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EPIDEMIOLOGICAL AND BIOCHEMICAL STUDIES ON PPR INFECTED GOATS

*El-Sheikh, W.M.A. and Sharawi. S. S. A.**

Dept of Pharmacology, Animal Health Research Institute, Tanta provincial lab.

*Department of Virology, Faculty of Veterinary Medicine, Banha University, 13736 Moshtoher, Egypt.

ABSTRACT

Peste des petits ruminant (PPR) is a highly contagious and economically important disease of small ruminants. This study aimed to investigate the seroprevalence of PPR disease in (Gemmaza Research Station) Gharbia governorate during winter of 2010, and monitoring the changes in blood biochemical parameters in goats suffering from PPR during natural field outbreaks. A total of 196 blood samples were collected from goats suspected to be infected by PPR and serum sample were separated by centrifigation. Competitive enzyme linked immune sorbent assay (cELISA) was used for determination of the seroprevalence of PPR, which revealed serologically the presence of the disease in 33.67% from all goats present in the station and the percentage of infection was higher (48.57%) in young (less than 6month) and (40 %) in dams (after recent parturition or in late pregnancy).

Ten serum samples from diseased animals and five from normal seronegative ones were examined biochemically. The mean values of most of biochemical parameters included in the investigation

significantly differed between healthy and infected animals ($p \leq 0.05$). The sero-monitoring findings of the study indicated PPRV infections are circulating among goat flocks and serum biochemical parameters play a part in the disease processes of PPR and that determining serum parameters concentrations may be used as a supplementary laboratory test in conjunction with clinical and laboratory findings when evaluating the incidence and prognosis of PPR.

Key words: peste des petits ruminants, biochemical, goats, seroprevalence, Egypt.

INTRODUCTION

A Peste des petits ruminant (PPR) is a highly contagious and infectious disease of domestic and wild small ruminants caused by PPR virus (PPRV) ***(Lefevre and Diallo, 1990)***. PPR is characterized by fever, gastroenteritis, pneumonia, conjunctivitis, necrotic stomatitis and purulent ocular and nasal discharges ***(Scott et al, 1990)*** PPRV is distinct form but closely related to rinderpest virus ***(Diallo, 2006)***; and both are members of the genus Morbillivirus in the family Paramyxoviridae, Order Mononegavirales ***(Gibbs et al., 1979)***; either PPRV-antigen or antibody assays are required to confirm the diagnosis of the PPR outbreak among sheep and goats ***(Abd El-Rahim et. al. 2005)***. Morbillivirus competitive ELISA detected anti-RPV and anti-PPRV antibodies; in all reference rinderpest virus (RPV) and PPRV antisera containing virus neutralization (VN) titers >=1:8; were developed, suggesting that the assay can simultaneously detect antibodies against RPV and PPRV ***(KangSeuk et. al. 2003)***. A competitive-ELISA (c-

ELISA) test; use monoclonal antibody to a neutralizing epitope of haemagglutinin protein of the PPRV; as well as serum neutralization test (SNT) were developed for detection of antibodies to PPRV in the serum samples of goats and sheep ***(Singh et. al. 2004)***.

In Gemmaza Research Station the problem began with introduction of new Demashky goats (in 25March2010) purchased from Siena region, after about 2 weeks the disease began as straw, symptoms of eyes inflammations began to appear in goats. First redness and not respond to local treatments then swelling in eyes with excess of purulent ocular and nasal discharges, redness and lesions in tongue in some cases, rise in body temperature with its general symptoms as depression, loss of appetite and recumbency. After that edematous swelling extended to all head and neck was appear with respiratory symptoms as cough, rapid respiration, dourness, stagger motions with recumbency, severe diarrhea appears in some cases. Young infected cases not respond to symptomatic treatments and finally die. The main P.M. lesions were present in lungs, different stages of lung inflammation especially hepitization, with presence of serous fluids in thoracic and abdominal cavities. High degree of inflammation in intestine with appearance of Zebra marks in some cases.

During the acute inflammatory conditions as well as subclinical inflammation in animals, the most sensitive proteins are serum haptoglobin and serum amyloid A, and concentrations of these proteins increases particularly as a subsequence response ***(Winter et al, 2006)***. So the determination of these proteins can be of value to the veterinarian in

helping to identify animals with inflammatory diseases ***(Karreman et al, 2000)*** and it is more sensitive, specific, and efficient and less likely to give false positive and negative results than haematological examinations ***(Skinner and Roberts 1994).*** Particularly, ***Rosenberg and Schengrund (1976)*** added proteins are sialylated at the terminus of the oligosaccharide chain of the glycoprotein in many acute-phase, and so ***Taniuchi et al (1981)*** proposed serum sialic acid has been as a marker of the induced acute-phase response that have been documented in several cattle diseases such as enzootic bovine hematuria ***(Singh et al, 1980)***, bovine leukosis ***(Sydow et al, 1988)***, bovine leptospirosis ***(Keles et al, 2000)***, rheumatoid artrhitis ***(Warabi et al, 1983)***, diabetes ***(Yokoyama et al, 1995)***, renal disease ***(Ozben, 1991)*** and cancer ***(Kokoglu et al, 1992).***

So, in the present study we determined the biochemical changes with clinical signs, epidemiology and competitive ELISA results in goats affected with PPR during natural outbreak and if can used as a aid in diagnoses and monitoring of prognosis of the disease.

MATERIALS AND METHODS

1. **Animals:** one hundred and ninety sex goats belonged to El-Gemmaza Research Station for Animal Production, were exposed to an outbreak during winter of 2010 suspected to be PPR. These animals were examined clinically and serologically using cELISA for disease. No previous PPR vaccination was given prior or during the stage of samples collection.

2. **Samples:** Blood samples were collected from all goats at the station during April of 2010. Blood samples were centrifuged. Serum samples were collected and transferred to screw capped serum tubes and stored at -20°C until analyzed immunologically and biochemically.

3. **Competitive ELISA:** was applied according to ***(Choi et al., 2005)*** using a virus neutralizing monoclonal antibody directed against the nucleoprotein (N) specific for PPR virus. All serum samples tested in the present study were processed in duplicates. A commercial competitive ELISA kit was used to detect sero-positive animals was applied according to manufacturer's instructions [Biological Diagnostic Supplies Ltd. (BDSL) ®, flow laboratories and Institute for Animal Health Pirbright, Surrey, England]. % inhibition (PI) = 100 - (OD of the sample/ OD of the control)%

4. **Biochemical studies:** Ten serum samples collected from diseased animals at different clinical stages and 5 from apparently normal sero-negative animals were taken for biochemical analysis by using commercial kits. Serum glucose and cortisol were analyzed using commercially available kits according to the maufactur's instruction. Urea and creatinine were estimated according to ***Patton and Crouch (1977)*** and ***Siest et al., (1985)*** respectively. while total proteins, albumin, total bilirubin, direct bilirubin and cholesterol were estimated according to ***Wotton and Freeman (1982)*** Serum calcium was determined as mentioned by ***Baron and Bell (1957)***. Serum inorganic phosphorous was estimated according to ***Tiez (1983)***.

Serum sodium and potassium were measured by using flame photometer according to ***Collins and Palkin-thorne (1952)***. Serum TSA concentration was measured by colorimetric Warren's method.

5. **Statistical analysis:** - statistical analysis was carried out using "t" test according to ***SAS (1987)***.

RESULTS

Clinical findings are strongly suggestive for PPR; severe diarrhea, dyspnoea, and mucopurelent discharge from eyes and nose, necrotic ulcers in mouth, fever and depression were shown.

Seroprevalence: The overall seroprevalence was 33.67% being higher in young goats (48.57%) (Table 1).

Biochemical parameters: The mean values of biochemical parameters in healthy animals and PPR affected goats are presented in (table 2). Most of parameters included in the investigation significantly differed between healthy and infected animals ($p \leq 0.05$).

Table (1): Serological survey for PPRV virus specific antibody in goats with different ages.

	age	Total no.	No. of +ve samples	%	fatality	
					no.	%
1	Less than 6 month	70	34	48.57	34	100
2	6 -12 month	71	10	14.08	---	0
3	More than 12 month*	55	22	40	22	100
	total	196	66	33.67	56	84.85

*Deaths were confined on advanced pregnancy and recent parturition

Table (2): Biochemical parameters in healthy and PPR affected goats.

	Parameters	Healthy (n =5) mean ± SD	Affected (n =10) mean ± SD
1	Glucose (mmol/L)	2.53 ± 0.53	1.59 ± 0.49*
2	Cholesterol (g/L)	1.12 ± 0.04	1.16 ± 0.05
3	Urea (mmol/L)	3.69 ± 0.69	4.91 ± 0.62*
4	Creatinin (Umol/L)	86.32 ± 4.01	184.49 ± 6.06**
5	Total bilirubin (mg/L)	2.29 ± 0.36	3.40 ± 0.35**
6	Direct bilirubin (mg/L)	1.72 ± 0.17	2.41 ± 0.24**
7	Indirect bilirubin (mg/L)	0.58 ± 0.25	1.61 ± 0.35**
8	Total protein (g/L)	73.40 ± 4.80	62.80± 6.18*
9	Albumin (g/L)	34.50 ± 1.90	24.10 ± 1.90**
10	Globulin (g/L)	39.90 ± 2.18	47.83 ± 2.73**
11	Albumin/ Globulin ratio	0.866 ± 0.07	0.492 ± 0.05**
12	Calcium (mmol/L)	2.49 ± 0.76	2.31 ± 0.37
13	Phosphorus (g/L)	0.046 ± 0.02	0.043 ± 0.02
14	Sodium (mmol/L)	129.06 ± 5.28	139.00 ± 6.78*
15	Potassium (mmol/L)	5.60 ± 2.33	8.90 ± 1.73*
16	Total Sialic Acid (g/L)	0.63 ±0.09	0.84 ± 0.09**
17	Cortisol (mmol/L)	0.17 ± 0.04	0.29 ± 0.01**

* P< 0.05; ** P≤ 0.001 when compared to healthy animals.

DISCUSSION

Peste des petits ruminant (PPR) is a highly contagious viral disease of domestic and wild small ruminants characterized by fever, erosive stomatitis, conjunctivitis, gastroenteritis and pneumonia. It mainly affects goats but involvement of sheep is not exceptional. The transmission of virus requires close contact between susceptible and infected animals in the febrile stage ***(Braide, 1981)***. The discharge from eyes, nose, mouth and the loose feces contain large amount of PPRV. Fine infected droplets

are released into the air from these secretions and execrations, particularly when PPR infected animals cough and sneeze. Animals in close contact inhale the droplets and are likely to become infected ***(Taylor, 1984 and Bundza et al., 1988).***

In the present study, examination of affected goats showed the same characteristic clinical signs of PPR. These clinical signs were in agreement with those in previous reported by ***(Gibbs et al, 1979,and Scott, 1990)***. Competitive ELISA (cEILSA) using specific monoclonal antibodies also confirmed the PPR *disease* ***(KangSeuk et al, 2003*** and ***Balamurugan, et. al. 2007).*** With regards to sensitivity of C-ELISA for PPR, the seroprevalence recorded in this study was 33.67%. Efficacy of c-ELISA compared very well with VNT having high relative specificity (98.4%) and sensitivity (92.4%) ***(Singh et. al., 2004)***.

The present study recorded significant increases in mean values of sodium and potassium while calcium, phosphorus and total serum protein values decreased although globulin concentration and total bilirubin, direct bilirubin and indirect bilirubin were increased, similar results were recorded by ***Arslan et al, (2007)*** and ***Kataria et al,(2007).*** This increasing in mean values of sodium and potassium reflecting haemoconcentration, decreasing total serum protein values and increasing globulin concentration indicating immune response towards infection and the higher globulin concentrations achieving to expense the compensatory fall in albumin levels ***Kataria et al,(2007)***. Furthermore, serum cortisol, creatinine and urea concentration were increased with

decreased glucose and albumin levels, similar results were recorded by ***Kataria et al, (2007)*** and they commended the higher serum cortisol indicating stress that must causes increase in blood glucose levels due to glycogenolytic property but the recorded decrease in glucose levels could have been due to animals not being fed since the onset of infection. They also added the higher levels of cortisol causing muscle wasting resulted in increased serum creatinine levels and an increased urea concentration reflected protein breakdown and haemoconcentration simultaneously ***(Kataria et al, 2007).*** The serum albumin/globulin ratio in the infected goats was significantly lower than that of the healthy but no significant differences were recorded for serum cholesterol concentration, similar results were recorded by ***Arslan et al, (2007).***

The results showed that serum total sialic acid (TSA) concentration in infected group (0.84g/L) was significantly higher than that in the control ones (0.63g/L) and it was positively-correlated with total protein, globulin, total bilirubin, direct bilirubin and indirect bilirubin while negatively-correlated with albumin and no significant correlation with cholesterol. This results are partially in agreement with Toplu's data ***(Toplu, 2004)***, that report by ***yarim et al, (2006)*** and ***Arslan et al, (2007)***. Previously ***Taniuchi, et al, (1981)*** considered the sialic acid concentration in serum as a marker of acute phase reactants which contain sialic acid residues of its oligosaccharide side chain. The elevation of TSA concentration may be explained by increased acute phase reactions associated with PPR infection and there are significant

correlations between TSA levels and clinical symptoms, hematological changes and liver function tests of the infected sheep and so the liver function tests are useful in the prognosis of PPR ***(yarim et al, 2006).*** In keeping with these lines, ***Ng and Dain, (1976)*** said that sialic acids are important component of cell membrane glycoproteins and glycolipids, ***Stefenelli, et al, (1985), Kokoglu, et al, (1992)*** and ***Keles, et al, (2000)*** added serum sialic acid concentration has been found to be elevated in chronic liver diseases, malignant tumors and bacterial infections.

In conclusion; preventive measures, particularly quarantine should be applied firmly to avoid PPR infection. The relationship between the biochemical changes and clinical signs, in goats affected with PPR are obvious and biochemical findings could be used as a helpful aid in the disease screening and follow-up, as well as in monitoring of prognosis in those animals. Furthermore, it revealed an inverse relationship between TSA and clinical, biochemical findings which including liver function tests that can be used in the prognosis of PPR as a supplementary laboratory test in combination with clinical and serological examination.

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دراسات وبائية وبيوكميائية على الماعز المصابة بطاعون المجترات الصغيرة

وجيه مصطفي عبد السلام الشيخ و سعد شعراوي علي شعراوي*

قسم الفارماكولوجيا – معهد بحوث صحة الحيوان (المعمل الفرعي بطنطا).

*أستاذ مساعد الفيرولوجي – كلية الطب البيطري بمشتهر – جامعة بنها

مرض طاعون المجترات الصغيرة مرض معدي ويسبب خسائر إقتصادية كبيرة في القطعان التي يصيبها. وقد أجريت هذه الدراسة على حالات للمرض ظهرت بمحافظة الغربية في محطة البحوث الزراعية بالجميزة التابعة لمعهد بحوث الإنتاج الحيواني في شتاء عام 2010م (الفترة من 25مارس إلى 30 إبريل) لدراسة التواجد السيرولوجي للمرض مع رصد التغيرات البيوكميائية المصاحبة للمرض ومقارنتها بنتائج الفحص السيرولوجي. وقد تم جمع 196 عينة دم من ماعز يشتبه في إصابتها بطاعون المجترات الصغيرة وفي مراحل مختلفة للمرض وتم فصل سيرم الدم حيث أظهرت الفحوص باستخدام طريقة الإليزا التوافقية وجود المرض بنسبة 33.67٪ من إجمالي الماعز الموجود بالمحطة وكانت نسبة الإصابة أعلي (48.57٪) في الماعز أقل من ستة أشهر يليها الأمهات (40٪) التي ولدت حديثا أو في مراحل العشار المتأخرة.

كما تم فحص عينات سيرم من 15 من الماعز (5 سليمة – 10 مصابة) لرصد التغيرات البيوكميائية بها حيث وجدت فروق معنوية واضحة في النتائج بين العينات التي أخذت من الحيوانات المصابة مقارنة بالعينات التي تمثل الحيوانات السليمة مما يدل على ملازمة هذه التغيرات البيوكميائية لوجود المرض وأنه يمكن استخدامها كوسيلة مساعدة في تشخيص المرض أو الكشف المبكر عنه.