Dept. of Biochemistry, Metabolic Diseases Disorders and Toxicology.
Animal Health Research Institute, Dokki, Giza.

SEROLOGICAL AND BIOCHEMICAL STUDIES IN SERUM AND MILK OF INFECTED IBR/IPV CATTLE

(With 9 Tables and 2 Figures)

By

B.G.A. FAHMY; NILLY A. ALI*
and S.A. ABD EL-FATHAH**

* Department of Virology, Animal Health Research Institute, Dokki, Giza.
**Giza Veterinary lab. Animal Health Research Institute, Dokki, Giza.

(Received at 13/9/2006)

دراسا ت سيرولوجيا وكيميائيه حيويه على مصل والبان الماشيه المصابه بمرض التهاب الانف والقصبه الهوائيه \ التهاب البثرى للفرج والمهبل

باسم جرجس فهمى ، نيللى عمر على ، شهاب عبد الوهاب عبد الفتاح

تم عزل وتحديد فيروس مرض التهاب الانف والقصبه الهوائيه \ التهاب البثرى للفرج والمهبل من أبقار تظهر الصوره المرضيه فى منطقه المهبل فى محافظه الدقهليه. شملت الاعراض المرضيه التهاب المهبل والفرج وحالات اجهاض واحتاس المشيمه والتهاب الرحم. وتم عزل الفيروس من المسحات المهبليه والتاكيد بواسطه اختبار التعادل. وتم قياس مستوى الاجسام المناعيه ضد الفيرس بواسطه اختار SNT و الاليزاء فى المصل والالبان. وكان مستوى الاجسام المناعيه و النتائج الايجابيه هى : ٦٣ (٧٠%) و ٧١ (٧٨%) فى المصل و ٣٥(٥٨,٣%) و ٤٠ (٦٦,٧%) فى الالبان بواسطه اختيارى SNT و الاليزاء. واظهرت نتائج التحاليل الكيميائيه زياده فى مستوبات البروتينات ١٩٣,٨٧ و ١٦٣,٩٧ و ١٥٦,٨١ و ١٢٩,٧٠ و ١٠٥,٠٣ و ١٠٠,٩٤ و ٨٧,٧٤ و ٧٤,٦٧ و ٧٣,٩٦ وزن جزئي وانخفاض فى مستوبات البروتينات ١٣٨,٦٩ و ٦٨,٥٥ و ٦٥,٠٩ و ٤١,٣٦ و ٤١,٣٦ وزن جزئي. كما اشارت النتائج التحليل الى ارتفاع عناصر الماغنسيوم والحديد ومعدل ارتباط الحديد يالبرونين فى امصال التى يها اجسام مناعيه منخفضه. والى ارتفاع عناصر الكالسيوم والقوسفورالغير عضوى والماغنسيوم والحديد ومعدل ارتباط الحديد يالبرونين مع وانخفاض فى مستوى الكلورقى امصال التى يها اجسام مناعيه متوسطه والى ارتفاع عناصرالكالسيوم والقوسفورالغير عضوى والماغنسيوم والحديد ومعدل ارتباط الحديد يالبرونين مع وانخفاض فى مستوى الكلورقى امصال التى يها اجسام مناعيه متوسطه والى ارتفاع عناصرالقوسفورالغير عضوى والماغنسيوم والحديد ومعدل ارتباط الحديد يالبرونين مع وانخفاض فى مستوى الكلورقى امصال التى يها اجسام مناعيه العاليه. ان الفيروس IBR/IPV مع المعدل الاصابه المرتفع يصاحيه تغيرات فى الايض ومايتبعه من نغيرات وتودى الى زياده المخاطر على حياه الحيوان ونسبب الخصويه وزياده معدل الاجهاض. ونشير الى اهميه الفحص للحيوانات الماشيه المستورده وان تكون خاليه منه. وان يخذ فى

الاعتبار IBR/IPV كمرض تناسلى وتتبع برامج التحكم للرصد والـتخلص مـن الماشـيه المصابه فى قطعان التربسه فى مصر.

SUMMARY

Infectious pustular vulvo vaginitis (IPV) was isolated and identified from cows showing the genital form of infection, located at Dakahlia Governorate. The clinical viral sign included vulvo-vaginitis, abortion, retained fetal membranes and metritis. IPV was isolated from vaginal swabs on MDBK cell line and confirmed by virus neutralization test (VNT). SNT and ELIZA detected IBR/IPV antibodies in serum and milk samples. SNT and ELISA revealed that 63 (70%) and 71 (78.1%) sera samples were positive respectively while 35 (58.3%) and 40 (66.7%) milk samples were positive respectively. The biochemical changes are more pronounced associated with sera containing high IBR/IPV antibody titer. There were significant increase protein bands with molecular weight 193.87, 163.97, 156.81, 129.70, 105.03, 100.94, 87.74, 74.67, 73.96 KD and significant decrease of protein band with molecular weight (KD) 138.69, 68.55, 66.63, 65.09, 41.36 and 30.39 KD in sera with low, moderate and high IBR/IPV antibody titer associated with several mineral changes including increase magnesium and iron protein binding capacity associated with serum with low IBR/IPV antibodies titer, while moderate IBR/IPV antibodies sera associated with significant increase in total calcium, inorganic phosphorus, total magnesium, iron and iron binding capacity levels and significant decrease in chloride levels, while significant increase in inorganic phosphorus, magnesium, iron and iron binding capacity levels and significant decrease in chloride level ions was determined in serum with high IBR/IPV antibody titer. The increase in IBR/IPV antibody concentration was associated with metabolic changes that lead to an increased individual animal risk of non-pregnancy and increased risk of abortion. All animals imported to Egypt should be free from IBR/IPV infections. IBR/IPV virus as cause of venereal disease must consider. Control programs for detection and removal of IBR/IPV-persisted cattle should be applied in cattle herds all over the country.

Key words: *IBR/IPV, Cattle, Serological, Biochemical, serum, milk*

INTRODUCTION

Cattle are the natural host of herpes viruses (Trapp *et al.*, 2003). Since now, four different bovine viruses are as members of family *Herpesvirida, (Varicllo virus, alpha herpes virinae, Herpes viridae family)*. The prototype of the bovine herpes viruses, bovine herpes virus type 1 (BHV-1), is a causative agent of infectious bovine rhino-tracheitis (IBR), infectious pustular vulvo-vaginitis (IPV) and infectious balano-posthitis (IBP).

Infectious bovine rhino-tracheitis/pustular vulvo-vaginitis (IBR/IPV) is a viral disease of domestic and wild cattle, characterized by various clinical manifestations (Gibbs and Rweyemamu, 1977 and Straub, 1990 and 1991). BHV-1 can be differentiated on the DNA level into three subtypes by restrictions endo-nuclease (Edwards *et al.*, 1991). Although there was no definite associations between subtype and clinical entity, subtype 1 strains cause respiratory infections, subtype 2 strains cause respiratory and genital manifestations and subtypes three strains have neuron-patho-genesis properties.

In 1886, the genital form of the diseases described, which called infections pustular vulvo vaginitis (IPV) is also called "Blaschenass chalg" or "Coital vesicular exanthema" (Madbouly and Hussein, 1997); while respiratory form was first recognized by Mckercher *et al.*, (1954) and isolated in Egypt, by Hafez *et al.*, (1974) several symptomatic disorders including conjunctivitis, encephalitis, abortions, enteritis, and systemic infections in neonate calves. Secondary bacterial infectious invadors that resulting with pneumonia development (Winkler *et al.*, 1999). The previous report of calves mortality due to IBR and/or Para-influenza alone or mixed with bovine viral diarrhea (BVDV) was observed in lower Egypt in El-Minofiya, ElBehira and EL Fayoum provinces with enteritis, either alone or with respiratory form during 2000 (ALy *et al.*, 2003).

Infectious bovine rhino-tracheitis (IBR/IPV) is belongs to family of infectious viral and bacterial diseases causes reproductive loss in beef herds (Waldner, 2005). Although he does not found any association between serological status for IBR and animal health status, pregnancy status, as well as the subsequent risk of abortion, or still birth. He found among 35 randomly herds where the proportion pregnant was > = 90%, 20.4% had positive titer to IBR > = 1:80.

The presence or absence of virus at entry did not predict subsequent illness (Fulton *et al.*, 2002). Our finding that, the herds with

a low morbidity rate, had lower level of IBR/IPV antibodies than herds with high morbidity rate. Also, for individual animals and the herd as whole, high level of antibody to IBR/IPV were related to decrease net value of the owner (carcass value minus total fed lot cost). The use of IBR viral vaccine either in a univalent (modified live IBR virus) or multivalent (modified IBR virus, Para-influenza, bovine viral diarrhea, and bovine respiratory syncytial virus) which protects and decreased losses comparative with un-vaccinated herds including loses as abortion, dead calves or premature born calves (Holzhauer *et al.,* 2003) and make difficult and aggravated the cases. Beside, No previous article was detected the correlation of infection of IBR/IPV with the changes associated with animal statues or milk character.

Infectious bovine rhino-tracheitis (IBR) can causes a venereal infection in adult cattle, acute diseases in dairy cattle is accompanied by severs and prolonged drop in milk production. In 1996, some, 70% of 360 tested dairy herds were positive for BHV-1 antibody in the bulk milk (Pritchard, 1996).

Dairy herds can either be screened by testing individual blood samples (IBS) or bulk milk sample (BMS). It has the following objectives: Caldow *et al.,* (1996) detection of infected herds and qualification of IBR-free herds. The choice for the screening methods depends not just on differences of sensitivity but on efficacy of the test and the consequences, costs and impact of an IBR infection.

The aims of this study were: 1. Clarify the incidence of IBR/IPV in local dairy cattle suffering from reproductive disorders. 2. Report biochemical profile including protein profile and minerals statues during IBR/IPV infection. 3. Reveal effect of virus infections on milk production and milk consumer.

MATERIALS and METHODS

1. Materials:

Animals: on December 2005, in private dairy farms in Dakahlia Governorate, the animals were suffering from inflammations and lesions in the genitalia, some with muco-purulent vaginitis, pyrexia and fever. Reduction, retardations in conceptions rate and vaginal, lacrimal and nasal discharge.

Samples: A. Vaginal swabs: 50 vaginal swabs were collected from cows showing clinical form of vulvo-vaginitis in sterile liquid transport media, preserved in crushed ice and sent to laboratory.

B. Blood, milk and Serum samples: 90 blood and 45 milk samples were collected and serum samples were prepared after complete collating from diseased animals for serological and biochemical investigations.

Cells and Virus: Madin-Darby bovine Kidney (MDBK) cells were grown in minimum essential medium (MEM) with 10% fetal calf serum, Egyptian (Abo- Hamed) strains isolated by Fathia (1974) was obtained from virology department, faculty of veterinary medicine, Cairo university. The virus was titrated according to Reed and Muench (1938).

Viral antigen: positive and negative antigen were locally prepared from infected animals and non-infected MDBK cell culture with reference virus, and using hydro-extraction methods according to Payment and Trudel (1993) and office of international epizootic (2004).

The viral antigen was used fro ELISA.

Hyper Immune sera: it was obtained from Virology Department, Faculty of Veterinary Medicine, Cairo University.

2. Methods:

Isolation: adaptation and propagations of viral isolates on monolayer culture of MDBK cell line from prepared vaginal swabs according to Hafez *et al.* (1974).

Virus identifications: virus neutralization test with reference anti-sera (VNT): equal volume of the isolated virus dilutions containing about 100 TCI D_{50} / 0.1 ml were mixed with serial 2- fold dilutions with IBR anti-sera in micro plates contains 96 wells. Then 100 ul of MDBK cells were added to all wells. The cytopathic effects (CPE) were microscopically observed till 7-9 days according to Shehab *et al.* (1996).

Serological procedures:

Virus neutralization test: virus neutralizing antibody was assayed according to Iman *et al.* (2005) using the micro-titration plate system with incubation of the serum virus mixtures for one hour at 37 °C before addition to the MDBK cell suspension titers were calculated by Reed and Muench, (1938).

ELISA test: indirect ELISA for detections of antibodies was done according to Iman *et al.* (2005). Equally volume of isolated virus dilution containing about 100 $TCID_{50}$/ 0.1 ml was mixed with serial 2-fold dilutions with IBR anti-sera in micro-plates containing 96 wells. Then 100 micro-litters of MDBK cells were added to all wells. Milk samples centrifuge at 2000 r.p.m. for 10 minutes, the creamy layer removed priors testing according to method of (office of international

epizootic, 2004). The cytopathic effect (CPE) was microscopically observed till 7-9 days, according to Shehab *et al.* (1996).

Statistical analysis: the optical density (OD) of the negative control micro-wells is subtracted from that of positive micro-wells sensitized by the viral antigen. The difference in optical densities is compared with that of cut-off value (COV) to determine whether the sample is to be considered positive or negative.

Biochemical parameters: 60 bulk milk samples and 90 serum samples were used. The sera were obtained after centrifuge at 3000 r.p.m. for 15 minutes, hemolysed and white-fatty and colored sera were discarded. The clear sera were used for further biochemical analysis. The positive sera for IBV/IPV virus classified into three main categories according ti IBV/IPV antibody titer into three main categories low , moderate and high according to ELISA results. The selected positive samples were examined by two serological tests. The serum samples were stored at -20 ° C for further biochemical analysis.

1. The electrophoresis pattern for protein using protein standard (range from 20-250 kDa), Bio-Rad, USA using Laemmeli methods (Laemmli, 1970) the separated protein bands were differentia using standard sigma protein band and according to total portion of sample which was analyzed using total protein determination, Henry, (1969).

2. Minerals analysis: The following biochemical parameters were done; total Calcium, Gindler and king, (1972); Inorganic phosphorus, El-Merzabani *et al.*(1977); total Chloride, Schales and Schales, (1941); total Magnesium, Teitz, (1983); Iron, Dreux, (1977); total iron binding capacity (TIBC), Piccardi *et al.* (1972).

The individual bulk milk samples was used to determination of Fat %, protein % and Ash % according to methods (Webb *et al.*, 1983). Using Milk samples, we compared between individual blood sample (IBS), individual bulk milk sample and 3 successive bulk milk sample for sensitivity and specificity apparent prevalence and real prevalence.

Statistical analysis: The statistical analysis of the biochemical results were preformed using T-test (student t test), (Farver, 1989).

RESULTS

Isolation from vaginal swabs was conducted on MDBK cells (Fig 1). The characteristic cytopathic effects (CPE) were observed 48-72 hours post- inoculations. The cells had marked changes consists of

rounding, shrinking and clumping of infected cells in grapes like formations. 3 viruses could be isolated from samples.

The identification of isolated virus using VNT, the results showed complete neutralization for thirteen isolates.

Prevalence of IBR/IPV antibodies was detected by SNT and ELISA. IBR antibodies were detected in 63 serum samples (70%) by SNT, in 71 serum samples (78.8%) by ELIZA (Table 1).

Table 1: Percentage of the positive serum samples detected by SNT and ELISA.

Test	Total number of serum samples		% of positive samples
	+ve	- ve	
SNT	63	27	70
ELISA	71	19	78.80

IBR/IPV antibodies were detected in 35 milk samples (58.3%) by SNT in 40 milk samples (66.7%) by ELISA (Table 2).

Table 2: Percentage of positive the positive milk samples detected by SNT and ELISA.

Test	Total number of milk samples		% of positive samples
	+ve	- ve	
SNT	35	25	58.3
ELISA	40	20	66.7

Table 3 demonstrated the comparison of positive serum and milk samples by SNT and ELISA. The highest incidence was found in serum samples (70%) by SNT and 78.8% by ELISA. while 58.3% by SNT and 66.7 % by ELISA in milk samples.

Table 3: Comparison between result percentages of positive serum and positive milk and positive milk samples detected by SNT and ELISA.

Samples	Total no	% of positive sample	
		SNT	ELISA
Serum	90	70	78.8
Milk	60	58.3	66.70

The cut off value was calculated as described by (Chris and Saif, 1993) so samples with OD of 0.213 or higher was classified as positive samples and any samples with less than 0.213 was considered negative.

Table 4: Detections of antibodies titer to IBR virus in tested sera and milk by SNT of diseased cattle.

Sample	No of samples		Antibody titer of +ve sample					
	Total	+ve	>8	>16	>32	>64	>128	>256
Serum	90	63	16	23	11	7	4	2
	%	70.00	17.80	25.50	12.20	7.80	4.40	2.20
Milk	60	35	11	14	6	3	1	-
	%	58.30	18.30	23.30	10.00	5.00	1.70	-

Table 5: Detection of antibodies titer to IBR virus in tested sera and milk samples by ELISA technique of diseased cattle.

Sample	No of samples		Antibody titer of +ve sample		
	Total	+ve	Low	Moderate	High
Serum	90	71	24	27	20
	%	78.90	26.70	30.00	22.20
Milk	60	40	14	20	6
	%	66.70	23.30	33.30	10.00

Low: 0.213: 0.250. Moderate: > 0.250 to 0.300. High: >0.300.

The biochemical changes are illustrated in the following two tables (6 and 7). The first not, no gross or significant change in physical, chemical and biochemical character of the milk collected from infected cows, except the decrease in milk yield during transit fever and return again to normal level.

Table 6: Milk profile associated with IBR virus in tested milk of control and diseased cattle.

Group / item	Fat (%)	Protein (%)	Ash (%)	Total Calcium (mg/dl)	Inorganic phosphorus (mg/dl)	Total Chloride (mg/dl)	Total Magnesium (mg/dl)
Control	3.96 ±0.17	3.67 ±0.11	0.72 ±0.10	123.00 ±0.10	95.00 ±0.19	119.00 ±3.35	12.00 ±1.11
IBR\IPV antibody titer of +ve sample	3.95 ±0.16	3.63 ±0.13	0.71 ±0.15	125.00 ±0.25	98.00 ±0.20	120.00 ±4.57	12.50 ±1.12

Mean ± standard Error.
*, **, *** significance at 0.05, 0.01 and 0.001 level of probability, using student t test.

Several preliminary estimates were done but failed to show any significant changes between control and IBR/IPV infected sera which give good judgment either to the weak of the infection and/or the high resistance of animals to this form of IBR/IPV infection. The only significant change observed in protein and minerals.

The serum samples were classified according the obtained serological dada (ELISA) into samples with low IBR/IPV antibody sera, others moderate and high IBR/IPV.

Table 7: Electrophoretic serum protein profile associated with IBR virus in tested sera of diseased cattle.

Item	Control			IBR antibody titer of +ve sera sample					
				Low and moderate			High		
	M.Wt.	%	amount	M.Wt.	%	amount	M.Wt.	%	amount
Gamma-2 globulin Bands	207.30	2.65 ±0.02	0.15 ±0.01	193.87	12.00*** ±0.60	0.62*** ±0.03			
Gamma-1 globulin Bands	177.30	3.56 ±0.20	0.19 ±0.05				163.97	28.5*** ±1.59	1.45*** ±0.09
Beta 2 globulin Bands	158.57	4.49 ±0.22	0.25 ±0.01	156.81	11.90*** ±0.59	0.62*** ±0.03			
Beta -1 globulin Bands	132.63	10.90 ±0.55	0.60 ±0.03	138.69	6.13*** ±0.31	0.32*** ±0.02	129.7	26.9*** ±1.70	1.37*** ±0.13
Alpha -2 globulin Bands	107.57	23.40 ±1.52	1.29 ±0.08	110.16	5.48*** ±0.36	0.29*** ±0.02	108.43	10.3*** ±0.67	0.53*** ±0.04
	105.03	9.32 ±0.61	0.49 ±0.03	100.94	7.32 ±0.48	0.38 ±0.02			
Alpha -1 globulin region Bands	87.74	5.66 ±0.37	0.29 ±0.02	87.743	6.34 ±0.48	0.32 ±0.02			
	74.67	4.39 ±0.29	0.23 ±0.01	73.96	6.11 ±0.40	0.31 ±0.02			
Albumin Bands	66.63	23.60 ±1.65	1.29 ±0.09	68.55	26.6 ±1.86	1.38 ±0.09	65.09	3.60*** ±0.23	0.18*** ±0.02
	60.71	13.44 ±0.87	0.74 ±0.05				59.04	3.80*** ±0.25	0.19*** ±0.02
Pre albumin Bands	49.61	11.7 0.53	0.64 0.03				41.36	9.40*** ±0.52	0.48*** ±0.05
	35.29	6.26 ±0.35	0.34 ±0.02	34.64	11.20*** ±0.78	0.58*** ±0.03	30.39	5.05* ±0.30	0.27* ±0.02
Total protein (mg/dl)			5.51 ±0.33			5.20* ±0.31			5.10* ±0.31

Mean ± standard Error. *, **, *** significance at 0.05, 0.01 and 0.001 level of probability, using student test.

No significant and gross changes between sera contain low and moderate IBR/IPV antibody sera, the real difference between control and high IBR/IPV antibody sera.

The obtained results (Table 6) (Fig. 2) demonstrated that:

1. There was significant increase in both percentage and concentration of 193.87, 163.97 KD levels (in gamma globulin region).
2. Significant increase of 156.81 KD (low and moderate IBR/IPV antibody sera), 129.70 KD (high IBR/IPV antibody sera) and significant decrease of 138.69 KD (low and moderate IBR/IPV antibody sera) and 108.43 KD (high IBR/IPV antibody sera) and increases of protein band with molecular weight 105.03, 100.94 KD (in low and moderate IBR/IPV antibody sera) (beta-2, bata-1 and alpha-2 globulin region).
3. Detection of 87.74, 74.67, 73.96 KD (alpha-1 globulin region) and significant decrease in albumin region (65.09, 66.63 and 68.55 KD) in (high IBR/IPV antibody sera).
4. Significant decreases in 41.36 and 30.39 KD (pre-albumin region) (in sera containing high IBR/IPV antibody sera).

Table 8: Serum mineral profile associated with IBR virus in tested sera of diseased cattle.

Group / item		Total Calcium (mg/dl)	Inorganic phosphorus (mg/dl)	Total Chloride (mmol/l)	Total Magnesium (mg/dl)	Total Iron (ug/dl)	Iron binding capacity (mg/l)
Control		8.79 ±0.10	4.45 ±0.09	150.81 ±2.92	1.98 ±0.01	32.64 ±1.47	0.59 ±0.05
IBR antibody titer of +ve sample	Low	8.89 ±0.15	5.58 ±0.10	144.89 ±3.57	** 2.06 ±0.02	34.32 ±3.41	* 1.45 ±0.51
	Moderate	** 9.52 ±0.17	*** 6.29 ±0.67	*** 100.29 ±2.83	** 2.09 ±0.05	*** 50.04 ±0.46	** 1.47 ±0.56
	High	9.48 ±0.34	*** 6.08 ±0.65	*** 101.45 ±2.59	*** 2.34 ±0.04	*** 60.44 ±0.63	** 1.88 ±0.28

Mean ± standard Error.
*, **, *** significance at 0.05, 0.01 and 0.001 level of probability, using student t test.

The low IBR/IPV antibodies sera characterized by double fold significant increase total magnesium level and triple fold significant in Iron protein binding capacity, while moderate IBR/IPV antibodies sera associated with significant increase in total calcium, inorganic

phosphorus, total magnesium, total iron and iron binding capacity levels while 1.5 fold significant decrease in total chloride level, while high IBR/IPV antibodies sera associated with significant increase in inorganic phosphorus, total magnesium, total iron and iron binding capacity levels while significant decrease in total chloride level ions.

Table 9: Efficacy of testing bulk sample versus individual blood sample.

Items	Sensitivity	specificity	Apparent prevalence *	Real prevalence **
Individual blood sample (IBS)	55%	99%	10.20%	17.50%
Individual Bulk milk sample	40%	99%	12.2%	17.00%
3 successive bulk milk sample	65%	98.7%	12.4%	17.4%

* based on bulk milk sampling. ** based on individual blood sampling.

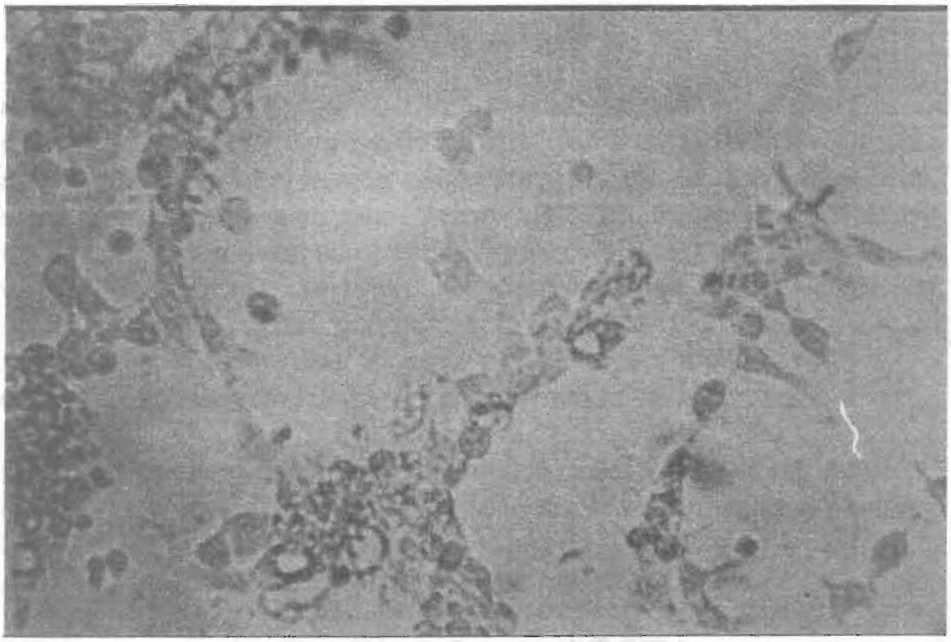

Fig. 1: Infect MDBK cells with suspected sample showing characteristic graphs like appearance (40X).

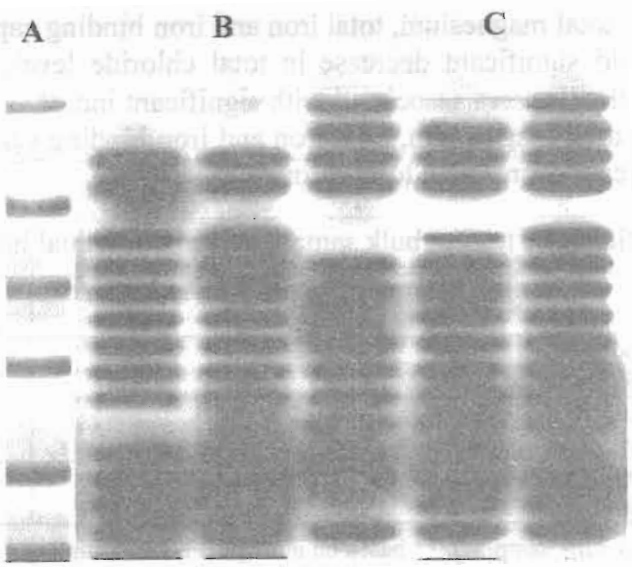

Fig. 2: Serum protein fraction in control and infected IBR/IPV cattle.
A: Standard molecular weight protein with range (30-220 KD).
B: protein fraction profile with of control cattle serum.
C: protein fraction profile with of infected IBR/IPV cattle.

DISCUSSIONS

Infections bovine rhino-tracheitis / infectious pustular vulvo vaginitis (IBR/IPV) is widely distributed all over the world. Although the diseases is name for the syndrome is named for the syndrome with which it was associated (IBR), several other diseases conditions have been attributed to (IBR/IPV) virus as infectious pustular vulvo-vaginitis infections pustular vulvo-vaginitis, conjunctivitis, fatal diseases of new born calves, mastitis, bovine epizootic abortion and encephalitis.

The high antibodies level in serum or milk samples, with clinical symptoms and virus isolation from vaginal swabs indicated the presence of IBR\IPV out breaks among farm animals.

A complication associated with IBR infection is the ability of the virus to establish latency (Pastoret *et al.*, 1984). The latent virus persists during the life of animal and may be reactivated under certain stress conditions. Shading of the virus may or may not be accompanied with clinical sign.

Isolations of IBR/IPV virus from diseased animals with genital form were reported among important friesian cows in Nahda (Hafez *et al.*, 1974). Regarding the virus isolations from prepared vaginal swabs

gave the typical CPE. These isolates were confirmed by virus neutralization test. This result agreed by (Madbouly and Hussein, 1997).

Parallel to the previous results, the two serological techniques (SNT and ELESA) confirmed the identifications and the presence of IBR/IPV according to Edwards *et al.,* 1985.

Virus neutralization (VN) test and ELISA are usually used for detecting antibodies against IBR in serum (Kramps *et al.,* 1993). VNT and EIISA are a prescribed test for international trade (office of international epizootic, 2004) but 24 hours incubation between serum and the virus is recommended to increase sensitivity of the test (Bitsch, 1978).

Serological study for IBR/IPV was carried out on 90 serum samples and 60 milk samples by SNT and ELISA. The gained results by SNT were 70% and 58.3% in serum and milk sample respectively. Result showed that the maximum titer of antibodies (>256) in serum samples and (>128) in milk samples, these results agree with EL-Hakim (2004) who detected IBR antibodies either in serum or milk samples.

On the other hand the result of ELISA was 78.8% in serum samples with high level titer in 22.2. % while 66.7% in milk samples with high level titer in 10%. These results agree with Nylin *et al.,* (2000).

In the present study, we could evaluate the developed indirect ELISA by comparing its results with that of VNT. The results revealed that VNT was less sensitive than ELISA. There fore ELISA was the most sensitive, in expensive, rapid and highly reproducible and these results are agreed with those obtained by Riegel *et al.,* (1987). The indirect ELISA doesn't require any reagents that are difficult to obtained or prepare (Hoff *et al.,* 1992). In addition to the more detected reactors for this assay than nature of some serum samples as mentioned before with Durham and Sillars (1986).

Although, lactating and pregnant cattle were suffering from inflammations and lesions in the genitalia, muco-purulent vaginitis, pyrexia, fever, Reduction and retardations in conceptions rate, vaginal, lacrimal and nasal discharge, no gross or significant change in physical, chemical and biochemical character of the milk collected from infected cows, except the decrease in milk yield during transit fever and return again to normal level. Our data is coincidence with Pritchard, *et al.,* (2003) whom detected that 70% of cows had sero-converted to IBR in large closed dairy herd of high health status with no clinical sign were observed apart from a light bilateral watery ocular discharge in few cows.

There was significant increase in both percentage and concentration of 193.87, 163.97 KD levels (in gamma globulin region) and significant increase of 156.81 KD, 129.70 KD and significant decrease of 138.69 KD and 108.43 KD and significant increase of protein band with molecular weight 105.03, 100.94 KD (beta-2, bata-1 and alpha-2 globulin region)and significant increase of 87.74, 74.67, 73.96 KD (alpha-1 globulin region) and significant decrease in albumin region (65.09,66.63 and 68.55 KD)in (albumin region)(high IBR/IPV antibody sera) and significant decreases in 41.36 and 30.39 KD (pre-albumin region)(high IBR/IPV antibody sera). In spite of the significant increases in gamma globulin levels which due to pronounced immune responses (Mathias and Monika, 2006), the changes in protein profile was associated either with liver (increase in alpha region protein band concentration) and/or kidney disturbance (increase in beta region of protein bands) was due to virus viremic stage and/or effect of genital form of the IBR/IPV infection on epithelia cell lining of both organs. This result can be supported with three facts; asymptomatic viral shading in genital tract (cervix, vulva, anal, uretheral) represents a major source of IBR/IPV transmission (Laurent, 2005). IBR virus was isolated from rhinitis as well as vaginitis (Bagust and Clark, 2005). There was no obvious correlation between pre-infection circulating virus neutralizing antibody titer and vaccine induced protection against virus shadings (Patel, 2005). The present results opposites the previous observation that decrease in total WBC, with a reduction in total numbers of lymphocytes (Trigo *et al.,* 1984), or the lymocellular organs were depleted of lymphocytes (ALy *et al.,* 2003). But on other hand, Kimman *et al.,* (1986) found increase neutrophil /large mononuclear cell ratio in wash fluid from IBR infected animals. This may explained to either to mixed infection or/and secondary infections.

The gross changes in minerals either as increase magnesium level and iron protein binding capacity in sera with low IBR/IPV antibodies titer, associated with significant increase in total calcium, inorganic phosphorus, total magnesium, total iron and iron binding capacity and while 1.5 fold significant decrease in chloride levels detected in moderate IBR/IPV antibodies sera, or significant increase in inorganic phosphorus, magnesium, iron and iron binding capacity levels while significant decrease in chloride level associated with high IBR/IPV antibodies sera. This result indicated the increase the severity of IBR/IPV virus infections as indicated with increase IBR/IPV antibody titer. The explanation of minerals disturbance can be associated with

massive degeneration, necrosis and erosions of lining epithelium of the alimentary (ALy *et al.*, 2003), or due to decrease albumin concentration associated with IBR/IPV infections, as observed with our cases which has important role in minerals distribution.

Cows with clinical IBR frequently suffer from a drop in milk production but return to full production when the diseases has run its course, average duration of drop in milk production be 5 days, Wiseman *et al.*, (1980) described losses in dairy herds with sever IBR, on the average, milk production in lactating cow with clinical IBR dropped by around 14 liters /cow/ day (range: 4-24 liters/cow/day), milk yield was usually only depressed for a few days.

CONCLUSIONS

The high antibodies level in samples, with clinical symptoms and virus isolation from vaginal swabs indicated the presence of IBR\IPV out breaks among farm animals.

It seem relatively high the increase in IBR/IPV antibody concentration was associated /and indicated serious and sever metabolic change leads to an increased risk to animal health demonstrated as protein and mineral profile disturbance, addition of protein and minerals source supply as syrup or electrolytes decrease individual animal health hazards, individual animal risk of non-pregnancy and increased risk of abortion.

Individual milk samples can be used to replace blood samples in lactating cows. Milk monitoring programs are based on the examination of milk samples at three months intervals for antibodies to IBR.

It can be concluded that by increasing the frequency of bulk milk sampling, the sensitivity of the test will improve and the apparent prevalence will be closer to the actual prevalence.

The epidemiological risk of herds had less than ten percent infected animals and the epidemiology situation within the herds is stabilized. So, the epidemiological consequence of the lower sensitivity of bulk sample testes compared to individual blood samples of the lower sensitivity of bulk milk sample tested compared to individual blood sample tests.

All animals imported to Egypt should be free from IBR/IPV infections. IBR/IPV virus as cause of genital disease must be considered. Unfortunately, the use of vaccines is only temporal and with limited value Control programs for detection and removal of IBR/IPV-persisted cattle should be applied in cattle herds all over the Egypt.

REFERENCES

Aly, N.M.; Shehab, G.G. and Abd El-Rahim, I.H. (2003): Bovine viral diarrhea, bovine herpes virus and parainfluenza-3 virus infection in three cattle herds in Egypt in 2000. Rev. Sci Tech., 2003. Dec; 22 (3): 879-892.

Bagust, T.J. and Clark, L.E. (2005): Pathogenesis of meningo-encephalitis produced in calves by infectious bovine rhino-tracheitis herpes virus. Arch. Virol. 2005. 61(1-2):175-181.

Bitsch, V. (1978): the $P_{37/24}$ modification of the infectious bovine rhino-tracheitis virus serum neutralization test. Acta Vet. Scand, 19: 497-505.

Caldow, G.L.; Buxton, D.; Spence, J.A. and Holisz, J. (1996): Diagnosis of bovine abortion in Scotland. XIX World microbiology congress, 8-12 July 1996, Edinburgh, 99, 191-194.

Chris, S.H. and Saif, Y.M. (1993): development of antigen capture enzyme linked immuno sorbent assay for detection of entero virus in commercial turkeys. Avian diseases, 37: 375.

Dreux, C. (1977): Colorimetric determination of iron using thio-glycolic acid. Ann. Biol. Clin. 35: 275.

Durham, P.J.K. and Sillars, H.M. (1986): Evaluations of an ELISA for sero-diagnosis of IBR infection with result of preliminary survey. New-Zealand Vet. J. 34(3): 27-30.

Edwards, S.; Newman, R.H. and White, H. (1991): The virulence of British isolates of bovine herpes virus 1 in relationship to viral genotype. Brit. Vet. J. 174: 216-231.

Edwards, S.; Woods, S.B.; Westcott, D.C.; Emmerson, M. Jones, P.C. and Phillips, A.J. (1986): An Evaluations of five serological testes for the detections of antibody to bovine herpes virus 1 in vaccinated and experimentally infected cattle. Research in Veterinary Sci., 41: 378-382.

EL-Hakim, V.A. (2004): An out break of bovine rhino-tracheitis (IBR) in Egypt. Assuit Vet. Med. J., 50 (100), 123-142.

El-Merzabani, M.; EL-Aaser, A. and ZakharySchales, S.S. (1977): Colorimetric determination of inorganic phosphorus using molybdic acid. J. Clin. Chem. BioChem., 15:, 715-718.

Farver, B.T. (1989): Concepts of normality in clinical biochemistry. In clinical Biochemistry of domestic animals, Kaneko, edit. P2-18 Academic Press. New York.

Fathia, M.M. (1974): Characterization and identifications of infectious bovine rhino-tracheitis virus of calves in Egypt. M.V.Sc. thesis, Fac. Vet. Med., Cairo University.

Frerichs, G.N.; Woods, S.B.; Lucas, H.H. and Sands, J.J. (1982): Saftey and efficency of live and inactivated IBR vaccines. Vet. Rec. 111: 116-122.

Fulton, R.W.; Cook, B.J.; Step, D.L.; Confer A.W.; Saliki bJ.T. et al., (2002): Evaluation of health statues of calves and importane of feed lot performance assessment of a retained ownership program of post waning calves. Can. J Vet. Res. Jul: 66(3):173-180.

Gibbs, E.P.J. and Rweyemamu, M.M. (1977): Bovine herpes viruses 1. Vet. Bull., 317-343.

Glindler, M. and King, J.D. (1972): determination of calcium ions. Am. J. Clin. Path. 58, 376-380.

Hafez, S.M.; Baz, T.L.; Zaharan and Ahamed, Y.A. (1974): Isolations and identifications of infectious pustular vulvo-vaginitis from local cattle and buffaloes in Egypt. J. Egypt. Vet. Med. Assoc., 38(4): 45-53.

Henry, H. (1969): determination of total protein "Hawks clinical chemistry" Lang Press. P38-70.

Hoff, H.S.; Mecham, J.D.; Schuttz, K.I.; Yuill, T.M.; Thompson, L.H. Mo., C.L. and Oviedo, M.T. (1992): Evaluations of diagnostic testes for the detections of central American and Caribbean blue tongue virus serotypes. Proceed. Of the 2nd Intern. Symposium: 626-632.

Holzhauer, M.; Dijk, R. and Mars, G.D. (2003): infectious bovine rhino-tracheitis outbreak on mostly BHV-1 free farm can result in great damage. Tijdschr Diergeneeskd. 2003. Oct: 1; 128 (19): 593-1595.

Iman, M. Bastawecy; Eweis, M.E.; Omayma, M. El-Desay and Ahamed, S.A. (2005): development of an- indirect ELISA for detection of antibodies against bovine herpes virus -1(BHV-1) bovine herpes virus 1 in cattle. Vet. Microbiol, 35:11-21.

Kimman, T.G.; Zimmer, G.M.; Straver, P.J. and De Leeuw, P.W. (1986): Diagnosisi of bovine respiratory virus infections improved by virues detection in lung lavage samples. Am. J. Vet. Res. 1986. Jan; 47(1):143-147.

Kramps, J.A.; Quaks, S. Weerd-meester, K. and Oirschot, J.I. (1993): Comparative study on sixteen enzymes linked immunp sorbent assays for the detections of antibodies. IBR-1 in cattle. Vet., Microb., 40, 125-128.

Laemmli, U.K. (1970): Electrophori tic separations of proteins. Nature, 227, 680-684.

Laurent, R. (2005): Herpes. EMC-medecine, 2005. June; 2(3): 265-275.

Madbouly, H.M. and Hussein, M.M. (1997): Isolation of Bovine herpes virus 1 from a dairy herd showing genital from infection. Vet. Science, Vol.13. No. 4: 439-446.

Mathias, A.J. and Monika, E.E. (2006): Pro and contra IBR-eradication. Vet. Microbil. 2006. March 113(3-4): 293-302.

McKercher, D.G.; Moulton, J.E. and Jasper, D.E. (1954): IBR spreading infections in Europe. Proc. U.S. Livestock san Assoc. 58: 260-266.

Nylin, B.; Stroger, V. and Ronsholt, I. (2000): A retrospective evaluation of a bovine herpes virues-1 (BHV-1) antibodies ELISA on Bulk-tank milk samples for classification of the BHV-1 status of Danish dairy herds. Preventive Vet., Med., 47(1/2): 91-105.

Office of international epizootic (OIE), (2004): Manual of diagnostic testes and vaccines for terrestrial animals, 5th editions.

Pastoret, P.P.; Thiry, E.; Brochier, B.; Derboven, G. and Vinde Vogel, H. (1984): the role of letancy in the epizootiology of infectious bovine rhinotracheitis. In Wittmann, G.;Gaskell, and Rziha, H.J.(Edt.) Latent herpes virus infectious in veterinary medicine. Martinus, Nijhoff, Publishers, the Hague, PP: 211-228.

Patel, J.R. (2005): relative efficacy of inactivated bovine herpes virus-1 (BHV-1) vaccines. Vaccine 2005. July 23(31):4054-4061.

Payment, P. and Trudel, M. (1993): Methods and techniques in virology Marcel Denkker, NewYork.

Piccardi, G.; Nyssen., Dorche, J. (1972): saturation method as a way to measure total iron binding capacity: J. Clin. Chem. Acta., 40: 219.

Prichard, G.C. (1996): Elimination of infection bovine rhinotracheitis virus infection from heavily infected dairy herd. XIX World microbiology congress, 8-12 July 1996, Edinburgh, 99,49-52.

Pritchard, G.C.; Banks, M. and Veron, R.E. (2003): sub-clinical break-down with infectious bovine rhinotracheitis virus infection in dairy herd of high health status. Vet. Rec. 2003. Jul; 26 153(4): 113-117.

Reed, L.J. and Muench, K. (1938): a simple methods of estimating fifty percent end points. Am. J. Hyg., 27: 493-497.

Riegel, C.A.; Ayers, V.K. and Collins, J.K. (1987): Rapid and sensitive, comparative serologic ELISA assay for detecting serum antibodies to BHV-1. J. Cl. Micro. 25(12): 2418-2422.

Schales, O. and Schales, S.S. (1941): The mercurious thiocyanate methods in chloride determination. J. Biol.Chem., 140, 879.

Shehab, G.J.; Rawhia, M.A.O.; Hosny, J.A. and Nawal, M.A. (1996): an outbreak of pneumoentritis in calves caused by IBR and rotavirus in Egypt. Virology and immunopathological aspects. Egypt, J. Comp. Path. and Clin. Path. 9(2): 15 -37.

Strub, O.C. (1990): Infectious bovine rhino-tracheitis virus. In Z. Dinter and B. Morein (Edt.) virus infections of Ruminants, 3Elisiever, Amsterdam, Pp. 71-108.

Strub, O.C. (1991): BHV-1 infectious: relevance and spread in Europe, Comp. Immun. Microbiology. Infect. Dis. 14: 175-186.

Teitz, N.W. (1983): "determination of total magnesium in serum" in "Clinical guide to laboratory tests". Saunders Co. New york: P238-266.

Trapp, S.; Beer, M. and Mettenleiter, T.C. (2003): biology of bovine herpes virues. Berl Munch Tieraztl Wochenschr, May-June: 116(5-6): 171-178.

Trigo, F.J.; Breeze, R.G.; Evermann, J.F. and Gallina, A.M. (1984): pathogenis of experimental bovine respiratory syncytial virus infection in sheep. Am. J. vet. Res. 1984. Aug 45(8):1663-1670.

Waldner, C.L. (2005): serological status for N. Caninum, bovine viral diarrhea, and infectious bovine rhinotracheitis virus at pregnancy testing and reproductive performance in beef herds. Anim Reprod. Sci., 2005 Dec: 90(3-4): 219-42.

Webb, B.; Johnson, A. and Alfoerd, J. (1983): Fundamentals of Dairy Chemistry. 2nd ed, p 120-182, AVI pub com, USA.

Winkler, M.T.C.; Dosters, A. and Jones, C. (1999): Bovine herpes virus 1 can infected CD4 $^{+T}$ lymphocytes and induced cell death during acute infection of cattle. J. of Viro. 37(10): 8657-8668.

Wiseman, A.; Msolla, P.M. and Selman, I.E. and Allan, E.M. (1980): clinical and epidemiological features of 15 incidents of severe infectious bovine rhinotrachities. Vet., Rec., 107: 436-441.