytium formation.**

**Fig 3: MDBK cells stained with FITC showing no intra-cytoplasmic fluorescence.**

**Table (1): Detection of BRSV antigens in nasal swabs and lung tissues from diseased cattle and buffaloes by ELISA :**

| Animal species | Nasal swab | | Lung tissue | |
|---|---|---|---|---|
| | No. of samples | % of +ve samples | No. of samples | % of +ve samples |
| Native cattle | 6 | 0% | 6 | 0% |
| Imported cattle | 24 | 0% | 34 | 0% |
| Buffaloes | 7 | 0% | 11 | 9% |
| Total | 37 | 0% | 51 | 1.1% |

**Table (2): Isolation of BRSV from diseased animal's samples using MDBK cells:**

| Animal species | Nasal swab | | Lung tissue | |
|---|---|---|---|---|
| | No. of samples | % of +ve samples | No. of samples | % of +ve samples |
| Native cattle | 6 | 0% | 6 | 0% |
| Imported cattle | 100 | 25% | 34 | 5.9% |
| Buffaloes | 23 | 13% | 11 | 45.5% |
| Total | 129 | 21.7% | 51 | 13.7% |

**Table (5): Identification of BRSV isolates using direct fluorescent antibody technique:**

| Animal species | Nasal swab | | Lung tissue | |
|---|---|---|---|---|
| | No. of samples | % of +ve samples | No. of samples | % of +ve samples |
| Native cattle | 6 | 33.3% | 6 | 0% |
| Imported cattle | 100 | 25% | 34 | 11.8% |
| Buffaloes | 23 | 13% | 11 | 45.5% |
| Total | 129 | 23.3% | 51 | 17.6% |

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## الملخص العربي

تمثل الأمراض التنفسية مشكلة خطيرة في تربية الأبقار الحلابة والتسمين علي مستوي العالم. والآن يعتبر الفيروس التنفسي التنخمى البقري من الأمراض التنفسية ذات الأهمية الاقتصادية لأنه يسبب خسائر فادحة في كل من العجول والأبقار البالغة. لذا تم عمل هذه الرسالة لعزل الفيروس التنفسي التنخمى البقري من الأبقار(المحلية والمستوردة) والجاموس التي تظهر عليها أعراض تنفسية مثل ( إفرازات أنفية ، كحة ، أخطار تنفسية، وارتفاع في درجة الحرارة) والتعرف عليه. في هذه الدراسة تم تجميع ١٨٠ عينة ( ١٢٩ مسحة أنفية و ٥١ نسيج رئوي ) من كل من الأبقار(المحلية والمستوردة) والجاموس من أماكن مختلفة في محافظة الشرقية. تم اختبار ٨٨ عينة فقط بواسطة اختبار الاليزا للتعرف علي الانتيجين الخاص بالفيروس التنفسي التنخمى البقري. وقد أظهرت نتائج الاليزا وجود الانتيجين في عينة واحدة فقط (نسيج رئوي ) من مجموع ٨٨ عينة التي قد تم اختبارها بنسبة (١,١% ) وكانت من الجاموس. تم اختبار ١٨٠ عينة في محاولة لعزل الفيروس على خلايا حيه وقد تم عزل الفيروس من ٣٥ عينة فقط علي خلايا MBDK بنسبة (١٩, ٤%). تم التأكد من تلك العينات المستخدمة في العزل بواسطة اختبار الفلورسينتي المناعي المباشر.