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ELISA AND OTHER TESTS IN DIAGNOSIS OF *PASTEURELLA MULTOCIDA* INFECTION IN CAMELS IN EGYPT

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SUMMARY

The bacteriological examination of internal organs from 14 dead camels had severe respiratory symptoms revealed the isolation of *P. multocida* (PM) from 86.6% of the collected organs (52 out of 60) *P. multocida* subspecies multocida (PMM) (serotype B) represented 85% of the isolates, while *P. multocida* subspecies septica (PMS) (serotype A) was isolated only from one lung specimen (1.7%). Nasal swabs and blood specimens from clinical cases and contact apparently healthy camels showed similar isolation patterns. PMM serotype (B) was isolated in 85.9%, 65.6% and 30.4%, 8.1% respectively, while PMS serotype (A) was isolated from clinical cases only, 3.1% and 1.6% respectively. PMM serotype (A) was also isolated from nasal swabs of contact apparently healthy camels (3%).
Indirect hemagglutination test (IH) showed higher PM antibody titers in the serum of clinical cases reached 1:512 in 17.2% of the clinical cases against 0% in contact apparently healthy camels. In Dot immunobinding assay (DIA), 95.3% of serum samples from the clinical cases were tested positive with an optical density (OD) range from 0.59 to 1.17, whereas only 4.7% were tested negative. Serum samples from contact apparently healthy animals showed 72.6% positive results, whereas 27.4% were negative. Serum IgG-ELISA revealed PM antibodies in 96.9% of the clinical cases with OD range from 0.66 to 1.63. Two clinical cases were tested negative (3.1%). In contact apparently healthy camels, 75.6% of the samples were tested positive with a lower OD range, while 24.4% of the samples were negative. Nasal secretion ELISA revealed the presence of PM antibodies in 95.3% of the nasal secretions from clinical cases with OD range from 0.62 to 1.27 while 4.7% of the samples were negative. Contact apparently healthy camels showed positive results in 74.1% of the samples, with OD range from 0.62 to 1.03, whereas the negative represented 25.9%.
Serum biochemical analysis showed significant decrease in total protein, albumin as well as A/G ratio, while the globulin fraction was increased. The enzyme activity of ALT, AST, alkaline phosphatasedase as well as the values of creatinine, urea and uric acid were significantly increased. Minerals profiles were also altered, calcium, phosphorus, magnesium, sodium, chloride were significantly decrease, whereas potassium increased. It was concluded that serum IgG-ELISA was superior to nasal secretion IgG-ELISA, DIA and IH which can also assess in the diagnosis of PM infection in camels in conjunction with serum biochemical parameters.

INTRODUCTION

Camel has been for long a neglected species of domestic animals, concerted efforts as well as intensive investigation on their disease problems yet has to be boosted for the greater potentials that can be manifested in the meat, milk and hide production. Beside the significant tolerance to water shortage in desert areas which constitute the majority of the Egyptian land Camel rearing might have the potential to become of pivotal economical importance. Pasteurella multocida (PM) in camel was considered a hazardous disease that drastically affect the productivity of camels. The bacterium was reported to affect a wide range of animals species as cattle , buffalo, sheep, goat, pig, wild animals as well as poultry and ducks (Berele, 1999; Sunder and Kumar 2001). PM infected camels had fever, depression, loss of appetite, watery nasal discharge that becomes mucopurulent and at late stages the camel becomes recombant to death, due to severe septicemia, congestion and oedema (Rana et al.1993; Berele, 1999).

Various serological procedures such as tube agglutination, Indirect hemagglutination (IH); Dot immunobinding assay (DIA) and enzyme linked immunosorbent assay (ELISA) have been used to quantify humoral antibodies against PM. Many antigen extracts were prepared from PM for such techniques as outer membrane protein (OMP), whole cell sonicate, formalin extract and KSCN extract. Contradictory results were repoted by many authors regarding the efficiency of the different techniques as well as the potential immunogenicity of the antigens as well as its solubility, ability to attach to different solid matrices and homogeniety (Kumar et al., 1985; Confer et al., 1996; Manoharan et al., 1997; Srivastava,1998; Singh and Jayprakasan, 2001). PM different antigenic proteins and toxines were found to act directly on the lung, liver and kidney cells altering their physiological mechanisms and indirectly affected the serum biochemical parameters. Total serum protein was decreased due to marked hypoalbuminia and increase in globulins. ALT, AST and alkaline phophatase activity were increased (Naser and El-Sayed, 1997 Ali et al. 1998)

The objective of this study was to investigate the PM infection in camels and it serotypes as well as to screen different serological and biochemical tests, that could be employed in controlling, and monitoring the infection in camels.

Materials And Methods

Specimens

The internal organs (lung, trachea, liver, spleen, heart and kidney) from 14 dead camels aged 1-6 years, which had a history of sever respiratory distress, pyrixia, and nasal mucopurulent discharge. Another 64 blood and 64 nasal secretion samples were collected from 64 clinically infected camels with respiratory infection. Also 135 blood and 135 nasal secretion/ swabs were collected from 135 contact apparently healthy camels. These samples were used for the bacteriological examination as well as for the serological investigations. Blood samples for serum biochemical analysis were collected on EDTA anticoagulant.

Impression smears from the internal organs, nasal secretions/ swabs as well as blood smears were prepared on microscope slides and stained with Gram's, Leishman's and Ziehl neelson's stains for direct microscopic study.

All these samples were collected during the period from juli 2001 till May 2002 from El-Basatin slaughter premises. Samples were packed separately in sterile plastic bags, labeled and transferred to the laboratory in ice box for bacteriological, biochemical and serological examination (Rana et al.1993).

Bacteriological examination of specimens
Organ specimens, nasal secretion and blood samples were cultured directly on blood agar plates containing 5% sheep blood and incubated at 37°C for 24hr. Nasal swabs were cultured indirectly by inoculation of 5ml of brain heart infusion broth (BHI) and incubation at 37°C for18hrs then streaked onto blood agar plates. Suspected colonies were morphologically and biochemically identified according to Mutters et al,. (1985)

Potassium thiocyanate extract (KSCN) antigen:
PM were grown on blood agar plates, harvested with 0.5M KSCN and 0.425M NaCl pH 6,3 and incubated in a water bath at 37°C for 5 hr. Cells were removed by centrifugation at 300xg for 30min. the supernatant was dialysed against 0.01M Tris-HCl and 0.32M NaCl. (Esslinger et al., 1994; Sing and Jayprakasan, 2001) The protein content in the extract was adjusted to 5µg/ml. (Lowry et al.,1951)

Indirect hemagglutination test
PM antigens were extracter by the heat extraction method at 100°C for 1hr. Blood was collected from human volunteers of blood group (O). Erythrocytes were washed in phosphate buffer saline (PBS) pH 7.4. Erythrocytes were then synthetized with the different heat extracted antigens and the serotype designation of each isolate as well as the antibody titer of the serum samples was determined (Carter 1972).

Dot immunobinding assay
KSCN extract antigen (100µl) was applied on nitrocellulose (diazobenzyloxymethyl) membrane of 0.45µm porosity (Bio Rad Laboratories) using the dot apparatus. The membrane was then removed and washed with Tris Buffered saline (TBS) pH 7.4 containing 0.1% bovine serum albumin and 0.05% Tween-20 (BSA-T) as a blocking solution. The membrane was then incubated 2 hr. at 37°C in TBS containing 3%BSA and washed three times with TBS-tween 20 containing 0.1% BSA. The membrane was then incubated with 1: 50 diluted serum samples and incubated at 37°C for 1hr. Nitrocellulose membranes were then washed three times with the same washing buffer. Then 100µl of 1:1000 Sheep anti-camel horse radish peroxidase conjugate (Kirkegaard and Perry Laboratories) were added and left to react at 37°C for 30 min. The membranes were then washed again three times and the colour was developed by adding 0.06% 4-chloro-1-naphthol containing 0.001% H_2O_2. The colour density which was the indicator of immunoreactivity was measured by scanning densitometer. Wells with antigen, serum, conjugate and substrate omitted were set up as appropriate controls. The antisera end point was calculated as the mean value of the negative sera plus two standard deviations. Positive and negative control sera were included based on the history of the animal, isolation of PM from the animal and indirect hemagglutination test (IH) results (Hawkes et al. 1982).

Serum ELISA
In microtiter plates the different antigen preparations were used after performing the checkerboard titration to determine the optimum antigen concentration and conjugate dilution. Plates were coated with 100µl of the KSCN extract antigen (5µg/ml) and left overnight at 4°C. After washing with PBS, the 1: 50 diluted serum samples were added and incubated at 37°C for 1hr and washed three times with the same washing buffer. Then 100µl of 1:3000 sheep anti-camel horse radish peroxidase conjugate (Kirkegaard and Perry Laboratories) was added and left to react at 37°C for 30 min (the optimum concentrations of the antigen and the conjugate were determined by checker board titration). The plates were washed again three times and finally 100µl of the substrate o-phenylenediamine conaining 0.001% H_2O_2 was added. After the colour developed 25µl of 8N H_2SO_4 were added to stop the reaction and

plates were read at 490nm. Antigen, serum, conjugate and substrate controls were set up as appropriate controls, end point was calculated as the mean value of the negative sera plus two standard deviations. Twofold dilutions 1:200 – 1:25,600 of the positive control serum and negative control sera were also included as in the case of dot immunobinding assay (Sing and Jayprakasan 2001).

Nasal discharge ELISA

Similar procedures to the serum ELISA were used to determine the antibody response in nasal discharges. The microtiteration plates were also coated with the forementioned coating antigen. Nasal discharge samples were diluted 1:2 in 1x NET (0.1M NaCl, 1mM EDTA, and 10mM Tris pH 8.0 then added to the wells and incubated at 37°C for 1hr. The washing steps, conjugate dilution and colour development by the substrate were the same as forementioned. Twofold dilutions (1:2 – 1:256) of the positive control nasal discharge samples and negative control were also included. The end point was determined as forementioned (Brennan et al., 1998).

Serum biochemical parameters

The following serum biochemical parameters were determined: total protein (Hoffmann and Richterrich (1970), albumin and globulin (Doumas et al. 1971), ALT and AST aminotransferases (Reitman and Frankel 1997), alkaline phosphatase (Kilichling and Freiberg 1951), creatinine (Hudson and Rapaport, 1968), Urea (Tabacco 1979), uric acid (Archibald, 1957), total calcium (Glinder and King 1972), Inorganic phosphorus (Kilchling and Freiburg 1952), Magnesium (Neil and Nelly, 1956), sodium and potassium by using flame photometer (Oser, 1979), chloride (Varley et al. 1980)

Statistical analysis

Statistical analysis of obtained serum values were carried out using the ‘t’ test according to the method of Snedecor and Cochran (1967)

RESULTS

The bacteriological investigations of the internal organs from the dead camels revealed the isolation of *P. multocida* (PM) from 86.6% of the collected organs (52 out of 60). *P. multocida* subspecies multocida (PMM) serotype (B) was the dominant type of the isolates (85%) while *P. multocida* subspecies septica (PMS) was isolated only from one lung specimen (Table-1). Nasal swabs and blood samples from the clinical cases and apparently healthy contact camels elucidated similar isolation pattern. PMM, serotype (B) was isolated in 85.9%, 65.6% and 30.4%, 8.1% respectively (Table-2), while PMS serotype (A) was isolated from clinical cases only, 3.1% and 1.6% respectively. Another PMM serotype (A) was isolated only from nasal swabs of 4 apparently healthy animals (3%). Microscopical examination of smear slides from dead animals as wll as clinical and apparently healthy cases revealed the typical morphological characteristics of PM.

Table 1: Recovery of *P. multocida* from the internal organs of camels had a history of respiratory infection.

Organ	No. of positive *P. multocida*	*P. multocida* subspecies	Serotype
Lung	13/14 (92.9%)	12/14 *P. multocida* multocida 1/14 *P. multocida* septica	B A
Trachea	12/14 (85.7%)	P. multocida multocida	B
Liver	10/14 (71.4%)	P. multocida multocida	B
Spleen	9/14 (64.2%)	P. multocida multocida	B
Kidney	8/14 (57.1%)	P. multocida multocida	B
Total Positive isolation **Negative isolation**	52/60 (86.7%) 8/60 (13.3%)	51/60 (85%)P. m. multocida 1/60 (1.7%) P. m. septica	B A

Table 2: Recovery of P. multocida from alive clinical cases and contact apparently healthy camels.

Animal status	Sample	No. of positive	P. multocida subspecies	Serotype
Clinical cases (respiratory infection)	Nasal swabs	57/64 (89.1%)	55/64 (85.9%) *P. m. multocida* 2/64 (3.1%) *P. m. septica*	B A
	Blood samples	43/64 (67.2%)	42/64 (65.6%) *P. m. multocida* 1/64 (1.6%) *P. m. septica*	B A
Contact apparently healthy camels	Nasal swabs	45/135 (33.3%)	41/135 (30.4%) *P. m. multocida* 4/135 (3%) *P. m. multocida*	B A
	Blood samples	11/135 (8.1%)	*P. m. multocida*	B

IH test recorded higher PM antibody titers in the serum of clinical cases than the apparently healthy. Antibody titers reached 1:512 in 17.2% of the clinical samples against 0% in apparently healthy camels (Table-3).

In DIA the cut off level of absorbance was 0.59 (0.11 + 0.48). 95.3% (61 0ut of 64) of the serum samples from the clinical cases were tested positive with an OD range from 0.59 to 1.17, whereas only 4.7% (3 out of 64) were tested negative. Serum samples from contact apparently healthy animals showed 72.6% (98 out of 135) positive results, whereas 27.4% (37 out of 135) were tested negative (Table-4).

In the case of serum IgG-ELISA, the cut off point was 0.66 (0.14 + 0.52). 96.9% (62 out of 64) of the clinical cases were positive for the presence of PM antibodies, with OD range from 0.66 to 1.63. Two clinical cases were tested negative (3.1%) with OD values less than 0.66. In contact apparently healthy camels, 75.6% (102 out of 135) of the samples were tested positive with a lower OD range from 0.66 to 1.3, while 24.4% of the samples (33 out of 135) were tested negative with OD less than 0.66. ELISA results of nasal secretion, revealed that the cut off value was 0.62 (0.12 + 0.50). Majority of nasal secretion collected from clinical cases (95.3%, 61 out of 64) were positive with OD range from 0.62 to 1.27 and 4.7% (3 out of 64) were negative. The incidence of positive in the nasal secretion of contact apparently healthy camels was 74.1% (100 out of 135) with OD range from 0.62 to 1.03, whereas the negative represented 25.9% (35 out of 135) with OD values less than 0.62 (Table-6).

Table 3: Detection of P. multocida antibody titers in the serum of clinical cases and apparenlty healthy camels by indirect hemagglutination test.

Animal status	Indirect hemagglutination titration					
	Negative	Positive				
	< 1:32	1:32	1:64	1:128	1:256	<1:256 (1:512)
Clinical cases (n = 64)	5/64 (7.8%)	5/64 (7.8%)	7/64 (10.9%)	15/64 (23.4%)	21/64 (32.8%)	11/64 (17.2%)
Contact apparently healthy (n = 135)	42/135 (31.1%)	29/135 (21.5%)	28/135 (20.7%)	20/135 (14.8%)	16/135 (11.9%)	0/135 (0%)

Table 4: Dot immunobinding assay carried out to detect P. multocida antibodies in the serum of clinically and apparently healthy camels.

Animal status	Dot immunobinding assay optical density (OD) values						
	Negative samples	Positive serum samples					
	OD< 0.59	OD 0.59 to 0.66	OD 0.67 to 0.75	OD 0.76 to 0.82	OD 0.83 to 0.90	OD 0.91 to 1.00	OD1.01 to 1.17
Clinical cases (n=64)	3/64 (4.7%)	3/64 (4.7%)	6/64 (9.4%)	15/64 (23.4%)	23/64 (35.9%)	11/64 (17.2%)	3/64 (4.7%)
Contact apparently healthy (n=135)	37/135 (27.4%)	34/135 (25.2%)	27/135 (20%)	19/135 (14.1%)	15/135 (11.1%)	3/135 (2.2%)	0/135 (0%)

Table 5: Optical density (OD) values of Serum samples from clinically infected and apparently healthy camels tested with ELISA

Animal status	Serum ELISA optical density (OD) values						
	Negative samples	Positive serum samples					
	OD< 0.66	OD 0.66 to 0.80	OD 0.81 to 0.95	OD 0.96 to 1.15	OD 1.16 to 1.30	OD 1.31 to 1.50	OD 1.51 to 1.63
Clinical cases (n = 64)	2/64 (3.1%)	8/64 (12.5%)	11/64 (17.2%)	12/64 (18.8%)	14/64 (21.9%)	13/64 (20.3%)	4/64 (6.3%)
Contact apparently healthy (n = 135)	33/135 (24.4%)	38/135 (28.1%)	28/135 (20.7%)	20/135 (14.8%)	16/135 (11.9%)	0/135 (0%)	0/135 (0%)

Table 6: Optical density (OD) values of nasal secretion samples from clinically infected and apparently healthy camels tested with ELISA.

Animal status	Optical density (OD) values of nasal secretion ELISA						
	Negative samples	Positive nasal secretion samples					
	OD< 0.62	OD 0.62 to 0.70	OD 0.71 to 0.81	OD 0.82 to 0.91	OD 0.92 to 1.03	OD 1.04 to 1.13	OD 1.14 to1.27
Clinical cases (n = 64)	3/64 (4.7%)	7/64 (10.9%)	9/64 (14.1%)	12/64 (18.8%)	15/64 (23.4%)	14/64 (21.9%)	4/64 (6.3%)
Contact apparently healthy (n = 135)	35/135 (25.9%)	46/135 (34.1%)	31/135 (23%)	14/135 (10.4%)	9/135 (6.7%)	0/135 (0%)	0/135 (0%)

Serum biochemical analysis showed significant decrease in total protein, albumin as well as A/G ratio, while the globulin fraction was significantly increased. The enzyme activity of ALT, AST, alkaline phosphatasedase as well as the values of creatinine, urea and uric acid were significantly increased. Minerals profiles were also altered, calcium, phosphorus, magnesium, sodium, chloride were significantly decrease, whereas potassium was significantly increased (Table-7).

Table 7: Serum biochemical analysis of camels infected with P. multocida and contact apparently healthy

Biochemical Parameters of serum samples	Infected camels (n=25)	Apparently healthy (n=15)
Total protein g/dl	6.80± 0.20*	7.60± 0.25
Albumin (A) g/dl	2.00± 0.10***	3.28± 0.13
Globulin (G) g/dl	4.80± 0.15*	4.32± 0.11
A/G ratio	0.42± 0.02***	0.76± 0.03
ALT (u/l)	25.08± 1.50**	19.87± 1.13
AST (u/l)	40.73± 2.63**	31.4± 1.65
Alkaline phosphatase (m.m u/l)	1.05± 0.02**	0.95± 0.03
Creatinine (mg/dl)	1.05± 0.04*	0.93± 0.02
Urea (mg/dl)	50.44± 2.68***	37.07± 1.80
Uric acid (mg/dl)	2.47± 0.15***	1.68± 0.10
Total calcium	7.80± 0.22*	9.10± 0.28
Inorganic phosphorus	4.90± 0.18***	6.78± 0.20
Magnesium	2.44± 0.13*	3.27± 0.15
Sodium	103.48± 3.17***	119.20± 2.70
Potassium	5.63± 0.38	4.29± 0.21
Chloride	243.68± 5.60	301.87± 5.79

* Significant ($p<0.05$) ** Significant ($p<0.01$) *** Significant ($p<0.001$)

DISCUSSION

P. multocida infection in camels was always accompanied most of the time with heavy economic losses. It was estimated that the losses incurred was due to fatalities, loss of productivity as well as the heavy costs of compulsory medication or vaccination required for the infected animals (Manoharan et al., 1997; Srivastava,1998; Singh and jayprakasan, 2001).
Camels infected with PM showed symptoms of pneumonia which might be accompanied by septicaemia, pyrexia and mucopurulent nasal discharge. In aggreviated stages the animal becomes recombantand end by death (Berele, 1999).

In our study 14 camels were reported dead after severe episode of pneumonia symptoms. The camels were 1-6 years old and fatalities included both sexes. The bacteriological investigations of the internal organs from these camels revealed the isolation of PM from 86.6% (52 out of 60) of the collected specimens. PMM serotype (B) was the dominant type of the isolates (85%) while PMS was isolated only from one lung specimen (Table-1). Nasal swabs and blood samples from the clinical cases and apparently healthy contact camels elucidated more or less similar isolation pattern. PMM, serotype (B) was isolated in 85.9%, 65.6% and 30.4%, 8.1% respectively (Table-2), while PMS serotype (A) was isolated from clinical cases only, 3.1% and 1.6% respectively. Another serotype of PMM (serotype A) was isolated only from nasal swabs of apparently healthy animals (3%). These results were found compatable with many other authors who could isolate different PM serotypes (A, B, D and E) from the blood liver, spleen kidney, lymph nodes and bone marrow of camels that had respiratory and septicemic symptoms. They also repoeted the isolation of PM from camels that were in close contact with the diseased. The wide range of serotype isolation (serotype, A, B, D, and E) was explained that dromedaries always lived in close association with the smaller ruminants and other farm animals that naturally harbour the organism in their respiratory system (Perreau and Maurice1968; Richard, 1975; Awad et al., 1976; Hassan and Mustafa, 1985).
Various serological procedures such as tube agglutination, IH, DIA and ELISA have been used to control and monitor the PM infection in different farm animals. Also many of these techniques were usedto assess the humoral antibodies in the serum to investigate the immune status of the flock and follow the course of infection as well as to gauge the efficiency of vaccination. For these serological techniques a wide diversity of antigens were prepared as OMP, whole cell sonicate, formalin extract, KSCN extract and subunit protein antigens. These antigens gave contradictory immunogenic results due to many factors as the genetic structure of the bacterial strains from which the antigen was prepared, methodological variations and due to host immune response variation. In our study we used KSCN extract which was recommended by many authors (Confer et al., 1996; Manoharan et al., 1997; Srivastava,1998; Singh and jayprakasan, 2001).
Results of IH test recorded higher P. multocida antibody titers in the serum of clinical cases than the contact apparently healthy camels. Antibody titers reached 1:512 in 17.2% of the clinical cases against 0% in apparently healthy camels(Table-3), which confirmed the incrimination of PM in causing the infection in camels beside the bacteriological isolation of the organism. In DIA the cut off level of absorbance was 0.59 (0.11 + 0.48) and 95.3% (61 0ut of 64) of the serum samples from the clinical cases were tested positive with an OD range from 0.59 to 1.17, whereas only 4.7% (3 out of 64) were tested negative. Serum samples from contact apparently healthy animals showed 72.6% (98 out of 135) positive results, whereas 27.4% (37 out of 135) were tested negative (Table-4).
When the serum samples were tested with IgG-ELISA, the cut off point was 0.66 (0.14 + 0.52). and 96.9% (62 out of 64) of the clinical cases were positive for the presence of P. multocida antibodies (with OD range from 0.66 to 1.63). Two clinical cases were tested negative (3.1% with OD values less than 0.66). In contact apparently healthy camels, 75.6%

(102 out of 135) of the samples were tested positive with a lower OD range from 0.66 to 1.3 compared to the clinical cases, while 24.4% of the samples (33 out of 135) were tested negative (with OD less than 0.66) This high titer of PM antibody was due to the active infection in clinical cases or due to either convalescent or chronic infection in the apparently healthy camels (Srivastava,1998; Singh and Jayprakasan, 2001)

For the detection of PM antibodies in nasal secretion by IgG ELISA, the cut off value was 0.62 (0.12 + 0.50) and 95.3% (61 out of 64) of nasal secretion collected from clinical cases were positive (with OD range from 0.62 to 1.27) and 4.7% (3 out of 64) were negative. The incidence of positive cases in contact apparently healthy camels was 74.1% (100 out of 135) with OD range from 0.62 to 1.03, whereas the negative represented 25.9% (35 out of 135) with OD values less than 0.62 (Table-6). These results confirmed the sureriority of Serum IgG-ELISA over the IH, DIA and nasal secretion ELISA which also supported the previous investigations which recommended serum IgG-ELISA in terms of convenience and sensitivity. (Manning 1984; Pathak et al., 1997; Brennan et al., 1998, Singh and Jayprakasan, 2001). DIA has only the advantage over ELISA in case of soluble protein antigen that has low affinity to adhere to polystyrene plates or those antigens that could be altered in configuration when adsorbed to the ELISA plates. Nevertheless the sensitivity of ELISA was much higher than the DIA (Kumar et al., 1985).

It was also believed that IgA constituted the major component of immunoglobulins in the nasal secretion. However in other studies the levels of IgG titers were found higher than the IgA titers in both nasal secretions as well as in the bronchoalveolar fluid. The high titration of IgG in the nasal secretion was explained, in part, by the fact that contineous existence of the bacteria in the respiratory passage stimulated the local IgG production at the site of the antigen colonization by the local interstitial plasma cells. Moreover, the antigen-sensitized cells may be disseminated to other tissues, resulting in production of antibodies in other remote sites (Smith et al., 1983; Mastecky 1987; and Brennan et al., 1998).

Serum biochemical analysis of PM infected animals elucidated a significant decrease in total protein, albumin and A/G ratio while globulin was significantly increased which was also coincided the results of Hassan1984, Nasser and El-Sayed 1997. This was explained as the direct effect of PM toxines on alveoli and hepatocytes attributed to failure in protein synthesis (especially albumin, hypoalbuminia). The globulin fraction was significantly increased due to the stimulation of the immune system and induction of immunoglobulins against the PM infection which was confirmed by the different serological tests IH, DIA, serum IgG-ELISA and nasal secretion IgG-ELISA (Koneka et al.1997 Brennan et al., 1998 Srivastava,1998; Singh and Jayprakasan, 2001). Also significant increase in AST, ALT, alkaline phophatase as well as creatinine, urea and uric acid (Table 7). These results supported previous investigations conducted by Jerre et al. 1963 and Ali et al., 1998.

The serum mineral profile of infected camels revealed significant decrease in calcium, inorganic phosphorus and magnesium. This was due to the hypoproreinaemia, as the serum calcium existed in protein-bound form that could not be repleanished by reabsorption due to renal cells damage. PM osteolytic toxines had also degenerative effect on bones due to the mitogenic stimulation of osteoblast which inturn stimulate osteoclast activity which destruct the bone cells (Mullan and Lax 1996; Hoskins et al. 1997). Hypomagnesimia was due to the direct correlation to hypocalcimea. Sodium and chloride were also decreases while potassium was significantly increased due to disturbance in the acid/base balance incurred by the septicemia and the destructionof alveoli and renal glumeruli which are primary organs for acid/base balance (Debonth and Sharma 1983; Koneka et al.1997).

It was concluded from this investigation that PM caused serious respiratory infection in camels, that can be diagnosed with different serological tests viz: IH, DIA, nasal secretion IgG-ELISA and serum IgG-ELISA with higher sureriority to the later test. Also serum biochemical profile can be beneficial aid in the diagnosis (Manning 1984; Pathak et al., 1997).

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