SERIOLOGICAL AND BIOCHEMICAL STUDIES ON VIRAL DISEASES CAUSING RESPIRATORY INFECTION IN CAMEL CALVES

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Abstract

The incidence of BHV-1 and PI-3 viruses in camel calves serum in Kaliobeia Governorate abattoirs were studied .Solid phase ELISA and SNT were used to detect antibodies against BHV-1in 120 camel serum , 27.5, 22.5 % were positive respectively . ELISA and HI were performed for PI-3 virus , the positive samples were 65% and 67% .

The study of some biochemical parameters revealed high significant decrease in the levels of total protein and albumin of diseased cases .Significant elevation in the values of AST, ALT, and AP activities , also in blood urea and serum createnine . The study comprises the effect of viral respiratory affection of camel calves on blood gases and acid base balance, there were marked decrease in blood PH values and base excess (B.E), while a significant decrease in PO2values as well as there was significant increase in PCO2 values in viral diseased calves in comparison with clinically healthy ones.

INTRODUCTION

Camels have long been imported to Egypt from Sudan for slaughter, for human consumption. Despite camel's importance the animal has been largely neglected by international agencies and local government as regards improvement of its health and productivity (Bekele, 1999 and Seleim et al., 2009)

Camels are susceptible to many viral diseases and play a role in amplification of some viruses (Eisa, 1998) infectious bovine rhinotracheitis (IBR) caused by bovine herpes virus -1 (BHV-1) of subfamily Alphaherpesvirinae within Herpesviridae family. The genome is single linear molecule of double stranded DNA (Intisar et al .,2009).Bovine Para influenza virus type (BPI-3) is an enveloped, non-segmented, negative-sense RNA virus within genus Respirovirus Paul, et al.,(2008)

Bekele (2004) recorded that the major clinical signs observed in camels suffering from viral respiratory diseases were cough, loss of appetite and watery nasal discharge which became mucoid.

Respiratory affections affect either directly the lung, liver and kidney cells altering their physiological mechanisms and indirectly affect some serum biochemical parameters. Oxygenation of the blood and gaseous exchange between the blood and lung tissue is the main function of the lung i.e. regulation of oxygen tension and carbon dioxide concentration. (Osman and Al-Busadah, 2000 and Al- Busadah, 2007).

MATERIALS AND METHODS

Animals: A total of 120 camel claves were used in this study which slaughtered at Kaliobia Governorate abattoirs. Camels showing signs of respiratory disturbance including moist cough, and rapid breathing and watery to mucoid nasal discharge,

Blood samples: Blood samples (10ml) were collected from jugular vein for serum separation. Serum samples were collected for detection of antibodies against IBR by solid phase ELISA and serum neutralization test and by heamagglutination test and ELISA for PI-3 virus .

Viral antigens : Positive and negative viral antigens were locally prepared from infected and non infected MDBK cell culture with reference virus.

Guinea pig erythrocyte for HI test for PI-3 virus : By puncture of heart of guinea pig ,then blood was taken on Alsever,s solution then suspended in PBS PH7.2 at 1% concentration .

Heamagglutination inhibition test : According to WHO (1980) ,all sera were heated with trypsin-periodate to remove non-specific inhibitors and tested for presence of parainfluenza antibody by HI test, two fold dilution of sera were prepared.

Tissue culture: Madin Darby Bovine Kidney (MDBK) . A permanent cell line of Madin Darby Bovine Kidney was maintained in animal health research institute . the cells were grown in MEM supplemented by 10% bovine serum .The cells were used for Serum neutralization test .

Serum neutralization test : Serum neutralization test was used for detection and titration of IBR viral antibodies in collected serum samples according to Edwards et al. (1986) .

Solid phase ELISA technique: It was used for detection of specific antibodies against IBR and PI-3 viruses in serum samples according to method described by Voller et al (1976) .

Protein –A peroxidase conjugate: It was supplied by Sigma Comp.-USA and used for Solid phase ELISA.

Determination of blood gases and acid- base balance: 10 blood samples were collected from both clinically healthy and diseased camels for determination of blood pH, PCO_2, PO_2 , HCO_3 ,TCO_2 and B.E by using corning pH-blood gas analyzer Model 168. The analyzer directly measured at 37^0C

Determination of biochemical parameters: Colorimetric determination of AST, ALT (Reitman and Frankle 1957), alkaline phosphatase (ALP) according to Szasz, 1976, total protein and albumin (Dumas et al., 1971). Determination of blood urea nitrogen (BUN) (Richterich, 1968) and blood serum creatinine according to Giorio, (1974).

Statistical analysis Data were expressed as means ± standard error (M±SE). The comparison between the three groups was conducted by one way analysis of variance (SPSS 1993).

RESULTS AND DISCUSSION

A number of microbial agents are involved as a primary or secondary infection of camel respiratory disease. Since the disease has multi etiological agents, this study gave special focus to the diseases having high spreading and contagious nature . In general, literature about camel pneumonia is scarce; however, some works having relevance with this paper were included for discussion. Bovine herpes virus-1 (BHV-1) and parainfluenza-3 (PI-3) one of the viruses causing respiratory infections in camels (Dioli and Stimmelmary,1992).

In this study the seroprevalence of BHV-1 were 27.5%, of 120 tested camel sera by ELISA as shown in table (1) .This result coincide with Intisar et al., (2009) .The demonstration of high percentage of positive reactors, beside the clinical symptoms of respiratory and reproductive manifestation or recent recovery from this signs. In addition due to the virus latency that is normal criteria of BHV-1. Serum neutralizing antibody titer against IBR tested camel sera(table 2) were 22.4% of total 120 samples .the titers detected in this study in camel sera were higher than that reported by Eisa (1998) which is mostly due to the increase in the disease prevalence. Most of the highest titers of positive titer (5%) showed only low titer. The results obtained in this study confirmed the pathogenic effect of BHV-1in camels reported

previously by Nawal et.al., (2003) and agreed with the conclusion raised by Werner and Kaaden (2002) .

Table (3) revealed the seroprevalence of antibodies against parainfluenza-3 virus in tested camel sera were 65% by ELISA which agreed with results concluded by Fekadu and Esayas (2010),Schwartz(1992) that prevalence of parainfluenza-3 is common and widely distributed in most camel rearing area . Heamagglutinating antibodies against parainfluenza-3 virus were 67%(table 4).The range of PI-3 HI titer estimated was 2-64. The HI unit comparing this result with that obtained by EL-Tarbilli (1979) revealed that titer ranged from 10-380 HIU. However the higher titer could be obtained from recent PI3 infection.

The viral respiratory affected camels showed significant decrease in total protein (table 5) specially in sever diseased camel similar results were obtained by Hassan 1984, Nasser and El-sayed 1997 ,Eman and Khamis 2009 these may be attributed to occurring inhibition of the liver to protein synthesis. Concerning to liver enzymes as shown in table (6) there were elevation of Aspartate amino transferase (AST), alanine transferase (ALT) and alkaline phosphates (ALP) in both moderate and severe respiratory affected camels. Such elevation in liver enzymes referred to the degenerative and circulating toxins. These results come in accordance with those reported by (Ali et al., 1998 and Seleim et al., 2003). Regarding to urea and creatinine as a mirror for kidney function there were significant increase in their levels. This increase may be attributed to the increased proteins catabolism, febrile respiratory diseases, impaired cardiac function and decreased renal blood flow (Radostits et al., (2002). This finding was in accordance with those reported by (Abdalla and Emam (2005). In pneumonia and bronchitis there were marked increase in Pco2 values associated with drop in blood pH values Rosenberger, (1979). The increased carbon dioxide tension of the blood and depletions of bicarbonate causes an increase in depth and the rate of respiration by stimulation of the respiratory center (Radostits et al., 2002).dealing with values of blood gases acid –base balance (table 7) (PH,pCo2 ,Po2,Hco3,Tco2 and base excess)our results raveled that in clinically diseased camels there were highly significant decrease in value of PH and base excess (B.E) while there was a significant decrease in value of PO2 and significant increase in PO2 these obtained results nearly similar with previously results recorded by Manna and Abd El-All (2011). El-sebaie et al., (1988) mentioned that pneumonia of calves associated with marked drop in blood pH and B.E while there were highly increase in values of PCO2 and significant decrease in PO2. also, a significant increase in power CO2 and a significant decrease in O2 tension could be attributed to defect in oxygenation process

of the lung during the course of pneumonia which leads to retention of CO2 in blood (Coles, 1980). Respiratory acidosis (Coles, 1986) occurs when CO2 elimination is decreased, and blood carbonic acid concentration and PCO2 are increased.

From this study we can concluded that PI3 and BHV-1 were found as primary causative agent for camel calves respiratory diseases. There for, Its recommended to develop vaccine containing responsible pathogens in order to protect this unique animal species from such kind of unpredictable disastrous diseases. Respiratory affections were accompanied with some reversible adverse effects on animal health represented by hepatic, renal dysfunction and disturbance of blood gases and acid-base balance. Adequate hygienic measures and proper management may reduce the degree of animal exposure to disease producing agents.

Table 1. Result of OD for detection of antibodies against IBR virus in tested camel serum samples .

No. of samples	No. of +ve samples	% of +ve samples	OD of +ve samples					
120	33	27.5	0.188-0.240		0.250-0.290		0.300-0.410	
			No.	%	No.	%	No.	%
			17	14.2	14	11.6	2	1.7

Mean OD +ve control = 0.172 Mean OD –ve control = 0.027

Table 2. Serum neutralizing antibody titer in tested camel sera against IBRV.

No. of samples	No. of +ve samples	% of +ve samples	Antibody titer of IBRv					
120	27	22.5	4		8		16	
			No.	%	No.	%	No.	%
			6	5	16	13.3	5	4.1

Table 3. Result of ELISA OD for detection of antibodies against PI-3 virus tested camel serum .

No. of samples	+ve samples	% of +ve samples	OD of +ve samples					
			0.210- 0.39		0.400- 0.500		0.510- 0.630	
120	78	65	NO	%	NO	%	NO	%
			33	27.5	31	25.8	14	11.7

Mean OD of +ve control = 0.189 Mean of –ve control =0.023

Table 4. Result of HI test of PI-3 in tested camel sera

No. of samples	No. of +ve samples	% of +ve samples	Titer of PI-3											
			2		4		8		16		32		64	
120	81	67.5	No	%	No	%	No	%	No	%	No	%	No	%
			1	0.8	16	13.3	17	14.2	34	28.5	8	6.7	5	4.1

Table 5. mean values of some biochemical parameters in clinically healthy and viral diseased camels.

Parameters Group of animals	Total protein	Albumin (g/dl)	Globulins(g/dl)
Clinically healthy	7.32±0.15	3.55±0.01	3.77±0.12
Camels with viral respiratory affection	5.65±0.23**	2.01±0.03***	3.64±0.12

= highly significant (P<0.01) *=highly significant (P<0.001)

Table 6. mean values of some biochemical parameters in clinically healthy and viral diseased camels.

Parameters Groups of animals	AST (u/l)	ALT (u/l)	ALP (u/l)	BUN (mmol/L)	Createnine (mg/dl)
Clinically healthy	31.5±1.07	15.12±0.42	0.95±0.02	22.19±0.86	0.83±0.01
Camels with viral respiratory affection	40.01±0.17**	20.55±1.45**	1.26±0.03**	46.15±0.60***	1.90±0.05**

Table 7. means values ± standard deviation of blood gases and acid – base balance in clinically healthy and viral diseased camels.

Group of animals	Number	pH	PCO_2 mm Hg	PO_2 mm Hg	HCO_3 mmol/L	TCO_2 mmol/L	B.E mmol/L
Clinically healthy	10	7.27±0.005	58.30±3.01	26.31±1.22	36.5±1.80	36.83±1.78	8.089±1.45
Camels with viral respiratory affection	10	7.01±0.07**	61.28±2.66**	21.03±1.55*	34.6±1.54	35.8±1.62	3.44±1.30**

*=significant (P<0.05) **= highly significant (P<0.01)

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دراسات سيرولوجية وبيوكيميائية علي بعض الفيروسات المسببة للعدوى التنفسية في صغار الجمال

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تم اجراء مسح مناعي لعدد ١٢٠ عينة من صغار الجمال من مجازر محافظة القليوبية للتأكد من وجود أجسام مضادة لفيروس التهاب الانف والقصبة الهوائية المعدي بإستخدام اختبار الاليزا واختبار الدم المتعادل وكانت النتائج ٢٧,٥ % و ٢٢,٥ % على التوالي . وقد أجري إختبار الاليزا وإختبار مثبط تلزن الدم علي فيروس البارانفلونزا وكانت النتائج بنسبة ٦٥ % و٦٧ % علي التوالى .

وقد أظهرت نتائج الفحوصات البيوكيميائية وجود نقص معنوي ملحوظ في نسبة البروتين الكلي والالبيومين في الحيوانات المصابة كما وجد زياده ملحوظة في مستوى انزيمات الكبد (الترانس أمينيز والفوسفاتاز القلوي) مع زياده معنوية في مستوي بولينا الدم والكرياتينين . وقد شملت الدراسة معرفة مدى تأثير الأمراض الفيرسية علي غازات الدم والاتزان الحمضي القاعدي وقد اظهرت النتائج وجود نقص شديد في كل من الأس الهيدروجينى - PO2- B.E بينما لوحظ ارتفاع في قيم pco2في الجمال المريضة عند مقارنتها بالمجموعة السليمة.