DIAGNOSIS OF SOME RESPIRATORY DISEASES IN CAMEL CALVES

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SUMMARY

A total of 53 lung tissues of slaughtered camel calves age about (9-12 months old) from Kerdasa abattoir-Giza Governorate were used in this study. The lung tissues were subjected to bacteriological, virological and pathological examinations. The bacteriological findings revealed isolation of many of gram +ve and gram -ve bacteria like Staphylococcus spp, Streptococcus spp, Actinomyces pyogenes, Diplococci pneumoniae, E. coli, Pasteurella haemolytica, Yersinia enterocolitica, Klebsiella spp. and Pseudomonas aeruginosa. The percentage of their isolations were 16.98%, 47.16%, 7.5%, 5.7%, 28.3%, 7.5%, 9.4%, 15.09% and 3.8% respectively. The isolates proved sensitive to enrofloxacin, gentamicin and ampicillin and resistant to amoxycillin and neomycin.

Direct fluorescent antibody technique (FAT) on lung tissue sections revealed that 4 cases of IBR (7.54%), 2 cases BVD (3.77%) and 2 cases BRS (3.77%) viral antigens.

The pathological examination showed 12 cases of non significant microscopic lesions and 41 cases suffered from different types of pneumonia (Haemorrhagic, necrotizing, fibrinous and bronchopneumonia) at the rate of 19 cases (35.8%), 6 cases (11.3%), 2 cases (3.8%) and 14 cases (26.4%) respectively. The pathological aspects of pneumonia in relation to bacterial and viral causes were studied and discussed.

INTRODUCTION

Camels in several countries including Egypt are still an important source of meat, milk and hide production, in addition to their secured trade and communication throughout the wide arid expanses.

The adaptability of camels to climatic extremes including world's hot zones and their natural resistance to certain deadly infectious diseases made the camel indispensable for the people of these regions where they are also known as the ships of desert (Wernery and Kaaden, 1995). Pneumonia in farm animals is a serious problem which hinders animal productivity and may results in great losses in animal farms. Many causative agents contributing to the occurrence of pneumonia in farm animals included bacteria, viruses, fungi and parasites (Fatma et al., 2001).

Bacterial pneumonia was previously studied in slaughtered camels by many authors. Bendary (1986) and Mahmoud et al. (1988) recorded the pathological changes occurred due to bacterial infection in camel's lung. Thabet (1993) and Amany (2000) isolated klebsiella species, Stapylococcus aureus, corynebacterium species, micrococcus species and citrobacter from pneumonic lungs of slaughtered camels. Fatma et al. (2001) isolated the same types of bacterial isolates from camel's lung suffered from haemorrhagic, necrotizing and bronchopneumonia while Pasteurella haemolytica was isolated from fibrinous pneumonic lungs. Viral pneumonia in farm animals has been attributed to various viral agents, mostly infectious bovine rhinotracheitis virus [IBRV], bovine viral diarrhoea virus [BVDV] (Bohrmann et al., 1988; Wernery and Wernery, 1990 and Zaghawa, 1988) and bovine respiratory syncytial virus [BRSV] (Olaleye et al., 1989 and Amal, 1998).

With the exception of the camel pox, a grave lack of information exists regarding viral diseases in slaughtered camels and its related pathological entities in general (Hafez et al., 1992).

The aim of the present study was to clarify the role of various bacterial and viral causative agents which are incriminated in common respiratory diseases with special reference to pneumonia in slaughtered camel calves and to correlate the lung lesions in relation to the etiology to give an obvious and complete picture for diagnosis.

MATERIAL AND METHODS

A total of 53 clinically pneumonic lung specimens of slaughtered camel calves were collected from Kerdasa abattoir, Giza governorate during the period from January to July 2001. The age of the animals ranged from 9-12 months old. The gross pathological lesions were recorded and representative portions from each pneumonic lungs were chosen and three necropsy specimens were taken. Two specimens were placed separately in a small sterile ice box for bacteriological and virological examinations while the third specimen was fixed in 10% formol saline solution for histopathological examination.

I. Bacteriological examination:

a-The examined specimens were cultured into nutrient broth for 24 hr. at 37 °C and subjected to bacteriological examination on the following media:

* Ordinary media: such as 5% sheep blood agar, MacConkey agar and nutrient agar.

The suspected colonies were picked up and subcultured onto:

* Specific media: such as Edwards medium EMB medium, Mannitol, salt agar, Pseudomonas selective media, Hektoen enteric media, XID media, Muller Hinton agar media (Oxoid) and Dnase agar. The isolates were identified biochemically according to Cruickshank et al. (1980).

b- Serotyping of the isolated strains by using diagnostic antisera :Lancifield group identification for streptococcal strains and staphytect test for the isolated staphyloccoccus stains.

C- Antimicrobial susceptibility of isolates were done by disc diffusion technique according to Quinn et al. (1994) using antibiotic disc (Oxoid) including erythromycin (15µg), enrofloxacin (30 µg), gentamicin (10 µg), amoxycillin (30 µg), Kanamycin (30 µg), oxytetracycline (30 µg), ampicillin (10 µg), Colistin sulphate (25 µg), Trimethoprin + sulfamtoxazol (1.25 +23.7 µg), and neomycin (30 µg).

II. Virological examination:

Samples preparation for virus isolation was carried out according to Payment and Trudel (1993). Median Darby bovine kidney (MDBK) cell line were used as a continuos cell for virus isolation, propagation and titration. The cell culture was supplied and maintained in virology department of Animal Health Research Institute (AHRI), Dokki, using minimal essential media (MEM) with 10% and 2% of bovine serum as growth and maintaince media respectively. Virus isolation was adopted according to Hierholzer and Killington (1996).

Direct immunofluorescent antibody technique (IFAT) using fluorescein isothiocynate (FITC) conjugated antibodies of IBRV/BVDV and BRSV was applied for detection of antigens of these viruses in lung tissue sections. The conjugates were supplied and applied as recommended by the source (Central Vet. Lab. New Haw, Weybridge U. K.).

III. Histopathological examination:

The fixed pneumonic lung specimens were processed by conventional paraffin embedding technique, sectioned (3-4 µm) and stained by hematoxylin and eosin (Harris, 1898) for histopathological examination.

RESULTS

I. Bacteriological examination:

Results of bacteriological examination were tabulated in tables 1, 2 and 3.

II. Virological examination:

Results of virological examination were tabulated in tables 4, 5 and 6.

Figures 1, 2 and 3 shows positive reaction of IFAT. Five out of 12 virus isolates were identified by using direct FAT on MDBK cell line. The results revealed that 4, 2, and one isolate were identified as IBR virus, BVDV and BRSV respectively.

III. Pathological findings:

The pathological examination of the 53 clinically pneumonic lung specimens revealed 41 cases (77.4%) suffered from different types of pneumonia and 12 cases (22.6%) of non significant microscopic lesions.

The microscopic examination of the pneumonic lung specimens (41 cases) showed haemorrhagic pneumonia in 19 cases (35.85), necrotizing pneumonia in 6 cases (11.3%), fibrinous pneumonia in 2 cases (3.8%) and bronchopneumonia in 14 cases (26.4%).

1. Haemorrhagic pneumonia (19 cases):

Gross findings:

The lung tissue appeared severely congested with the presence of multiple large haemorrhagic areas on the pleural surface. The cut section of the lung tissue ozzed bloody exudate.

Histopathological findings:

The bronchial and bronchiolar lining epithelium showed partial stratification in most cases and focal desquamation in few cases. Multiple and extensive areas of heamorrhages were observed in the alveolar lumina and in the interstitial tissue throughout the lung tissue (Fig.4). In addition, perialveolar blood capillaries appeared congested, dilated and engorged with blood (Fig. 5). Although thickening of the alveolar walls by mononuclear cell infiltration and septal cell proliferation was detected in most of the pulmonary tissue alveolar emphysema was seen in few areas. The pleura showed oedema and congested blood capillaries. Types and numbers of bacteria and viruses isolated from haemorrhagic pneumonic cases were given in table 8.

2. Necrotizing pneumonia (6 cases):

Gross findings:

The pulmonary tissue appeared pale in colour with the presence of multiple focal areas of consolidation which appeared greyish in colour.

Histopathological changes:

The epithelial lining of the bronchi and bronchiols showed degenerative changes and/or desquamation into the lumen in most cases and it revealed focal stratification in few cases. The alveolar lumina were distended mainly with denuded necrotic alveolar lining cells and sometimes studded with mononuclear cells mostly lymphocytes and few neutrophils. Moreover multifocal areas of necrosis associated with lymphocytic and histiocytic cell aggregations were detected through the lung tissue (Fig. 6). The pleura of most cases revealed foci of fibroplastic proliferation.

The bacterial isolation of these cases revealed mixed infection only:

E. coli + *Actinomyces pyogenes* (2 cases).
E. coli + *Staph. albus* (1 case).
Yersinia enterocolitica + *Strept. pyogenes* (1 case).
Yersinia enterocolitica + *Actinomyces pyogenes* (1 case).
Strept. pyogenes + *Pasteurella haemolytica* (1 case).

The viral isolation of these cases revealed single infection only of IBRV (1 case), while the mixed infection was noticed only between IBRV + RBSV (1 case).

3. Fibrinous pneumonia (2 cases):

Gross findings:

The lung tissue showed multiple moderate congested patches. Focal areas of fibrosis were observed on the pleural surface.

Histopathological changes:

Most of the alveolar lumina showed the presence of oesinophilic exudates consisted principally of faintly pink fibriller network of fibrin. Sometimes this network admixed with lymphocytes and histiocytes. Perialveolar blood capillaries appeared severely congested (Fig. 7) with the presence of multiple haemorrhagic areas scattered through the pulmonary tissue. Mononuclear cells lymphocytes and histiocytes were diffusely infiltrated in the interstitial tissue and surrounding some bronchi and bronchiols (Fig. 8). Diffuse alvealar emphysema was noticed in most cases.

The bacterial isolation of these 2 cases showed Pasteurella haemolytica in both. In addition one case showed E. coli and the other revealed Strept. pyogenes. No virus isolation was noticed in these cases.

4. Bronchopneumonia (14 cases):

Gross findings:

The entire surface of the lungs revealed mild to moderate congestion and on cut section serous exudate was seen.

Histopathological changes:

The epithelial lining of the bronchi and bronchiols either revealed degenerative changes or may be desquamated into the lumen or exhibited partial stratification. The bronchial and bronchiolar lumina showed the presence of cellular exudate consisted of lymphocytes and histiocytes admixed with exfoliated necrotic bronchial and bronchiolar epithelium (Fig. 9). Marked proliferation of the bronchus associated lymphoid tissue (BALT) was observed in most of the bronchi (Fig. 10). Peribronchial, peribronchiolar, perivascular and perialveolar moderate to severe infiltration of lymphocytes, histiocytes and few neutrophils together with the congestion of interstitial blood capillaries were seen. Pluritis was indicated by inconsistent edematous thickening of the pleura with lymphocytic cell infiltration was observed. Types and numbers of bacteria and viruses isolated from bronchopneumonia cases were given in table 9.

DISCUSSION

It is clear that the bacteria and viruses have been overlooked as potential causes of pneumonia in camel calves when compared with other etiological agents. Table (1) revealed that 40 cases (75.5%) out of 53 clinically pneumonic lungs showed bacterial isolation, from which 9 cases (22.5%) of single isolates and 31 (77.5%) of mixed bacterial isolates. Our results nearly seem to agree with that observed by Fatma et al. (2001). The results showed that not all pneumonic lungs of slaughtered camels yielded positive results for bacterial isolation as pneumonia may have been due to other causes.

The prevalence of many bacterial species isolated from pneumonic lungs of slaughtered camel calves, table (2). The isolates were differentiated by biochemical tests into gram positive bacteria [S. aureus, 5 (9.4%); S. albus, 4 (7.5%); Str. Pyogenes, 15 (28.3%); Str. Pneumoniae, 10 (18.8%); Actinomyces pyogenes, 4 (7.5%) and Diplococcus pneumoniae, 3 (5.7%)] and gram negative bacteria as [E. coli, 15 (28.3%); P. haemolytica, 4 (7.5%); Y. enterocolitica, 5 (9.4%); P. aeruginosa, 2 (3.8%); K. oxytoca, 6 (11.3%) and K. pneumoniae, 2 (3.8%)]. These results in general partially agree to those recorded by Gobrial et al. (1991), Rana et al. (1993), Bekele (1999), AL-Rawashed et al. (2000) and Fatma et al. (2001).

The antimicrobial susceptibility of different bacterial isolates were determined in 10 commercial antibiotic discs (Finegold and Martin, 1982). Table (3) proved that most of gram +ve and gram -ve bacterial isolates were sensitive to enrofloxacin and gentamicin except Klebsiella species were sensitive to kanamycin, oxytetracycline and colistin sulphate, our results concurred with those described by many authors as Riad (1989), Thabet (1993), Amany (2000) and Fatma et al. (2001).

The resistance of some bacterial isolates was mostly to colistin sulphate, trimethoprim and neomycin except P. haemolytica was sensitive to erythromycin and kanamycin. These findings were reported by Thabet (1993), Amany (2000) and Fatma et al. (2001).

Seroepidemiological viral studies on camels during the last two decades were performed mainly on camels for slaughter with little or no background regarding either the origin or condition of the animals (Allen et al., 1992). Serological results have a limited predictive value since they only confirm that the animal has come in contact with a viral agent and has produced antibodies (Dallinig et al., 1966).

In this study, the test of choice for viral diagnosis was direct immunoflurescent (IFAT) which has been considered one of the reliable, sensitive, specific and rapid technique for detection of IBR, BVD and BRS viral antigens in lung tissue of camel calves. These viruses have been incriminated as causative agents for respiratory disorders in camel calves as described by many authors (Bornstein and Musa, 1987 and Bohrmann et al., 1988).

Our results revealed that the detection of viral antigens in lung tissues were 4/53 (7.54%), 2/53 (3.77%) and 2/53 (3.77%) for IBRV, BVDV and BRSV respectively, (table 4 and photos 1,2,3). The dromedary does not seen to be susceptible to the IBR virus. Hedger et al. (1980), Bornstein et al. (1988) and Wernery and Wernery (1990) were not able to detect any antibodies to the causative BHV-1 and only Burgemeister et al. (1975) found low antibody titres in 5.8% of Tunisian dromedaries. BHV-I antibodies were found by Rosadio et al. (1993) in Perwian Lamas and alpacas and he also found the highest prevalence when the herds grazed on the same pasture together with cattle, sheep and goats. When these animals separated from other ruminants, the prevalence was decreased from 16-17% to 5.1%.

With regard the BVDV, Wernery and Wernery (1990) explained the higher incidence of mucosal disease (MD) in breeding animals (9.2%) when compared to racing dromedaries (3.6%) with the greater size of the breeding herds and their closer contact to cattle herds.

The results of serological studies identifying MD antibodies in dromedaries which recorded by Burgemeister et al. (1975), Bohrmann et al. (1988) and Wernery and Wernery (1990) were 3.9%, 3.4% and 3.6% respectively seemed to concur with our results.

The detection of the presence of MD in camels was primarily based on serological methods (Wernery, 1992). He recommended the use of FAT as routinely screened technique for diagnosis of the presence of MD as viral causes in adults dromedaries and calves that have died in U.A.E.
On the other hand, Olaleye et al. (1989), recorded BRSV among slaughter camel (Camelus dromedarius) in preliminary survey. Amal (1998) clarified that 31 out of 120 camel sera samples were positive for BRSV with antibody titer ranged from 2-8 with percentage about 25.83%.

Table (5) showed the relation of mixed infection with different viral agents. The results clarified that there were 7 cases of camel calves suffered from mixed infection of IBRV, BVDV and BRSV, 3 cases of IBRV and BVDV, 3 cases of IBRV and BRSV and one case of BVDV with BRSV. Our results indicated that significant problems still exist for the route of transmission by aerosol infection (Baker, 1987) who mentioned that entry of the virus is probably through the lungs or orally. In order to evaluate how the serology for BRSV compares with other potential viral pathogens, the responses for the same animals were compared for serum neutralizing titres against IBRV, BVDV and Parainfluenza (PI_3) (Mock, 1987).

With regard the virus isolation, a trial for isolation by blind passage on MDBK cell line for 3 successive passages showed a clear CPE in the form of rounding and gaps formation due to the shrinkage of cells sheet after 3-4 days post inoculation. Our results revealed that 12 out of 53 lung tissues were positive reactors while the other 41 samples were negative. Only 5 out of 12 isolates were identified positive (4 IBRV, 2 BVDV and 1 BRSV) by using direct flourescent antibody technique [2 single isolates of IBR, one isolate BRSV and 2 mixed isolates IBR with BVDV.]. These characteristic CPE and serological identification seemed to agree with those described by Baker (1987) and Wernery and Kaaden (1995). Although the other 7 isolates gave a clear CPE, not identified as IBRV or BVDV or BRSV. These may be due to other viral causes like adenovirus or parainfleunza 3 or others.

The seroepidemiological studies have confirmed that the camel produces antibodies to great number of pathogenic viruses without developing the disease and only few viruses appeared to cause disease in camel as recorded by El-Gayoum (1986). In addition, newer findings have indicated, however, that the camel is resistant to most of the respiratory disorders viruses.

Table (7) showed that E. coli appeared to be the most bacterial isolates mixed with viral infection. While P. haemolytica gave no relation to viral infection. The correlation between viral and bacterial infections may be attributed to the presence of opportunistic bacteria which were stimulated as secondary invadors due to viral infection as reported by Chauhan et al. (1985).

Concerning pathological findings, it was observed that the present study showed four types of pneumonia in lung of camel calves due to bacterial and viral infections. Haemorrhagic pneumonia was seen in 19 cases (35.8%). Histopathologically, these cases characterized by presence of extensive areas of haemorrhages in the alveolar lumina and interstitial tissue associated with thickening of the alveolar wall by mononuclear round cells infiltration and septal cells proliferation. The most isolated bacteria from such cases were K. oxytoca, Str. pneumoniae and E. coli. Similar observations were reported by Mahmoud et al. (1988), Jubb et al. {1993) and Fatma et al. (2001). The highly toxigenic bacteria infecting the pulmonary tissue particularly the gram +ve bacteria play important role for the occurrence of pathological alterations observed in the lung tissue as recorded by Fatma et al. (2001).

Necrotizing pneumonia was found in 6 cases (11.3%). Histopathologically, it was characterized by desquamation and necrotic changes involving the bronchial and bronchiolar epithelium accompanied by presence of necrotic areas scattered in the lung tissues. Bacterial isolation of these cases revealed Actinomyces pyogenes, Staph. albus, Past. haemolytica and E. coli. These results come in accordance with Mahmoud et al. (1988) and Fatma et al. (2001). These necrotic changes may be due to the effect of toxins released by some isolated bacterial species (Jubb et al., 1993).

Fibrinous pneumonia was noticed in 2 cases (3.8%). Histopathologically, these cases characterized by presence of faintly pink fibriller network of fibrin together with infiltration of mononuclear cells in the interstitial tissue. Past. haemolytica was the main bacteria isolated from such cases. These findings come in agreement with Bendary (1986) and Fatma et al. (2001). Jones et al. (1997) attributed the pathological picture detected in cases of Past. haemolytica infection to the endotoxins released by Past. species.

Bronchopneumonia was observed in 14 cases (26.4%). Histopathologically, it was characterized by desquamation and degeneration of the bronchial epithelium associated with infiltration of lymphocytes and histiocytes in the bronchial lumina and diffusely in the interstitial tissue. The isolated bacteria from such cases mostly were Str. pneumoniae, Staph. aureus, Str. pyogenes, Pseudo. aeruginosa and E. coli. These findings coincide with that of Jubb et al. (1985) and Donald et al. (2001).

The pathogenic bacteria could cause these inflammatory alterations in the lung tissue. Pathological alterations occurring in the different types of pneumonia in the present study concur with the findings of Pinkette et al. (1966), Brunstetter et al. (1971) and Abo-El-Lial (1997) due to BVDV infection in the camel calves. These findings were also recorded by Narita et al. (1982), Jubb et al. (1985) and Abo-El-Lial (1992) due to IBR infection and by Pirie et al. (1981), Jubb et al. (1985) and Abo El-Lial (1992) due to BRSV infection in cattle calves. The viral diseases of camels may directly affected these animals as primary causes or as predisposing causes through stimulation of other microbial agents as second invadors as reported by Chauhan et al. (1985).

Histopathological results showed 12 cases of non significant lesions. The bacterial isolates of few of which were pathogenic bacteria which were opportunistic pathogens that occasionally cause infection in animals under stress factors or drop in immunity in camel calves (Quinn et al., 1994). Some of the non significant lesions cases gave positive results for IBRV, BVDV and BRSV either single or mixed infection by FAT. The presence of IBRV in these animals may be attributed to latency or carrier status as reported by Pastoret et al. (1982 & 1986). While BVDV infection in these animals may be due to persistent infection (Bolin et al., 1985 and Niskanan, 1995). Concerning BRSV infection in these camel calves either due to early infection or during incubation as mentioned by Hirsh and Yun-Zee (1999) who recorded not all cattle infected with BRSV showed clinical signs of the disease and the infected animals serve as the reservoir of the disease. The viral antigen can be detected by FAT in nasopharyngeal cells and in the epithelial cells of bronchioles and alveoli.

Our study conclude that the viral infection among camel calves do not seem to reveal a true problem with exception of secondary invadors, mostly bacterial infection. Pathological examination complemented with microbiological investigations appear to more reliable for diagnosis of many respiratory disorders. The epidemiological significance of our study in camel in the endemic areas still need further investigations.

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Table (1): Positive samples revealed single and mixed bacterial isolates.

Total samples	+ ve samples		-ve samples		single bacterial isolates		mixed bacterial isolates	
	No.	%	No.	%	No.	%	No.	%
53	40	75.5	13	24.5	9	22.5	31	77.5

Table (2): Prevalence of bacterial isolates from pneumonic lung of slaughtered camel calves.

Types of bacterial isolates	No. of isolates	% of total number of samples (53)
Gram +ve bacteria		
Staphylococcus aureus	5	9.4
Staphylococcus albus	4	7.5
Streptococcus pyogenes	15	28.3
Streptococcus pneumoniae	10	18.8
Actinomyces pyogenes	4	7.5
Diplococcus pneumoniae	3	5.7
Gram -ve bacteria		
E.coli	15	28.3
Pasteurella haemolytica	4	7.5
Yersinia enterocolitica	5	9.4
Pseudomonas aeruginosa	2	3.8
Klebsiella oxytoca	6	11.3
Klebsiella pneumoniae	2	3.8

Table (3): Antimicrobial susceptability of bacterial isolates from pneumonic lung of slaughtered camel calves.

Isolated bacteria / Antibiotic disc	Erythromycin (15 µg)	Enrofloxacin (30 µg)	Gentamicin (10 µg)	Amoxycillin (30 µg)	Kanamycin (30 µg)	Oxytetracycline (30 µg)	Ampicillin (10 µg)	Colistin sulphate (25 µg)	Trimethoprin + Sulfamoxazol (1.25 + 23.7 µg)	Neomycin (30 µg)
S. aureus	+	++	+++	-	-	++	+++	-	-	-
S. albus	+++	+++	+++	+	+	+++	+++	-	-	-
S. pyogenes	+	++	+++	-	-	-	+++	-	-	-
S. pneumoniae	++	++	+++	-	-	-	++	-	-	-
Actinomyces pyogenes	+	++	+++	-	-	-	++	-	+	-
Diplococcus pneumoniae	++	++	+++	-	+	-	+++	+	+	++
E. coli	-	+++	-	-	++	-	+	++	++	++
P. haemolytica	+	-	-	-	+	-	-	-	-	-
Y. enterocolitica	-	+++	-	-	-	-	-	+++	-	-
Ps. aeruginosa	+	+++	-	-	-	-	+	-	-	-
K. oxytoca	-	-	-	-	+++	++	-	++	++	+
K. pneumoniae	-	-	-	-	++	+++	-	++	-	+

Table (4): Results of direct IFAT for detection of single infection of IBR, BVD and BRS viral antigens in lung tissues of camel calves.

Results	IBRV		BVDV		BRSV	
	No.	%	No.	%	No.	%
Positive	4	7.54	2	3.77	2	3.77
Negative	49	92.45	51	96.22	51	96.22
Total	53		53		53	

Table (5): Relationship of mixed infection with IBR, BVD and BRS viral antigens in lung tissue sections of camel calves examined by direct FAT.

Results	IBRV	BVDV	BRSV
IBRV	4 (single)	3 (mixed)	-
BVDV	-	2 (single)	1 (mixed)
BRSV	3 (mixed)	-	2 (single)
Total of mixed	3	3	1

Table (6): Trials for virus isolation on MDBK cell culture and identification by direct FAT.

Samples	Total	CPE	Non CPE
Lung tissues of camel calves	53	12	41

Table (7): Prevalence of mixed isolation of viral and bacterial agents.

Viral isolates / Bacterial isolates	Gram +ve bacterial isolates					Gram -ve bacterial isolates							
	Staphylococcus		*Streptococcus*		*Actinomyces Pyogenes*	*E. coli*	*P. haemolytica*	*Y. enterocolitica*	*Ps. aeruginosa*	*Klebsiella*			
	aureus	*albus*	*pyogenes*	*pneumoniae*							*Oxytoca*	*Pneum -oniae*	
	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	No.	
-	-												
IBRV	0	2	5	3	3	10	-	-	-	1	1	1	
BVDV	1	2	5	1	-	8	-	1	1	1	1	-	
BRSV	-	2	4	2	-	6	-	1	1	1	1	1	

Table (8): Types and numbers of bacteria and viruses isolated from haemorrhagic pneumonic cases either single or mixed.

Bacterial infection		Viral infection	
Single	Mixed	Single	Mixed
E. coli (2 cases)	+ S. aureus (1 case) + S. albus (1 case) + S. pneumoniae (2cases)	IBRV (1 case) BVDV (2 cases)	IBRV + BVDV (1 Case) IBRV + BVDV + BRSV (6 Cases)
S. pyogenes (3 cases)	+ Past. haemolytica (1 case)		
S. pneumoniae (1 case)	+ K. oxytoca (3 cases) + S. albus (2 cases) + Actino. Pyogenes (1 case)		
	S. aureus + K. oxytoca (1 case)		

Table (9): Types and numbers of bacteria and viruses isolated from bronchopneumonia cases either single or mixed.

Bacterial infection		Viral infection	
Single	Mixed	Single	Mixed
E. coli (1 case)	+ K. oxytoca (1 case) + P. aurogenosa (1 case)	IBRV (1 case) BRSV (1 case)	IBRV + BVDV (2 Cases) IBRV + BRSV (1 Case)
S. pyogenes (1 case)	+ Y. enterocolotica (3 cases)		
	S. pneumoniae + S. aureus (2 cases) S. pneumoniae + Diplococcus pneumoniae (1 case)		

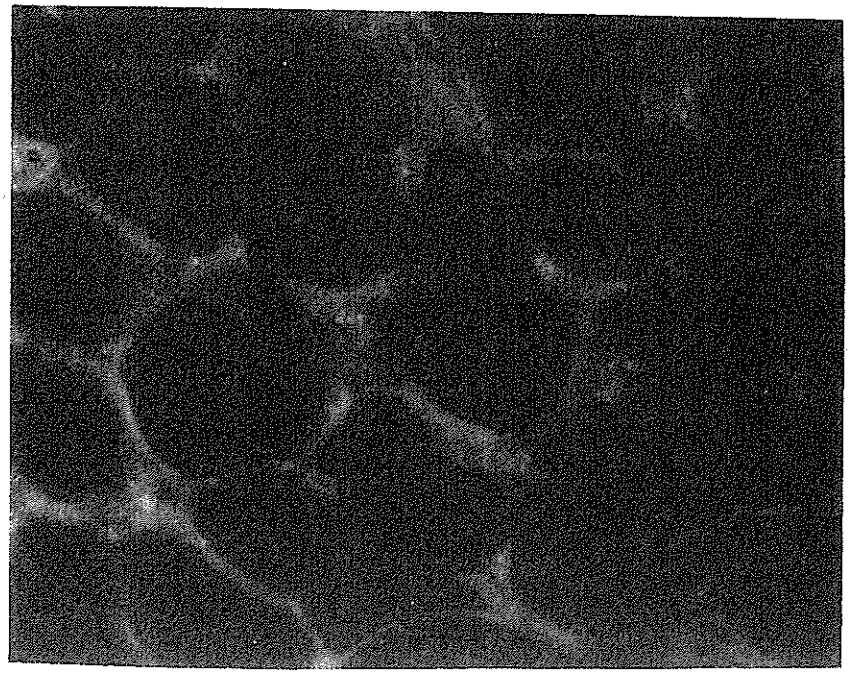

Fig. (1): Emission of green yellowish fluorescence in lung tissue sections with gap formation grape-like clusters of round, refractile enlarged cells stained by FITC conjugated with IBR antibodies (x: 400).

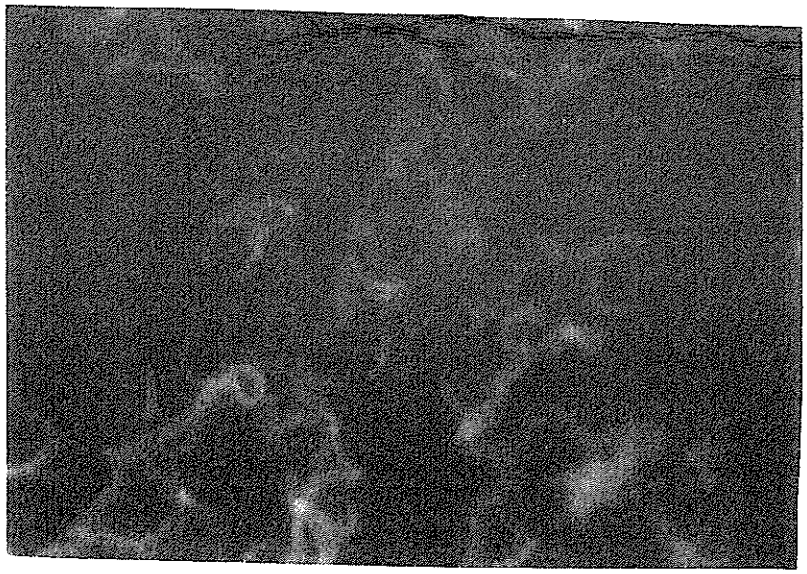

Fig. (2): Gradual degeneration with granulation of cytoplasm and slowly lytic CPE, notice the diffuse flurescent in the cytoplasm of infected cells stained by FITC conjugated with BVD antibody (x: 400).

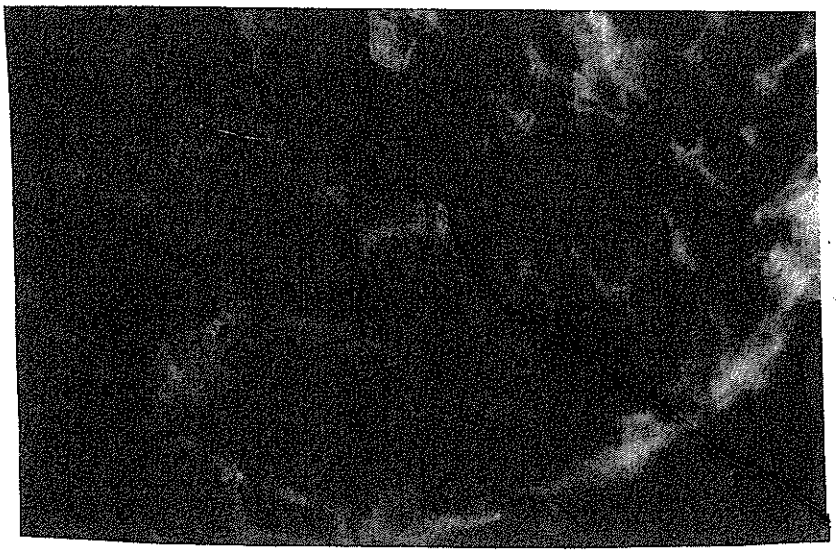

Fig. (3): Notice the arrangment of the tissues in the form of syncytial appearance or like a net of patches with immunofluorescent emission. The section stained by FITC conjugated with BRSV antibodies (x: 400).

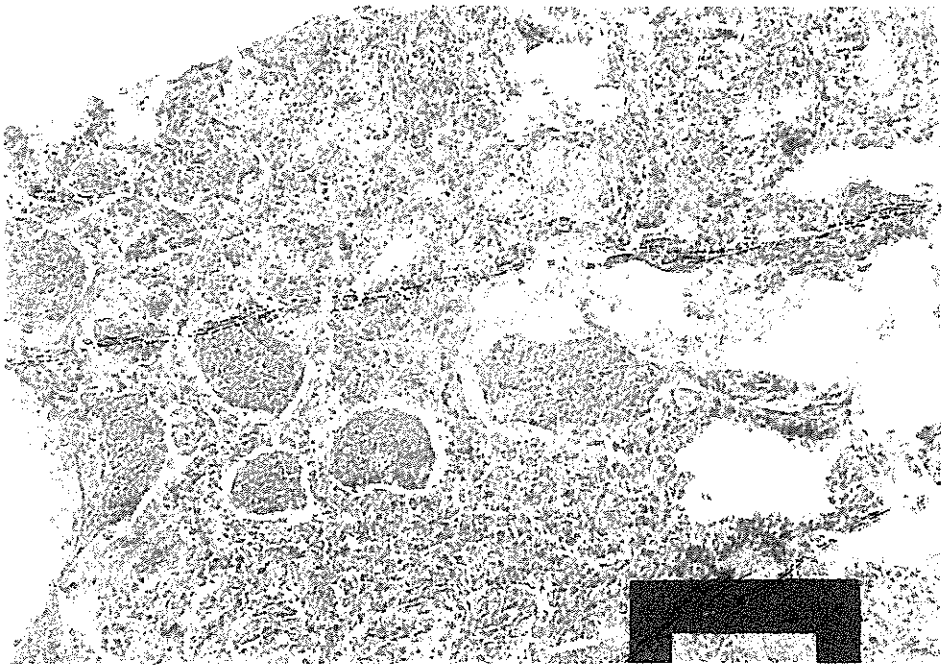

Fig. (4): Lung of camel calf suffered from haemorrhagic pneumonia showing multiple and extensive areas of haemorrhages in the alveolar lumina and interstitial tissue (X: 200).

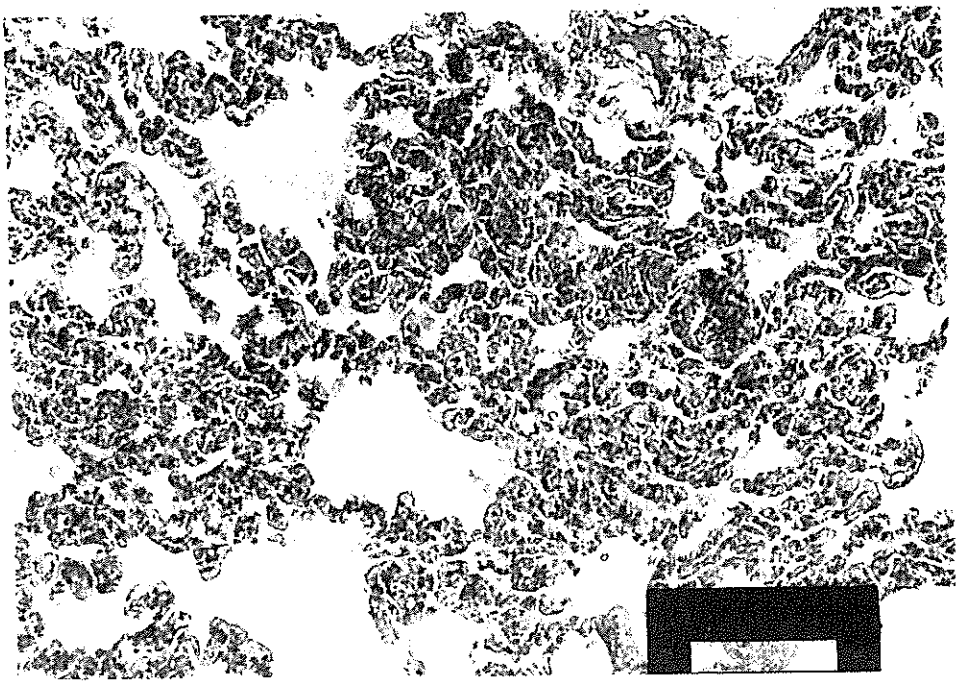

Fig. (5): Lung of camel calf suffered from haemorrhagic pneumonia showing congested perialveolar blood capillaries (X: 100).

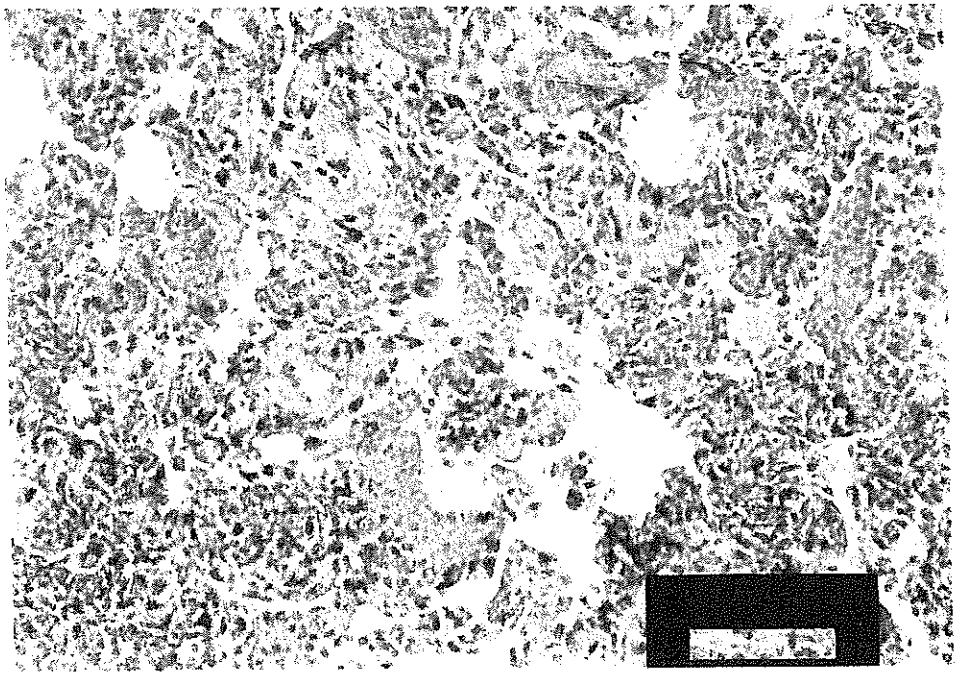

Fig. (6): Lung of camel calf suffered from necrotizing pneumonia showing focal areas of necrosis in the lung tissue (X: 400).

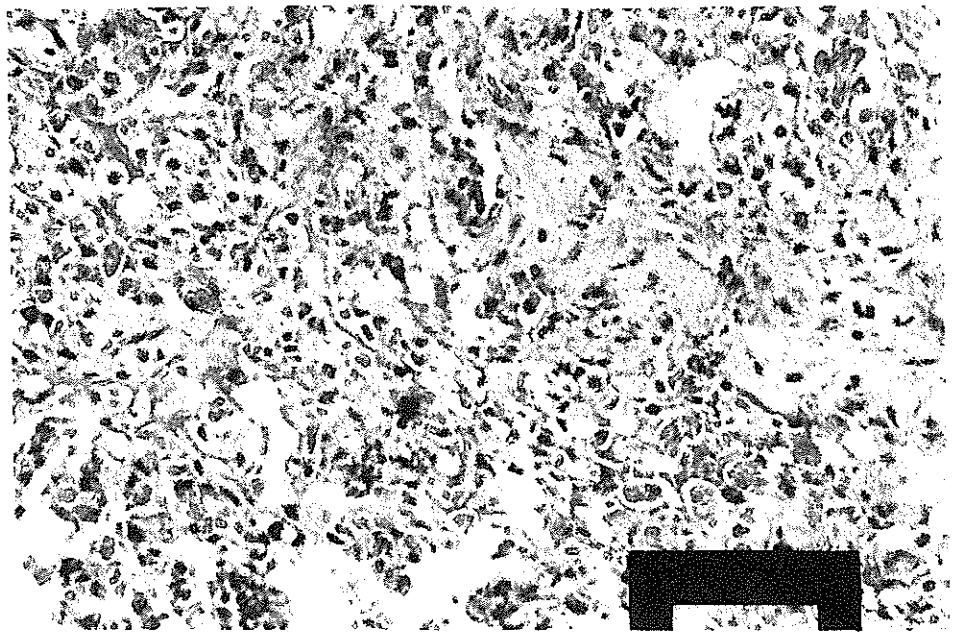

Fig. (7): Lung of camel calf suffered from fibrinous pneumonia showing faintly pink fibriller network of fibrin and congested perialveolar blood capillaries (X: 400).

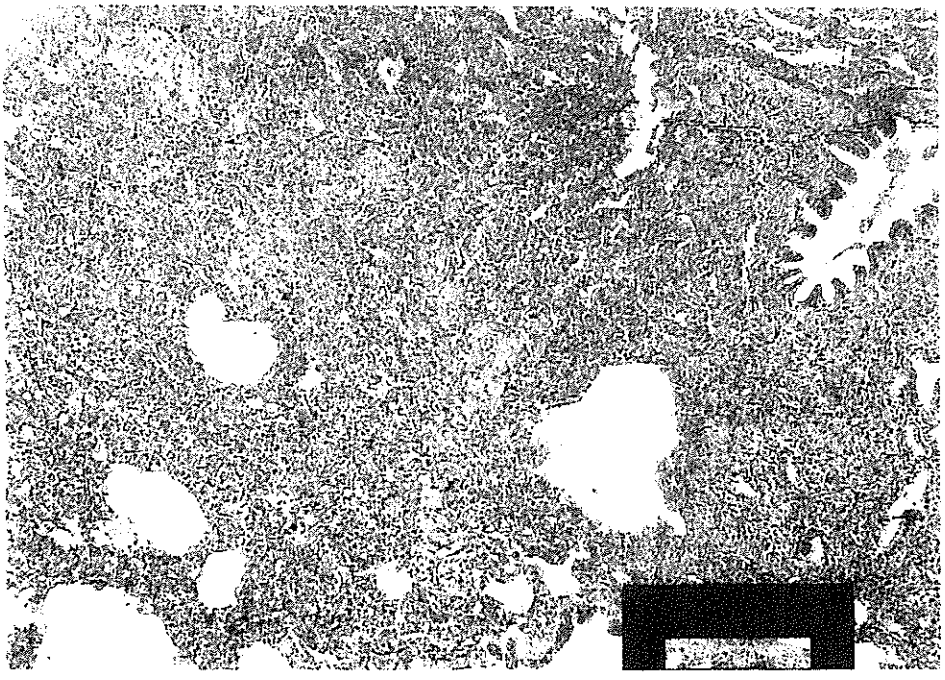

Fig. (8): Lung of camel calf suffered from fibrinous pneumonia showing diffuse infiltration of mononuclear inflammatory cells in the interstitial tissue and surrounding some bronchi and bronchiols. (X: 100).

Fig. (9): Lung of camel calf suffered from bronchopneumonia showing presence of cellular exudate consisted of mononuclear inflammatory cells admixed with necrotic bronchiolar epithelium in the bronchiolar lumina (X: 200).

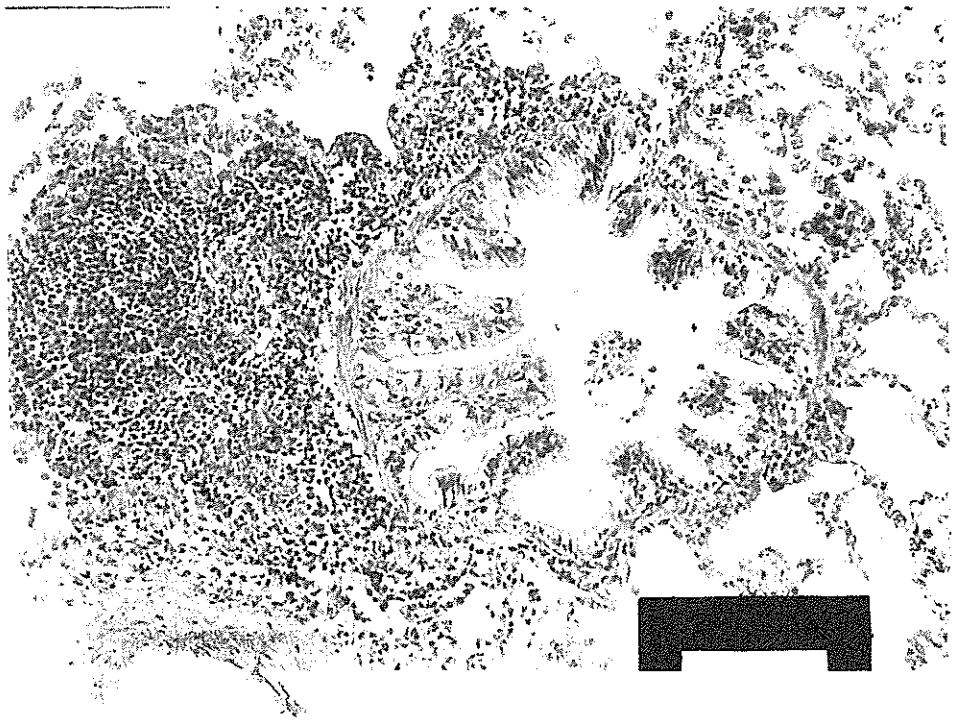

Fig. (10): Lung of camel calf suffered from bronchopneumonia showing proliferation of the bronchus associated lymphoid tissue (BALT). (X: 200).

الملخص العربى

تشخيص بعض الأمراض التنفسية فى صغار الجمال

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أجريت هذه الدراسة على عدد ٥٣ أنسجة رئة صغار جمال عند عمر ٩-١٢ شهر مذبوحة فى مجزر كرداسة التابع لمحافظة الجيزة. حيث تم اجراء الفحوص البكتريولوجية والفيرولوجية والباثولوجية لهذه الأنسجة. وقد أوضح الفحص البكتريولوجى عزل بكتريا موجبة وسالبة الجرام مثل بكتريا المكور العنقودى والمكور السبحى والأكتينوميسس بيوجينس ودبلوكوكس نيومينى والأشيريكية والباستريلا هيموليتيكا واليرسينيا إنتيروكولتيكا و الكليبسيلا والسودوموناس ايروجينوزا بنسب ١٦,٩٨% و ٤٧,١٦% و ٧,٥% و ٥,٧% و ٢٨,٣% و ٧,٥% و ٩,٤% و ١٥,٠٩% و ٣,٨% على التوالى وكانت هذه الميكروبات حساسة للمضادات الحيوية الآتية: إنروفلوكساسين وجنتاميسين والأمبسيلين بينما كانت مقاومة للأموكسيسيلين والنيومايسن. وأظهر الفحص الفيرولوجى باستخدام الميكروسكوب الفلورسنتى المباشر عزل فيروس IBR من ٤ حالات بنسبة ٧,٥٤% وفيروس BVD, BRS من حالتان بنسبة ٣,٧٧% لكل منهما. أما عن الفحص الباثولوجى لهذه العينات فقد بين أن هناك ١٢ عينة رئة طبيعية و ٤١ عينة رئة تعانى من التهابات رئوية مختلفة (التهاب رئوى نزيفى فى ١٩ حالة بنسبة ٣٥,٨% والتهاب رئوى تتكرزى فى ٦ حالات بنسبة ١١,٣% والتهاب رئوى فيبرينى فى حالتان بنسبة ٣,٨% والتهاب رئوى شعبى فى ١٤ حالة بنسبة ٢٦,٤%). وقد تم دراسة ومناقشة الصورة الباثولوجية لهذه الحالات بالنسبة لعزلها البكتيرى والفيروسى.

serological diagnostics have been developed to detect leptospiral infections of these, Microscopic Agglutination Test (MAT) is acknowledged as the WHO standard reference test (Faine, 1982). The drawbacks of this method are the necessity of permanent cultivation of the reference leptospira strains and time-consuming laboratory procedures, particularly when serological studies have to be done on different serovars.

An alternative serological method is the ELISA technique, associating easy performance with high sensitivity and specificity (Voller et al., 1978).

The aim of the present study was to apply an ELISA system for the first time in Egypt for serodiagnosis of Leptospirosis in naturally infected cattle serum in a comparison with the results obtained by MAT and ST.

MATERIALS AND METHODS

Samples:

Blood samples were collected from 289 cows at different private farms in Egypt and sera were harvested. These animals were non pregnant but suffering from reproductive disorders.

Leptospiral strains

Leptospiral reference serovars:

hardjo, grippotyphosa, pomona, canicola and icterohaemorrhagiae with specific rabbit reference antisera (as control positive) were kindly obtained from C. Sulzer, C.D.C, Atlanta, U.S.A.

Serological diagnosis

1) Microscopic Agglutination Test (MAT) (Alexander et al., 1970):

The MAT was performed with living reference leptospira strains cultivated for 7 days in Ellinghausen Modified by Johnson & Haris medium at 30 ^{0}C. For serological studies a serial double fold serum dilution is done using Phosphate Buffer Saline (PBS) beginning with dilution 1:100. 0.025 ml of each leptospiral serovar was mixed with 0.025 ml of serum sample, the mixture was subsequently incubated in a microtitre plate for 2 h at 30 ^{0}C then one drop of each sample was placed onto a clean glass slide and examined using a dark field microscopy. Positive titre was considered at dilution 1:200 or more.