PREVALENCE OF ANTIBODIES TO BOVINE RESPIRATORY SYNCYTIAL VIRUS, INFECTIOUS BOVINE RHINOTRACHEITIS AND PARAINFLUENZA-3 IN CATTLE AND BUFFALO - CALVES

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Abstract

Serum samples were collected from 155 cattle calves and 40 buffalo-calves aged 2 weeks-6 months old, belonging to different farms in which there were some reported cases of pneumonia. These animals included 50 apparently healthy and 145 cattle and buffalo calves in acute and convalescent stages of pneumonia. Cattle and buffalo-calves were tested for antibodies to bovine respiratory syncytia virus (BRSV), infectious bovine rhinotracheitis (IBR) and parainfluenza-3 (PI-3) by serum neutralization test (SNT). Immunocomb IBR, PI-3 and BRSV antibody test kit and haemagglutination inhibition test were applied on some of the tested serum samples.

The prevalence of neutralizing antibodies in cattle calves to RSV was 16%, to IBR virus 53% and to PI-3 virus 63%. In buffalo-calves, it was 7.5% to RSV, 45% to IBR and to PI 53%. The prevalence of antibodies to IBR, PI-3 and BRS viruses was greater in calves (>3 months) than in calves (<3 months). There was a high correlation between immunocomb IgG and SN IBR, PI-3 and BRS virus antibody titers for all samples tested and also to HI titer to PI-3.

INTRODUCTION

Respiratory diseases of young calves are of extreme importance on account of the losses and high death rates. They cause annual losses among animal population according to the annual report of Veterinary Department of the Ministry of Agriculture in Egypt for the last 10 years. Respiratory diseases in calves ranged between 17.9 to 85% (Eweis 1992).

From the other side, calves which begin their life with pneumonia, their capabilities render their keeping economically unprofitable. Enzootic calf pneumonia

is a respiratory disease which affects calves 2 weeks to 5 months of age (smith 1990). The cause is complex and the common virus agents in Egypt include infectious bovine rhinotracheitis (IBR) (Hafez 1973, Fatehia 1974, Hassan 1993), Parainfluanze virus type 3 (PI-3) (Singh and Baz 1966, Fedida *et al.* 1976) and recently syncytial virus (BRSV). According to the recent pathological studies in Egypt, BRSV was detected in many Egyptian Governorates (Tawfik 1992).

Studies of the present work were conducted as follows:

1. Estimation of the specific antibodies to some common bovine respiratory tract viruses such as BRSV, IBRv and PI-3.

2. To evaluate the application of the immunocomb IBR/IP-3/BRSV antibody test kit as a quick sensitive test which detects antibody levels in the serum.

MATERIALS AND METHODS

Blood samples

Blood samples were collected from 115 Fresian calves and 30 buffalo-calves in acute and convalescent stages of pneumonia. Blood samples were also collected from 40 apparently healthy Fresian and 10 buffalo-calves. The blood samples were refrigerated at 4°C overnight; the serum was then separated by centrifugation at 1500 rpm for 10 minutes and the clear sera were collected and inactivated at 56°C for 30 minutes, then, kept in the deep freeze at -20°C. Age factor was not included during sampling, but was considered when the results were analysed.

Reference viruses

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

2. Infectious bovine rhinotracheitis virus (IBR) Abou-Hammad strian isolated by Hafez (1973) was used.

3. Bovine respiratory syncytial virus (BRSV) standard 375 L strain of BRS was kindly supplied by Maryland University, Microbiology Department, USA.

Tissue culture

Median Darby Bovine Kidney (MDBK) cell line was adopted.

Serological techniques

1. Serum neutralization test (Carbery and Lee 1966):

Antibodies to BRSV, PI-3 and IBR viruses were titrated by serum neutralization test in MDBK cell line.

2. Haemagglutination and haemagglutination inhibition tests (Lennetle and Schmidt 1964):

Antibody to PI-3 was titrated against 4 HA units by HI test.

3. Immunocomb IBR/PI-3/BRSV antibody test kit:

This was designed to determine cow serum IgG antibody titers for Infectious Bovine Rhinotracheitis (IBR), Parainfluenza-3 (PI-3) and Bovine Respiratory Syncytial Virus (BRSV) (BIOGAL GALED LABS, Israel).

The positive control (c+) was calibrated to S-3 on the comb scale value and calibrated to 200 ELISA units (0.2 OD). Specimens with an identical or higher colour intensity than the positive control were considered positive. Specimens with a colour intensity lower than the positive control were considered negative or non-immunized. When a test colour was darker than S-6, it may indicate either an acute or recent infection.

RESULTS

Table 1 showed that neutralizing antibody titers to IBRV ranged from 4 to 128, to PI-3 from 4 to 256, to BRSV from 4 to 16 in acute, convalescent cattle calves. In apparently healthy ones, the titer to IBRV ranged from 4-16, to PI-3 from 4-32, to BRSV from 4-8. In acute, convalescent buffalo-calves, neutralizing titers to IBRV ranged from 4-64, to PI-3 from 4-128 and to BRSV they were 4. In the apparently healthy ones, the titers to IBRV ranged from 4-16, to PI-3 from 4-32 and to BRSV they were 8.

In table 2, the greatest prevalence of antibody to IBRV, PI-3 and BRSV was

Table 1. Neutralizing antibody titers to IBR, PI-3 and BRSV in acute, convalescent and apparently healthy cattle and buffalo-calves.

Animal case	Species	Total No.	Virus	Neutralizing antibody titre							
				4	8	16	32	64	128	256	512
Acute and convalescent	Cattle calves	115	IBR	12	10	7	16	19	4		
			PI-3	18	11	6	7	12	8	9	
			BRSV	6	4	4					
Apparently healthy	Cattle calves	40	IBR	7	5	3					
			PI-3	7	9	8	3				
			BRSV	4	6	1					
Acute and convalescent	Buffalo-calves	30	IBR	4	-	3	5	3			
			PI-3	3	1	-	3	5	3		
			BRSV	1							
Apparently healthy	Buffalo-calves	10	IBR	2	-	1					
			PI-3	3	1		2				
			BRSV	-	2						

SNT: SN titres were expressed as a reciprocal of the highest serum dilution that completely neutralized 1oo TCID50 of (IBRV, PI-3 V and BRSV) in MDBK.

Table 2. Distribution of neutralizing antibodies to IBR, PI-3 and BRS viruses among calves <3 and calves > 3 months old.

Age	Species	No.	Animals with antibody %		
			IBR	PI-3	BRSV
< 3 month	Cattle calves	91	62	71	18
< 3 month	Cattle calves	64	42	52	14
< 3 month	Buffalo-calves	22	50	59	9
< 3 month	Buffalo-calves	18	39	44	5

among cattle calves and buffalo-calves <3 months old; the percentages were 62%, 71% and 18%, respectively in cattle calves. In buffalo-calves, it was 50%, 59% and 9%, respectively. The percentages among cattle calves >3 months old were 42% to IBR, 52% to PI-3 and 14% to BRSV, while, in buffalo-calves, they were 39% to IBR, 44% to PI-3 and 5% to BRSV.

Table 3 showed that 30 serum samples were examined by immunocomb test kit, SNT and HI to PI-3. In some cases, lower antibody levels were only measurable by SNT and HI, but, there was a high correlation between comb scale's S positive values and SN, IBR, PI-3 and BRS virus antibody titers in all samples, and also the HI titer to PI-3.

DISCUSSION

Respiratory diseases are often cited as the most significant causes of economic losses in feedlot cattle. In addition, great ecomic losses occurred due to the deaths of animals from respiratory diseases, cost of treatment, weight loss, prolonged feeding periods and prevention programmes (Leukeux *et al.* 1985). The appearance and severity of respiratory diseases can be attributed to stress factors as bad weather, transportation, accumulation of ammonia and excessively high relative humidity in closed barns which lowerd the resistance of the animal and enhanced the multiplication of the the microorganisms. A number of viruses including Parainfluanza-3 (PI-3) virus, Infectious Bovine Rhinotracheitis (IBR) virus are involved in the production of respiratory tract damage (El-Shantory 1979, El-Tarabili 1983, Baker *et al.* 1986 and Hassan 1989).

The present work included serological studies carried out on the sera samples collected from pneumonic, convalescent cattle and buffalo-calves (group I) and apparently healthy ones (group II). From table 1, it is clear that the neutralizing antibody titers in group I to IBR virus reached to 128, to PI-3 to 256 in cattle calves and 128 in buffalo-calves; the titers to BRSV reached to 16 in cattle calves and 4 in buffalo-calves, while, in group II, the neutralizing antibody to IBRV reached to 16, to PI-3 32 in both cattle and buffalo-calves, to BRSV 8 in cattle calves and 8 in buffalo-calves. The demonstration of high antibodies titers against IBR and PI-3 in group I, besides the clinical symptoms of pneumonia or history of recent recovering from it, was indicative for active virus exposure. This work was supported by Adair (1986).

Table 3. Comparative study between Comb scale's "S" value and SN, HI titers to PI-3 virus and SN titer to IBR and BRSV.

No of samples	Comb scale's "S" value			Comb scale's "S" value			HI titer PI - 3
	IBR	PI-3	BRSV	IBR	PI-3	BRSV	
1	S-0	S-5	S-0	-	64	0	160
2	0	6	4	0	128	16	160
3	0	3	0	0	16	0	40
4	0	5	0	8	32	0	80
5	0	0	0	0	0	0	0
6	0	3	0	0	16	0	20
7	0	>6	4	0	256	16	640
8	0	6	3	4	128	8	320
9	3	>6	3	16	128	8	320
10	3	>6	3	16	256	0	640
1	3	0	0	16	8	0	0
2	3	0	0	32	16	0	0
3	5	0	3	128	0	16	10
4	4	6	0	64	128	0	160
5	3	0	0	16	0	4	0
6	0	3	0	4	16	4	40
7	3	0	0	32	0	0	0
8	5	0	0	64	4	4	10
9	5	6	0	64	128	0	320
20	4	0	3	32	8	16	0
1	4	5	0	64	64	0	160
2	3	>6	3	32	256	8	640
3	3	>6	4	16	128	16	640
4	4	5	0	32	128	0	320
5	0	0	0	0	8	0	0
6	3	0	0	32	0	0	0
7	4	3	0	32	16	0	40
8	0	0	0	0	0	0	0
9	4	0	0	0	0	4	0
30	0	0	0	0	0	0	0

The weak results obtained by the same test for the detection of antibody to BRSV referred to the insignificant role played by this virus in the induction of enzootic pneumonia in our investigated calves, although, it is well known that this virus is capable of inducing serious respiratory problems among calves in other localities (Prie *et al.* 1981, Bohlender *et al.* 1982 and Harrison and Pursel 1985). The neutralizing antibody to this disease may be due to natural exposure to BRSV, or probably, due to maternal immunity derived from mothers previously infected with the virus because there is no vaccination policy adopted in Egypt against these respiratory viruses. The age of calves plays an important role in increasing the susceptibility to respiratory syndromes. The age of investigated calves in this study ranged from 2 weeks up to 6 months. This is the age at which maternal antibody became in critical levels due to their considerable decrease. In addition, at this age, the persisting maternal antibody blocks the active response specialized against the causative agents. Similar observations were recorded by Dawson *et al.* (1966), who confirmed this finding, and added that, most of the affected animals were newly born as their age ranged between 2 weeks to 4 months.

The positive serological findings to more than one viral agent in serum sampes collected refer to the presence of double infections of the same animals. Bakos and Dinter (1960) was the first to mention this fact. This has been followed later by findings of several authors authors who came to the same conclusion that, respiratory syndrome in calves can be included by several microbial agents besides the presence of some stress factors (Hamdy *et al.* 1983).

The present study offers an opportunity for a comparative investigation between the different serological tests used. Concerning their sensitivity and validity as diagnostic tools, Table 3 shows a trial where the results of SNT and HI have been compared in relation to S-value of immunocomb test kit. The immunocomb negative, but SNT positive sera might be as a result of early infection where the IgM appears earlier than IgG. IgM is the kind of antibodies detected by SNT, where immunocomb detected only IgG antibodies. It is clear that the increase in the S values was corresponding with high titer of SNT and HI; this means that the immunocomb is a sensitive test as SNT and HI in measuring the antibodies.

The SNT is a specific sensitive test depending on tissue culture and the using of living virus. It takes three days at least to provide a result, while, the immunocomb test kit is quick sensitive test detecting antibody levels in the serum or blood within 35 minutes. In conclusion, it has to be metioned that incidence of

enzootic calf pneumonia (PI-3, IBR, BRSV), as well as the presence of corsiderable levels of antibodies against these affections, must receive more attention for deeper and planned investigation on the national level. It is of great importance to throw some light in this field to realize this problem among the Egyptian calves, and to explain the role played by other etiological agents which are incriminated in the enzootic pneumonia syndrome. This will facilitate the epidemiologists for tracing and controlling such problem with the aid of other specialized in this field.

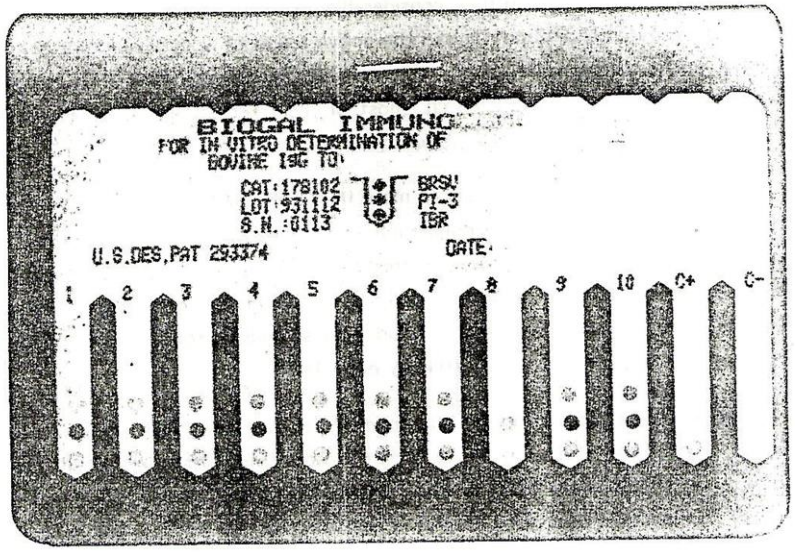

Fig. The immunocomb is a plastic card shaped like a comb on which purified BHV-1 (IBR), P1-3 and BRVS antigens are attached.

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الكشف عن وجود الأجسام المناعية ضد كل من فيروس التهاب الجهاز التنفسى البقرى، التهاب الأنف والقصبة الهوائية المعدى فى الأبقار والبارانفلوانزا نوع ٣ فى العجول البقرى والجاموس

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تم اختبار أمصال بعض العجول البقرى والجاموسى السليمة ظاهريا والمصابة بالالتهاب الرئوى فى مراحله المختلفه لوجود الأجسام المناعيه المضادة لكل من فيروس التهاب الجهاز التنفسى البقرى والتهاب الأنف و القصبه الهوائيه المعدى فى الأبقار والبارانفلوانزا نوع ٣ .

وقد اثبتت النتائج وجود اجسام التعادل لفيروس (RAS) بنسبة ١٦٪ ولفيروس (IBR) بنسبة ٥٣٪ ولفيروس (PI-3) بنسبة ٦٣٪ بينما كانت فى العجول الجاموسى ٧,٥٪ لفيروس (RSV) ، ٤٥٪ لفيروس (IBR) و ٥٣٪ لفيروس (PI-3).

وقد كانت الأجسام المضادة لهذه الفيروسات أكثر شيوعا فى أمصال العجول التى كانت أقل من ثلاثة شهور اذا ما قورنت بالعجول الأكثر من ثلاثة شهور. كما أثبتت نتائج المنشط المناعى وجود علاقة قوية بين نتائجه ونتائج كل من اختبارى التعادل فى الخلايا ومنع التلزن.