Clinicopathological studies on experimentally infected rabbits with bovine herpesvirus -1

Walaa M. Sayed[1], H. H. Kamel[1], Azza H. Abd-El-Rahman[1], K. A. El-Nesr[2], H. M. Madbouly[3], Amira H. Mohamed[4]

[1]*Department of Clinical Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt*
[2]*Department of Pathology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt*
[3]*Department of Virology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt*
[4]*Department of Clinical Pathology, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt*

Forty-eight pathogen free New Zealand rabbits were divided into two groups, the first group contained eighteen rabbits served as normal control and the second group of thirty rabbits were received 1 ml bovine herpesvirus-1 (BHV-1) virus suspension (10^7 TCID $_{50}$) by intraperitoneal route. Rabbits both groups were subjected to hematological, serum biochemical, different serological and histopathological examination 3,7,10,14,21 and 28 days post infection. Clinical observation of infected rabbits showed febrile response and mild conjunctivitis after 24 and 48h. of inoculation, respectively. The hemogram revealed no significant alteration in the erythrogram while leucogram showed leucocytosis accompanied with heterophilia, lymphopenia and monocytopenia at the 3rd and 7th days post infection. Serum biochemical analysis showed significant elevation in the activity of AST, ALT and AP and in blood urea nitrogen and creatinine concentration along the experimental period. Serum total proteins, albumin, α, β and δ globulin significantly increased at different periods of the experiment. BHV-1 antibodies were detected in the sera of infected rabbits by Dot ELISA and ELISA from the first week until the forth week post infection. Histopathological examination revealed that the most affected organs were the trachea, lungs and liver while adrenals, kidneys, and spleen showed mild pathological alterations.

Bovine herpesvirus-1 (BHV-1) is a member of the subfamily *alpha-herpesvirinae* Roizman *et al.,* (1992). It is the aetiological agent of number of diseases such as infectious bovine rhinotracheitis (IBR), infectious pustular vulvovaginitis (IPV) and infectious balano-posthitis (IBP). Infection with BHV 1 occurs worldwide and causes serious economic losses due to death of animals, abortions, decreased milk production, and loss of body weight (Zhou *et al.,* 1999). IBR was first confirmed in Great Britain in 1961 (Darbyshire *et al.,* 1962 and Dawson *et al.,* 1962). IBR is an important cause of bovine respiratory disease (Martin *et al.,* 1999). BHV-1 may spread in the infected host by viraemia gaining access to a broad range of tissue and organs and causing a variety of diseases (Monika and Ackermann, 1996). Leucopenia accompanied with lymphopenia was observed with BHV-1 (Sandeep and Sharma, 1995; Aly and El-Kanawati, 2000 and Gaber *et al.,* 2000). Blood serum biochemical changes associated with infection with BHV-1 in animals have been studied by some authors. They included increased activities of hepatic enzymes and significant decrease in total protein, albumin and globulin were reported (El-Sawally *et al.,* 1995). ELISA test can detect very small quantities of BHV-1 antibodies (Bratanich *et al.,* 1990). Histopathological alteration of IBR affected animals showed that the virus affected different organs with a cellular destructive power especially in the parenchymatous organs (Nafie *et al.,* 1996).

The present work was carried out to demonstrate the effect of the experimental infection of rabbits with BHV-1 on hematology, serum biochemistry, serodiagnosis of BHV-1 using ELISA and Dot- ELISA as well as histopathology.

Material and Methods

Experiment and design. Forty- eight white Newzealand rabbits of 2-2.5 kg body weight and 2-3 months old were used. Animals were kept under observation for 2 weeks before experimental infection. They received anticoccidial drug, vitamins and minerals. Rabbits were divided into two groups. The first

group (18 rabbits), were inoculated intraperitoneally with 1ml sterile saline and served as normal control. In the second group (30 rabbits) each animal received 1ml IBRV suspension (10^7 $TCID_{50}$) by intraperitoneal route. All rabbits were kept under observation where clinical symptoms and rectal temperature were recorded. Rabbits of the two groups were subjected to haematological, serum biochemical, histopathological and serological examinations.

Virus antigen. BHV-1 was obtained from Animal Health Research Institute, Dokki, Cairo. The virus was prepared in cell culture and has a titer of 10^7 $TCID_{50}$/ml.

Diagnostic kits. Commercial diagnostic kits were supplied by El-Nasr Pharmaceutical Chemicals Co., Egypt and Bio-Merieux, France. They were used in determination of serum alanine aminotransferase (ALT), serum asparate aminotransferase (AST), alkaline phosphatase (AP), blood urea nitrogen (BUN), creatinine and serum total protein. ELISA kit was obtained from Idexx Co.

Sampling.

Blood samples. Blood samples were collected by heart puncture at 3, 7, 10, 14, 21 and 28 days post exposure. In each time blood samples were collected from five infected rabbits and three controlled rabbits. Blood used for determination of the hemogram was collected into clean dry tubes containing EDTA. Blood samples for biochemical determination were collected into clean dry centrifuge tubes without anticoagulant for serum separation.

Tissue specimens. After blood sampling the rabbits were sacrificed. Post mortem findings were recorded and tissue specimens from trachea, lungs, liver, spleen, and kidney were obtained for histopathological examination and virus detection.

Hematological studies. Total erythrocytic (RBCs) and leucocytic (WBCs) counts were done using improved Neubauer hemocytometer. Hemoglobin concentration (Hb) was determined colorimetrically using the cyanomethemoglobin method. Packed cell volume (PCV) was estimated by the microhematocrit technique and differential leucocytic count was performed on the stained blood smear (Jain, 2000).

Serum biochemical studies. Transaminases (ALT and AST) and alkaline phosphatase (AP) activities were determined according to (Reitman and Frankel, 1957 and Kind and King, 1954) respectively. Blood urea nitrogen (BUN) was determined by an enzymatic method after (Reale and Croft, 1961). Kinetic determination of serum creatinine was done colorimetrically according to (Houot, 1985). Serum total proteins and their electrophoretic pattern were estimated according to (Peters, 1968 and Kohin, 1958), respectively.

Histopathological studies. Tissue specimens collected from liver, kidneys, lung, trachea, spleen and adrenal glands from all infected and control rabbits were prepared and stained with haematoxylin and eosin according to (Bancroft and Stevens, 1996).

Serological examination.

Preparation of organ samples for detection of virus antigen. Collected tissue samples (liver- lung- spleen- trachea and kidney) from experimentally infected and control rabbits were around separately in sterile morter using sterile PBS and prepared as 50% homogenate. The homogenates were freezed and thawed for 3-5 successive times then centrifuged for 20 minutes at 3000 r.p.m. The supernatant aliquots were collected in sterile vials and stored at –20 ºC until used.

Dot ELISA. The test was done according to Hawkes *et al.,* (1982).

ELISA. Antibody titration by ELISA kit for collected sera was done according to (Calvo *et al.,* 1994).

Statistical analysis. The obtained data were statistically analyzed for the mean and the standard error of the mean according to (Snedecor and Cochran 1976).

Results

Clinical signs. Clinical observation of the BHV-1 infected rabbits showed febrile response (40- 40.9ºC) after 24 h of infection. The temperature subsided within 3-4 days. Rabbits developed mild conjunctivitis within 48 h. after inoculation. Control rabbits showed no abnormal clinical signs.

Clinical pathology.

Hemogram. Table (1) showed that there is no significant alteration in values of RBCs, Hb and PCV as well as in erythrocytic indices (MCV, MCH, MCHC) of infected rabbits as compared with those of control rabbits. In Table (2), leucogram revealed significant leucocytosis at the 3rd and 7th days post infection accompained with heterophilia and lymphopenia. Monocytes showed significant decrease during the

whole experimental period except at the 3rd and 7th days post infection. Eosinophils revealed significant increase at the 7th day post infection.

Serum biochemistry. Determined activities of ALT, AST, and AP in serum samples of rabbits infected with BHV-1 are shown in Table (3). Significant increase in the activity of all enzymes along the experimental period. Results of serum blood urea nitrogen and creatinine concentrations was recorded (Table 3) revealed significant increase along the experimental period. Determination of serum total protein showed significant increase at the 7th day post infection along the experimental period. Determination of the protein fractions is shown in Table (4). Results showed significant increase in the α globulin at the 10th and 14th day post infection. β globulin revealed a significant increase at the 21st and 28th days post infection.. γ globulin showed significant increase at the 10th, 14th, 21st and the 28th days post infection. The albumin/globulin ratio showed significant decrease at the 10th day, 2nd and 4th weeks post infection.

Pathological findings.

Lung. Gross examination revealed the presence of some areas of congestion and hemorrhage in the lungs of animals sacrificed 3 and 7days post-infection. Histopathological examination of the lung tissue revealed focal interstitial pneumonia in some cases (Fig.1) especially in those sacrificed 3,7 and 10 days post infection. Peribronchial lymphoid hyperplasia was found in same groups. Bronchial epithelium showed mild degree of degenerative changes, with the presence of catarrhal exudates containing erythrocytes and desquamated epithelial cells in the the lung tissue of animals at 14 days post-infection. Congestion and hemorrhage were seen in some areas of the lung tissue after 3 days of infection. Emphysema was commonly found. No inclusion bodies could be seen.

Trachea. Grossly, the trachea of animals' sacrificed 3, 7, 10 and 14 days post-infection showed areas of congestion compared with those of other treated or control animals. Microscopically, the surface epithelium lining the trachea showed degenerative changes, represented by vacuolar degeneration in most cases. Hyperplasia of goblet cells was recorded in all cases especially those associated with pathological alterations. Submucosal blood vessels were dilated and congested in animals sacrificed 3, 7 and 10 days post-infection and were accompanied with oedematous changes with leucocytic infiltrations (Fig.2). Neutrophils infiltrating the wall of the trachea were found in animals sacrificed 10 days post infection. No inclusion bodies were found in any case.

Liver. Grossly, minute pale areas were found on the hepatic capsule and extended on hepatic parenchyma 3 days post-infection only. Vacuolar degeneration (Fig.3) was the most common pathological alteration in the liver of animals sacrificed 3, 7 and 10 days post-infection. Central veins were slightly dilated and congested 3 and 7 days post infection. Early necrobiotic changes were found in the hepatocytes after 3 days of infection. No inclusion bodies could be observed in specimens of all animals. Hyperplastic activation was obvious in Kupffer cells in most cases. Multifocal leucocytic infiltration was seen in the portal area and hepatic parenchyma 3 and 7 days post infection (Fig.4). Hepatic sinusoids of the same animals were dilated. Typical granulomatous reaction represented by necrosis and cellular infiltration of epithelioid cells, lymphocytes and giant cells surrounded by connective tissue capsule was seen (Fig.5).

Kidneys. Degenerative changes were found in the epithelial cells lining renal tubules at the same level of cortex and medulla. Pyknosis with coagulative necrosis of the cytoplasm of the renal epithelium in some areas of cortex were found in the kidneys of animals sacrificed 3, 7 and 10 days post infection (Fig.6). Oedema of Bowman's capsule and atrophy of the capillary tufts were obvious in rabbits slaughtered 3 and 7 days post infection. Congestion of some cortical blood vessels was seen in a few cases. No inclusion bodies could be observed.

Spleen. Grossly, the spleen of most animals was in the normal size except for animals that sacrificed 14 days post infection, which was enlarged. Microscopically, hyperplasia of the lymphoid follicles and widening of sinusoids were found in most animals that obviously observed at 3 and 7 days post infection. No inclusion bodies were seen.

Adrenal glands. Grossly, no detectable pathological alterations were found. Histopathologically, the adrenal glands showed moderate to severe vacoulation of the cytoplasm of the chromofin cells of medulla in animals sacrificed 3, 7, 10 and 21 days post infection. The cortex of all cases showed no pathological alteration.

Serology.

Dot ELISA. Table (5) shows the colour intensity of the reaction. Liver, trachea, lung, kidney and spleen showed blue colour (positive colour) which differ in its intenstiy. Dark blue dots appeared in the lung samples while the liver, kidney and some of spleen samples showed moderate blue dots. Samples of trachea showed faint blue colour.

ELISA for antibody detection. Table (6) shows the IBR antibody titer (represented by ELISA sample/positive % [S/P %]. The titer began to increase from the first week post infection, reached its peak by the third week post infection and persisted until the fourth week post infection.

Table (1): Values of erythrogram of rabbits infected with BHV-1 virus (Mean ± S.E.)

Time of exam. PI*		RBCs ($\times10^6$µl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (Pg)	MCHC (%)
3 days	Control	6.33±0.05	11.20±0.55	38.30±0.33	60.50±0.12	17.67±0.87	29.17±1.45
	Infected	6.66±0.13	11.80±0.18	39.00±0.45	58.58±1.23	17.82±0.35	30.46±0.16
7 days	Control	6.17±0.17	10.73±0.59	39.00±0.58	63.40±2.74	17.50±1.50	27.50±1.15
	Infected	6.20±0.16	11.42±0.15	39.00±0.63	62.98±1.45	18.46±0.59	29.29±0.50
10 days	Control	6.17±0.06	11.06±0.27	39.67±0.33	64.30±1.25	17.93±0.54	27.90±0.64
	Infected	6.13±0.09	11.39±0.19	39.00±0.63	63.61±0.92	18.52±0.49	29.14±0.56
14 days	Control	6.18±0.11	11.20±0.17	40.00±0.58	64.67±0.72	18.13±0.13	28.01±0.40
	Infected	6.21±0.09	11.62±0.24	39.40±0.59	63.58±1.61	18.70±0.31	29.54±0.83
21 days	Control	6.21±0.08	12.00±0.05	39.67±0.33	63.90±1.24	19.33±0.34	30.26±0.14
	Infected	6.20±0.14	12.02±0.26	39.40±0.51	63.64±1.66	19.40±0.65	30.48±0.45
28 days	Control	6.35±0.05	12.00±0.05	40.00±0.58	62.97±0.66	18.87±0.23	30.03±0.50
	Infected	6.31±0.11	12.14±0.13	40.20±0.38	63.80±1.04	19.28±0.33	30.22±0.30

*Time of examination post infection.

Table (2): Values of leucogram of rabbits infected with BHV-1(Mean ± S.E.).

Time of exam. PI.		Total WBCs ($\times10^3$µl)	Differential leucocytic count ($\times10^3$µl)			
			Heterophil	Lymphocyte	Monocyte	Eosinophil
3 days	C	8.40 ±0.08	2.53±0.12	5.36±0.09	.0.43±0.07	0.03±0.03
	I	10.93 ±0.38*	7.20±0.42**	3.32±0.38**	0.38±0.04	0.02±0.02
7 days	C	8.50 ±0.25	2.70±0.20	5.30±0.10	0.43±0.07	0.06±0.03
	I	11.34 ±0.50*	7.20±0.35**	3.42±0.19**	0.48±0.06	0.24±0.05*
10 days	C	9.27 ±0.08	2.83±0.13	5.80±0.21	0.53±0.03	0.06±0.03
	I	9.26 ±0.28	3.62±0.25	5.38±0.18	0.24±0.04**	0.02±0.02
14 days	C	9.14 ±0.17	2.43±0.11	6.00±0.10	0.63±0.03	0.03±0.03
	I	9.20 ±0.29	2.76±0.22	6.10±0.13	0.20±0.03**	0.16±0.05
21days	C	9.25 ±0.06	2.76±0.24	5.90±0.11	0.53±0.03	0.06±0.07
	I	8.82 ±0.25	3.06±0.13	5.40±0.18	0.22±0.05**	0.10±0.04
28 days	C	9.04 ±0.09	2.90±0.10	5.73±0.12	0.50±0.06	0.10±0.06
	I	8.86 ±0.28	2.94±0.12	5.26±0.15	0.22±0.04**	0.14±0.05

*Significantly different at ($p < 0.05$).
** Significantly different at ($p < 0.001$).

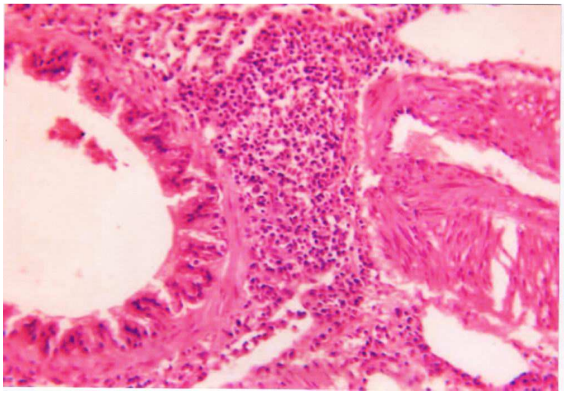

Fig. (3): Liver of rabbit infected with BHV-1showing severe vacuolation of hepatocytes (H&E x 400).

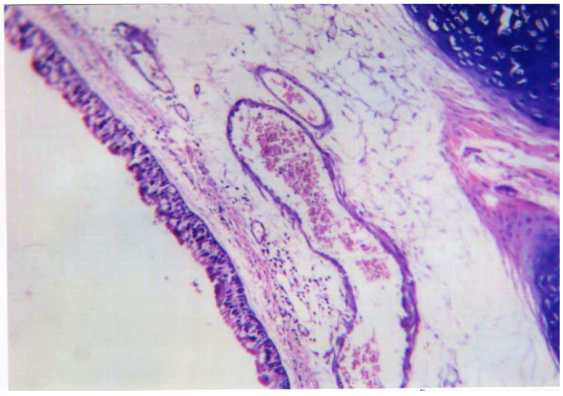

Fig. (4): Liver of rabbit infected with BHV-1 showing leucocytic infiltration in the portal area (H&E x 200).

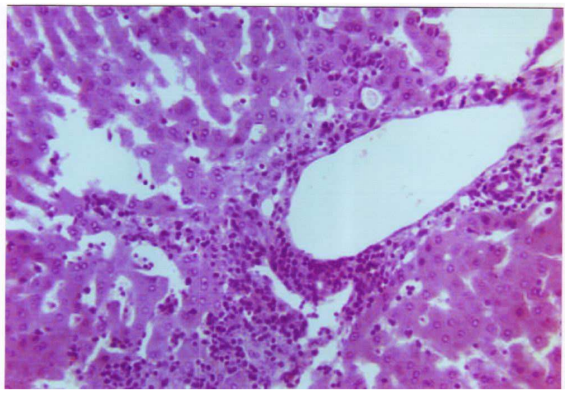

Fig. (3): Liver of a rabbit infected with Herpes virus type I showing severe vacuolation of hepatocytes. (H&E x 400).

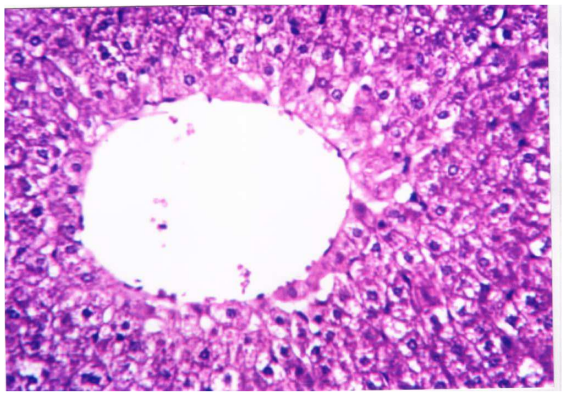

Fig. (4): Liver of a rabbit infected with Herpes virus type I showing leucocytic infiltration in the portal area. (H&E x 200).

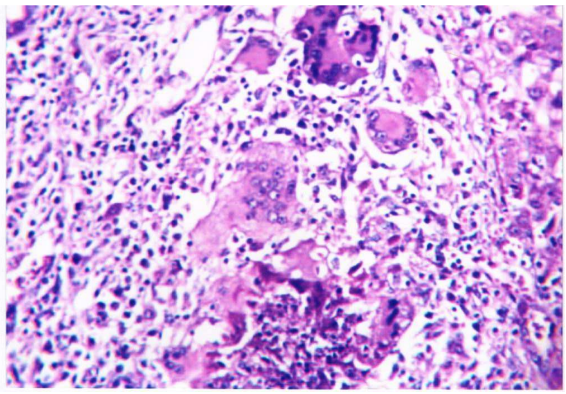

Fig. (5): Liver of rabbit infected with BHV-1showing typical granulomatous reaction (H&E x200).

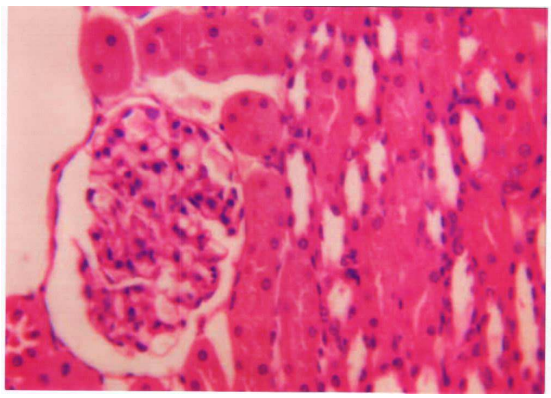

Fig. (6): Kidney of rabbit infected with BHV- I showing nuclear pyknosis and coagulative necrosis (H&E x 400).

Table (3): Values of some serum biochemical parameters of rabbits infected with BHV-1(Mean ± S.E.)

Time of exam. PI		ALT (u/L)	AST (u/L)	A.P (u/L)	BUN mg/dl	Creatinine mg/dl
3 days	C	67.80±1.11	24.70±0.75	55.40±0.55	28.75±0.77	0.79±0.04
	I	100.82±1.79**	67.20±1.34**	89.02±1.88**	46.25±1.13**	1.42±0.04**
7 days	C	75.30±0.88	27.33±1.07	51.76±0.82	28.78±0.87	0.76±0.03
	I	138.00±1.97**	71.80±1.80**	97.04±1.93**	42.62±0.83**	1.40±0.01**
10 days	C	81.20±0.19	27.33±0.43	63.50±1.38	29.89±1.15	0.87±0.03
	I	146.60±1.65**	94.94±1.77**	97.82±1.42**	56.78±0.97**	1.27±0.04**
14 days	C	83.40±0.87	23.6±1.02	61.00±1.13	33.10±2.44	0.83±0.01
	I	183.10±1.39**	91.00±2.16**	87.06±1.08**	58.54±1.05**	1.32±0.03**
21 days	C	86.30±1.47	28.50±1.27	72.43±1.29	31.27±1.20	0.78±0.02
	I	230.90±1.57**	93.20±1.52**	103.30±1.86**	60.99±1.46**	1.21±0.04**
28 days	C	87.3±1.22	32.43±1.33	64.36±1.53	30.65±0.92	0.80±0.01
	I	157.5±1.95**	111.27±1.75**	107.36±1.52**	62.49±1.06**	1.28±0.03**

**Significantly different at ($p < 0.001$).

Table (4): Values of total and electrophoretic pattern of serum proteins in rabbits infected with BHV-1 (mean ± S.E.).

Time of exam. PI.		T.P (g / dl)	Albumin (g / dl)	Globulin g / dl			A/G ratio
				α- globulin	β- globulin	γ- globulin	
3 days	Control	7.00±0.06	5.00±0.09	1.00±0.06	0.43±0.14	0.53±0.07	2.57±0.16
	Infected	7.63±0.33	5.40±0.18	0.80±0.12	0.83±0.14	0.63±0.24	2.39±0.16
7 days	Control	6.80±0.05	5.00±0.09	0.96±0.03	0.30±0.07	0.57±0.09	2.65±0.09
	Infected	8.20±0.17*	5.60±0.31	1.10±0.06	0.50±0.17	1.10±0.23	2.56±0.38
10 days	Control	6.50±0.23	4.60±0.20	1.20±0.03	0.23±0.13	0.50±0.06	2.41±0.10
	Infected	8.20±0.07*	5.00±0.09	1.43±0.09*	0.50±0.10	1.20±0.17*	1.61±0.05*
14 days	Control	6.70±0.09	4.90±0.10	1.03±0.03	0.23±0.03	0.50±0.06	2.80±0.09
	Infected	8.20±0.43*	5.30±0.27	1.60±0.12*	0.30±0.06	1.06±0.09*	1.80±0.01**
21 days	Control	6.50±0.20	4.60±0.21	1.00±0.07	0.20±0.03	0.63±0.03	2.57±0.21
	Infected	8.40±0.27*	5.90±0.12*	0.90±0.22	0.50±0.03*	1.06±0.03**	2.37±0.10
28 days	Control	6.60±0.09	4.80±0.12	1.06±0.09	0.20±0.03	0.50±0.06	2.79±0.12
	Infected	8.50±0.12**	6.00±0.10*	1.20±0.12	0.30±0.03*	1.03±0.09*	2.35±0.04*

*Significantly different at ($p< 0.05$). ** Significantly different at ($p< 0.001$).

Table (5): Result of Dot ELISA in different organs from rabbits infected with BHV-1 at different periods post infection.

Organs / Time of exam. PI.	Liver	Lung	Trachea	Kidney	Spleen
3 days	++	+++	+	++	+
7 days	++	+++	+	++	++ +
10 days	++	+++	+	++	++
14 days	++	+ ++	+	++	+++
21 days	++	+ ++	+	++	++
28 days	++	+++	+	++	++

+ = faint blue dots. ++ = moderate blue dots. +++ = dark blue dots.

Table (6): Values of ELISA S/P % in rabbits infected with BHV-1(Mean ±S.E.)

Time of exam. PI. / Groups	3 days	7 days	10 days	14 days	21 days	28 days
Control	9.3±0.07	10.06±0.35	9.93±0.27	10.17±0.86	10.00±0.31	9.86±0.43
Infected	31.8±0.63	56.2±0.05**	58.2±0.06**	146.6±0.07**	182.8±0.05**	60.1±0.05**

**Significantly different at (P< 0.001).

Discussion

Infectious bovine rhinotracheitis "IBR" is a disease caused by bovine herpesvirus-1 (BHV-1) which belongs to the family *Herpesviridae.* The first detection of this disease in Egypt was done by Hafez and Frey in 1973.

Experimentally infected rabbits with BHV-1 through the intraperitoneal route showed febrile response with mild conjunctivitis. This rise of body temperature corresponds to the viraemic period of the disease in rabbits (Lupton *et al.,* 1980 and Arab *et al.,* 1984). Data of the hemogram revealed no significant alteration in RBCs, Hb, PCV, and erythrocytic indices. This observation agreed with Aly and El-Kanawati (2000). A marked increase of total leucocytic count due to elevation of heterophil cells accompanied with lymphopenia was noticed at the 3rd and 7th days post infection with BHV-1. This result agrees with Baker *et al.* (1960) and disagrees with Mehrotra *et al.,* (1987a,b), Sandeep and Sharma (1995) and Aly and El-Kanawati (2000) who showed leucopenia with increase neutrophils and decrease lymphocyte count. The observed changes in leucocytes count can be considered as a response against the viral infection and / or stress. Eosinophilia was observed only at the 7th day post infection. Elevation of eosinophils has been reported to occur in antigen-antibody reactions (Kelly, 1984). Monocytopenia is not a clinically useful feature of leucograms (Duncan *et al.,* 1994). Activities of ALT, AST and A.P are used for evaluating the liver function (Kaneko *et al.,* 1989).The present results revealed significant increase in their activities in rabbits infected with BHV-1 along the experiment. These results indicated liver affection (both hepatocytes and biliary system) and come in line with previous studies by (El-Sawalhy *et al.,* 1995; Aly and El-Kanawati 2000 and Gaber *et al.,* 2000). The present results are confirmed histopathologically. The most affected organs were the lungs, trachea, and liver comparing to adrenal, kidneys, and spleen that showed mild pathological alterations. The liver showed degenerative and necrobiotic changes in the hepatocytes and hyperplastic activation of surface epithilium lining bile ducts. Values of BUN and creatinine were increased in rabbits infected with BVH-1from the beginning till the end of the experiment which agree with Gaber *et al.* (2000). Increase in BUN concentration may be due to accelerated catabolism of body protein which could have been occurred in the present experiment as a response to viral infection as suggested by (Wallach, 1979). The increase in serum BUN and creatinine, may also be attributed to renal damage after infection (Miller *et al.,* 1991). Histopathological findings showed degenerative changes in the kidney tissue and proved explanation for the previous results. In the present work, serum total protein concentration was significantly increased from the 7th day post infection till the end of the experiment. This agrees with Gaber *et al.,* (2000) and disagrees with Zurita *et al.*, (1988) who observed no variation in total protein and its fractions. α and β globulins are increased in acute inflammatory conditions and infections because they are an acute phase proteins (Jain, 2000).There was an increase in γ globulin starting from the 10th day of infection and continued through the period of the experiment. This may be due to an increase in production of immunoglobulins as a result of infection (Kaneko *et al.,* 1997). Mean values of A/G ratio were decreased at the 10th, 14th and 28th days post infection which may be due to increase in globulins concentration (Pratt, 1997). Microscopically, in the respiratory system, the lung and trachea were affected. Presence of neutrophils infiltrating the wall of the trachea of the animals at the 10th day post infection may indicate subjection of these animals to secondary bacterial infection. There were no inclusion bodies which coincide with Allan *et al.,* (1980); Obi *et al.,* (1981) and Aly and El-Kanawati

(2000) who did not observe IBR inclusions and attributed this to the stage of the disease and

the strain of the virus. The main changes that were seen in the liver in rabbits infected with IBR virus consisted of degenerative changes especially vacuolar degeneration. There was hyperplastic activation in Kupffer cells and epithelium lining the bile ducts with dilatation of central veins and sinusoids. Similar pathological picture was described by Mahmoud (1991) and Arab *et al*. (1984). Granulomatous reaction consisted of necrosis surrounded by several layers of epitheliod cells, lymphocytes and giant cells was found in the liver of one. The kidney showed degenerative changes in epithelial cells lining renal tubules. The hyperplasia of the lymphoid follicles of the spleen observed in the present experiment may be explained as an immune defense mechanism against the virus (Nafie *et al.*, 1996). Concerning the pathogenesis of BHV-1 in internal organs like liver, lung, trachea, kidney and spleen, the BHV-1 antigens were detected at different days post infection (3, 7, 10, 14, 21 and 28 days). The viral antigens were detected in these tissues at these different days by Dot ELISA. Although the virus was injected intraperitoneally, the virus was detected intensively in the lung and this mean pneumotropic nature of the virus. The virus spread from the site of infection to the other tissues through circulating macrophages or lymphocytes. The pathogenesis of the injected BHV-1 strain (IBR virus) is not varied even when the route of infection is changed.

Determination of BHV-1 antibodies revealed that the S/P % in sera of infected rabbit was increased gradually from the 7th days post infection till the 21st day post infection (from 56.2 to 182.8) then decreased to 60.1 at the 28th days post infection which is an expected S/P pattern for microbial infection.

From the present results, it can be concluded that, BHV-1 induced significant changes of some hematological and biochemical parameters in infected animals and it altered liver and kidney functions, in addition Dot-ELISA is considered very sensitive test for diagnosis of BHV-1.

References

Allan, E. M.; Priie, H. M.; Meolla, P. M.; Selman, I. E. and Wiserman, A. (1980): The pathologic feature of severe cases of infectious bovine rhinotracheitis. Vet. Res., 107: 441- 445.

Aly, A. A. and El-Kanawati, Z. R. (2000): Studies on infectious bovine rhinotracheitis in Red Sea Governorate. Egypt. J. Comp. Path. Clin. Pathol., 13 (2): 249-258.

Kelly, W. R. (1984): Veterinary Clinical Diagnosis. 3rd ed., Bailliere Tindall, London.

Gaber, A. S.; Mohamed, O. M.; A. M. Samy and El-Sayed A. F. M. (2000): Serological and biochemical changes in sheep sera infected with either parainfluenza-3 (PI3) or infectious bovine rhinotracheitis (IBR) viruses. Egyptian Journal of Comparative Pathology and Clinical Pathology, 13 (1): 134 -143.

Arab, R. M.; Marzoub, M. S.; Bakeer, A. M.; Karim, I. A. and El-Nakashly, S. A. (1984): Some studies on infectious bovine rhinotracheitis (IBR) virus in rabbits. Vet. Med. J., 32 (3): 160-169.

Baker, J. A.; Mc Entee, K. and Gillespie, J. H. (1960): Effects of infectious bovine rhinotracheitis-infectious pustular vulvovaginitis (IBR-IPV) virus on newborn calves. The Cornell Veterinarian, 50: 156-170.

Bancroft, J. D. and Stevens, A. (1996): Theory and Practice of Histological Techniques. 4th ed Chukhll living stone, New York, USA.

Bratanich, A.; Sardi, S.; Smitsaart, E.; Estevez Madero, J. and Schudel, A. A. (1990): Comparison of three serological techniques for the diagnosis of bovine herpesvirus type 1: serum neutralization, enzyme-linked immunosorbent assay and indirect immunofluorescence. Revista Argentina de Microbiologia, 22 (4):192-8.

Calvo, T.; Cordiou, P.; Coudep, T.; Perrin, B.; Edwards, S.; Eloit, M.; Guerim, B.; Kramps, J. A.; Lenihan, P.; Paschaleri, E.; Perrin, M.; Schon, J.; Thibier, M.; Van Oirshot, J. T.; Vanopdenbosch, E.; Wellwmans, G. and Wizigmann, G. (1994): Selection of European Union standard reference sera for use in the serological diagnosis of infectious bovine rhinotracheitis. Revue scie. Tech., 13 (3): 947- 960.

Darbyshire, J. H.; Dawson, P. S. and Paterson, A. B. (1962): The isolation of infectious bovine rhinotracheitis virus in the United Kingdom: A preliminary report. Vet. Rec. 74: 156-157.

Dawson, P. S.; Darbyshire, J. H.; Loosemore, R. M.; Paterson, A. B. and Faull, W. B. (1962): Infectious bovine rhinotracheitis (IBR). A clinical condition of cattle occurring in the United Kingdom. Vet. Rec. 74: 1379-1383.

Duncan, J. R.; Prasse, K. W. and Mahaffey E. A. (1994): Veterinary Laboratory Medicine, 3rd ed., Iowa State University Press Ames.

El-Sawalhy; El-Bagoury, A. A.; Salem, S. A. W. and Youssef, M. A. A. (1995): Bovine viral diarrhea complicated with rota virus infection among foreign breed herd in Sharkia governorate. Proc. 3rd Sci. Cong., Egypt. Soci. Cattle Dis. 1 (3-5) 66-77.

Hafez, S. M. and Frey, H. R. (1973): Serological evidence for the occurrence of bovine viral diarrhea-mucosal disease (BVD-MD) and infectious bovine rhinotracheitis (IBR) in Egypt. Bull. Epiz. Dis. of Africa, 21: 5-10.

Hawkes, R.; Niday, E. and Gordan, J. (1982): A dot–immunobinding assay for monoclonal and other antibodies. Anal. Biochem., 119:142-147.

Houot, O. (1985): Interpretation of Clinical Laboratory Tests. Cited by Siest, G.; Henny, J.; Schiele, F. and Young, D. S. in Pamphlete of Bio Merieux, France.

Jain, N. C. (2000): Schalm's Veterinary Hematology 4th ed. Lea and Philadelphia, U.S.A.

Kaneko, J.; Cornelius, C.; Finco, D.; Charles, C. and Rosol, T. (1989): Clinical Biochemistry of Domestic Animals. 4th ed., Academic Press Limited, London.

Biochemistry of Domestic Animals. 5th ed. Academic press, SanDiego Boston, New York Sydney Tokyo Toronto.

Kind, P. R. and King, E. G. (1954): Estimation of plasma phosphatase by the determination of hydrolysed phenol with aminoantipyrine. J. Clin. Pathol., 7: 322.

Kohin, J. A. (1985): Micoelectrophoretic Method. Nature, 181: 838.

Lupton, H. W.; Barnes, H. J. and Reed, D. E. (1980): Evaluation of the rabbit as a laboratory model for infectious bovine rhinotracheitis virus infection. The Cornell Veterinarian, 70: 77-95.

Mahmoud, A. H. M. (1991): Extensive studies on pathological investigations of IBR virus in rabbits. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ., Egypt.

Martin, S.; Nagy, E.; Armstrong, D. and Rosendal, S. (1999): The association of viral and mycoplasmal antibody titers with respiratory disease and weight gain in feedlot calves. Can. Vet. J., 40 (8): 560-567.

Mehrotra, M. L.; Shukla, D. C. and Sharma A. K. (1987a): Reactivation of bovine herpes virus-1 following corticosteroid treatment of vaccinated animals. Ind. J. Comp. Microbiol.-Immunol. Infec. Dis., 8 (1): 33-36.

Mehrotra, M. L.; Shukla, D. C. and Sharma A. K. (1987b): Experimental bovine herpes virus 1 infection in hill bulls. Ind. J. Anim. Sci., 57 (5): 359-365.

Miller, M. W.; Mobbs, N. T. and Williams, E. S. (1991): Spontaneous pasteurellosis in captive rocky mountain bighorn sheep (*Ovis canajensis*) clinical laboratory and epizootiological observations. J. Wildlife Dis., 27 (4): 534-542.

Monika E. M. and Ackermann, M. (1996): Pathogenesis of ruminant herpesvirus infections. Vet. Microbol., 53: 3-15.

Nafie, E. K.; Doghaim, R. H. M.; El-Nimer, M.; Agag, A. E. and Hamouda, M. (1996): Pathological and immunological changes in rabbits infected with infectious bovine rhinotracheitis virus (IBR). Assiut Vet. Med. J., 35 (70): 248-257.

Obi, T. U.; Wiseman, A.; Selman, I. E.; Allan, E. M. and Nettleton, P. F. (1981): An infectious bovine rhinotracheitis like respiratory syndrome in young calves. Vet. Rec., 108: 400-401.

Peters, T. (1968): Proposals for standardization of total protein assays. Clin. Chem., 14: 1147-1159.

Pratt, P. W. (1997): Laboratory Procedures for Veterinary Technicians. 3rd ed. Mosby company New York, Philadelphia, Toronto.

Reale, R.N. and Croft, D. J. (1961): Colorimetric determination of blood urea nitrogen. Clin. Pathol., 14: 418.

Reitman, S. and Frankel, S. (1957): A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases." Am. J. Clini. Pathol., 28: 56-63.

Roizman, B.; Desrosiers, R. C.; Fleckenstein, B.; Lopez, C.; Minson, A. C. and Studdert, M. J. (1992): The family Herpesviridae: an update. Arch. Virol., 123: 425-426.

Sandeep, G. and Sharma, R. (1995): Experimental infection of lambs with bovine herpes virus-1 (BHV-1). Int. J. Anim. Sci., 10 (1): 73-75.

Snedecor, G. and Cochran, W. (1976): Statistical Methods. 6th ed. Ames Iowa State Press, USA.

Wallach, J. (1979): Interpretation of Diagnostic Tests. 2nd ed., Little, Drown, M.A.

Zhou J.; Lyaku J.; Fredrickson, R. A. and Kibenge, F. S. (1999): Improved detection of bovine herpesvirus 1 in artificially infected bovine semen by protein amplification. J. Virol. Meth., 79 (2): 181-9.

Zurita, A.; Rudolph, R. W.; Sotomayer, M. E.; Moraga, B. L.; Berrlos, F. P. and Palaviccino, H. I. (1988): Experimental infection with infectious bovine rhinotracheitis virus. II- Haematological and serum protein response. A Vances en Ciencias Veterinarias, 3 (1): 38-4.