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Pathological Study On Experimental Infection With *Mycoplasma Mycoides* Subspecies *Capri* In Different Age Groups of Goats

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Abstract: Introduction: To determine clinico-pathology caused by *Mycoplasma mycoides subspecies capri (PG3)* in goats of different age groups and antibody titers. **Methods:** Twenty-one goats of three age groups viz; 1, 2 and 3 years with 7 goats of each age. Goats were divided in four groups i-e; G1, G2, G3 each contain 5 goats of each age while G4 comprise 6 goats; 2 from each group. Goats in G1, G2 and G3 were infected with *Mycoplasma mycoides* (PG3) dose (1×10^7 CFU/ml/kg) whereas, G4 was kept as control. Clinical examinations were recorded at 12-h intervals. The blood samples collected were tested through cELISA and the specimen of trachea, lungs, kidney, and liver were collected at the end of experiment for gross and histopathology. **Results:** Temperature, respiratory rate, pulse rate nasal discharge, coughing and lacrimation were noted higher in G1 compared to G2 and G3 groups. Gross pathology showed severe multifocal and diffused necrosis G1 compared to G2 and G3 groups. Histopathology showed sloughing of tracheal mucosa in all groups while hypertrophic secretary glands in G1. Lungs showed emphysema in all groups except G4. Kidneys showed glomerulonephritis while Liver showed congestion and hyperemia in all groups. cELISA, revealed the antibody titers rose from 1st to 3rd week post infection afterwards, reduced slowly. Antibody titers were higher in G1 compared to G2 and G3 groups. **Conclusion**: *Mycoplasma mycoides* subspecies *capri* (PG3) can cause infection in goats of all ages, yet the infection is more severe in young animals compared to old.

Keywords: Pathology**,** *Mycoplasma mycoides*, Susceptibility, Age, Antibody titers, cELISA

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Introduction

The livestock is rapid growing sector with contribution of 11.8% to the national GDP and 55.9% to the agriculture sector. Goats are important mammals which are reared for dual purpose of milk and meat. Goats contribute about 275 thousand tons of meat, 851 thousand tons of milk 25 million skins and 21.4 thousand tons of hair annually to the national economy [1]. At present Pakistan have more than 60 million heads of goats, which consist near about 37 well- known breeds found in different areas of Pakistan [2]. Goat population facing several challenges mainly rough climatic conditions, deprived management, food scarcity & numerous diseases mainly contagious caprine pleuropneumonia (CCPP).

CCPP is highly contagious and fibrinous pleuropneumonia in goats caused by *mycoplasma capricolum subspecies. Capripneumoniae (mccp).* Mycoplasmas are the smallest free living fussy bacteria having diameter of 300 nm, do not possess a rigid cell wall of murin, a membrane of three layers bounded. The genomic size is nearby one third to one sixth of *E. coli* [3]. The mccp have incubation period of about 3-5 days in lungs, however it could be extended up to 3-4 weeks depending upon the influencing factor [4]. CCPP remained for 2 days in initial stage of infected goats with higher mortality [5] in spite the fact in further cases it may last for more than a few days [6].

In Pakistan CCPP causes economic losses due to high morbidity, mortality, and reduced production of goats [7, 8]. CCPP caused high morbidity and mortality in both sexes and all age groups are vulnerable but as related to adult the mortality rate is higher in young kids, in natural conditions mortality rate is 60-70% and morbidity reach up to 100%. It is associated with pyrexia, respirational warning signs such as increased lacrimation and nasal discharge. Inhalation is sore due to forceful and recurrent coughing. Further clinical symptoms in advance stages of disease are animal stand with abducted fore limbs, unable to move, diarrhea, lameness, firm neck and kneeling on ground with lateral recumbency [6].

Due to limited laboratorial diagnosis of CCPP, some conventional serological and biochemical test are performed. There is scarce data available on different age-related disease diagnosis, so this research is designed to investigate the clinical findings, gross pathological and histopathological lesions produced in different age groups of goats experimentally infected with CCPP and to detect the antibodies of CCPP through cELISA.

Material And Methods

Place of Study

The study was conducted at the Department of Veterinary Pathology and Department of Veterinary Medicine, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam.

Mycoplasma Seed Propagation

The seed of mycoplasma cultured in pleuropneumonia -like organism (PPLO) broth was obtained from Vaccine Production Unit (VPU), Tandojam. Bacterial counts were determined from culture media and calculate dosage of CCPP.

Growth Medium for Mycoplasma

Modified hay flick culture medium was used for cultivation of Mycoplasma. Both agar and broth mediums were prepared as suggested by manufacturers. Samples were inoculated and streaked according to standard operating procedure [6].

Fresh Cultures

The pure bacterial seed of *Mycoplasma mucoides subsp, capri* (Mmc) was cultivated and activated by giving 04 passages in mycoplasma agar Medium and Mycoplasma broth medium at vaccine production unit (VPU), Tandojam. Challenge organisms were calculated by plate count method to determine colony forming units (CFUs) / ml. The authentic mean dose was determined, post inoculation, looking back from viable/plate counts as is described by [9].

Latex Agglutination Test

Capri-LAT reagents were purchased from Animal and Plant Health Agency- Weybridge, United Kingdom, which were cold chain maintained at Central Veterinary Diagnostic Laboratory Tandojam The test was performed as per manufacturer instructions [10]. All goats were tested

through CapriLAT Latex Agglutination Test and seronegative goats were further tested for CCPP experimental infection.

Experimental Infection of Goats

A total of 21 CCPP seronegative goats were kept at experimental station, Department of Veterinary Medicine Sindh Agriculture University Tandojam for 7 days of adaptation period. The animals were divided in 4 group such as G1, G2 and G3 with 5 animals in each experimental group. G1 of about 1 year old goats while G2 and G3 are of 2- and 3-years old goats respectively. Group G4 comprise 06 goats including 2 goats from each age group. The goats in group G1, G2 and G3 were infected with CCPP at dose of 0.3 ml/kg body weight of 1×10^7 CFU/ml of PG3 strain through intra-tracheal route by using syringe of 18-gauge needle.

Blood Collection

Subsequent blood was collected from Juglar vein using sterilized syringe of 18-gauge needle weekly basis (W1, W2, W3, W4, W5 and W6) from all experimental animals in red top vacutainers. Then the samples were sent to laboratory for serum separation and confirmation of CCPP antibody titers through cELISA.

Competitive Enzyme Linked Immunosorbent Assay (cELISA)

The sera collected were tested for detection of *Mycoplasma capricolum* subsp. *capripneumoniae*through monoclonal antibody cELISA Test Kit (IDEXX CCPP, 0656231-01). The 1st batch of cELISA Kit was developed at CCPP reference laboratory CIRAD-Montpellier, France which was purchased, and the serum samples were tested as per protocols of manufacturer's instructions.

Clinical Evaluation

The course of disease was observed by clinical examination of infected goats. Clinical examination like rectal temperature, pulse rate and respiration rate were inspected at 12-hour intervals from the day of inoculation till 42 days of experimental trial.

Pathological Evaluation

All animals along with control group were euthanized at the end of experiment. Gross pathological lesions in organs i-e; trachea, lungs, liver, kidney, was recorded and graded as +++ = severe infection, ++ = moderate infection and + = mild infection. Furthermore, the samples were taken for histopathology, stain with H&E and microscopic changes were examined.

Ethical Approval

This study is approved by Institutional Ethical Board of Sindh Agriculture University, Tandojam.

Statistical Analysis

The data obtained was tabulated on MS-Excel Sheet, represented as mean ± standard deviation. The analysis of variance (ANOVA) was used and pairwise comparison through Tuckey test was performed at significance level ($p < 0.05$) by using statistical tool Statistix 8.1. student version software.

Results

Frequency of Clinical Signs

The most prominent clinical signs including pyrexia in G1, G2 and G3 such as 77, 70 and 60%. Lacrimation in G1, G2 and G3 i-e; 74, 72 and 55%. Coughing in G1, G2 and G3 was noted as 78, 75 and 68. Nasal discharge G1, G2 and G3 i-e; 72, 68 and 62% in goats of all infected groups as illustrated in Figure 1.

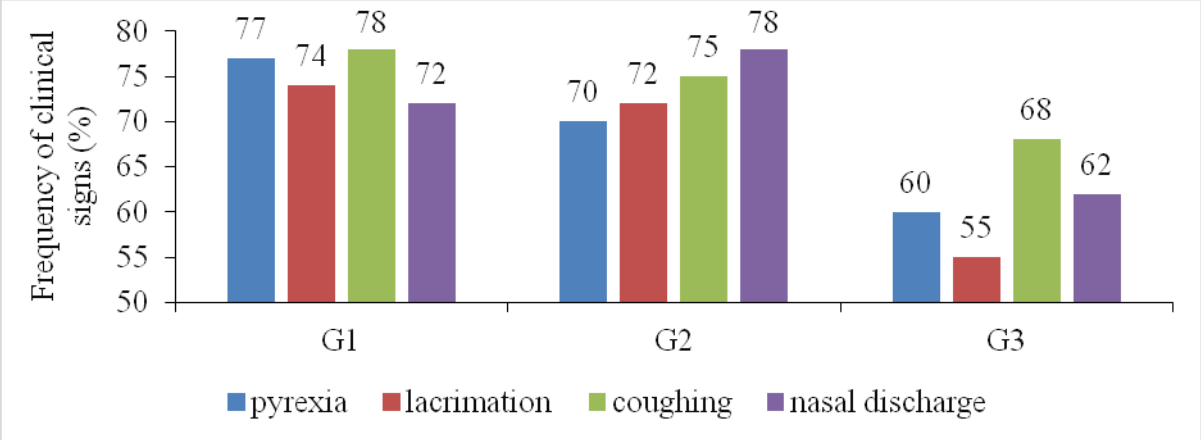

Figure 1. Frequency of clinical signs (%) at peak of infection in different age groups post-inoculation of CCPP infection.

Clinical signs

Clinical signs such as rectal temperature was non-significantly different (P<0.05 and LSD=0.86) between week and within groups. Temperature was normal to subnormal in all groups in all weeks. However, pulse rate was significantly increased (P<0.05, LST=12.4) in 1st, 2nd and 3rd weeks G1, G2 and G3 whereas non-significantly different later on. Likewise, respiratory rate was comparatively higher (P<0.05, LSD=6.68) in G1 in 1st and G2 in 3rd week while non-significantly different in all groups and weeks vice versa as illustrated in Table 1.

Table 1 Clinical signs of goats on different week infected with CCPP

Clinical Signs	Groups	Weeks					
		W1	**W2**	**W3**	**W4**	**W5**	**W6**
Rectal Temperature	G1	103.2^{ac}	103.0^{a}	102.6^{ab}	102.5^{ab}	102.4^{bc}	102.3^{ac}
	G2	103.3^{a}	103.2^{a}	103.0^{ab}	102.4^{a}	102.3^{a}	102.2^{ab}
	G3	103.4^{ab}	103.1^{a}	103.0^{ab}	102.5^{a}	102.4^{ab}	102.2^{ab}
	G4	102.4^{a}	102.5^{a}	102.5^{a}	102.4^{a}	102.5^{a}	102.5^{a}
Pulse rate	G1	92.6^{a}	91.9^{dg}	87.0^{eg}	75.0^{g}	74.9^{g}	72.4^{fg}
	G2	88.4^{ab}	86.7^{be}	82.0^{bf}	77.9^{eg}	75.1^{g}	72.6^{fg}
	G3	87.0^{cd}	85.0^{ad}	83.1^{dg}	80.6^{g}	76.3^{g}	72.9^{g}
	G4	76.6^{ad}	75.4^{ab}	72.0^{ac}	74.1^{ac}	74.7^{ac}	76.5^{a}
Respiratory rate	G1	25.7^{a}	22.4^{ab}	21.9^{ab}	19.0^{ab}	18.3^{b}	17.0^{b}
	G2	24.9^{ab}	23.0^{ab}	22.4^{a}	20.1^{ab}	19.1^{b}	16.1^{b}

	G3	23.6^{a}	22.1^{ab}	21.3^{ab}	20.1^{ab}	19.1^{b}	18.0^{b}
	G4	17.9^{ab}	17.9^{ab}	16.9^{ab}	16.9^{ab}	16.7^{ab}	16.6^{ab}

Table 2 Gross pathological findings.

Organ	Lesion observed at 06-week post infection of CCPP	Grading of lesions
Trachea	Lesions in tracheas include severe foamy material in lumen of trachea along with moderate haemorrhagic lines in goats of 1 year whereas moderate haemorrhages with foamy material in goats of 2 and 3 years old respectively.	G1= +++ G2= ++ G3= ++
Lungs	Lungs infected with CCPP showed unilateral and bilateral involvement. Groups G1 have severe pulmonary consolidated with multifocal and diffuse lesions with accumulation of serous fluid while moderate multifocal and diffused lesion in G2 and G3.	G1= +++ G2= ++ G3= ++
Kidney	Kidneys showed slight congestion and necrotic foci at cortex. While congestion and edematous swelling of medulla were observed in all animals	G1, G2 & G3 = ++
Liver	Liver was usual in size with distended gall bladder and minute hemorrhages found in all infected groups	G1, G2 & G3 = +

+++ = severe infection, ++ = moderate infection and + = mild infection

Histopathological Findings

The trachea of infected goats showed mild to severe sloughing of epithelial mucosa in all infected groups with ciliated columnar epithelial lining up to lamina propia in parallel trends except in group G4. Obvious hypertrophic secretary glands and edematous inflammation was observed in the muscular layer in G1 as illustrated in figure 2.

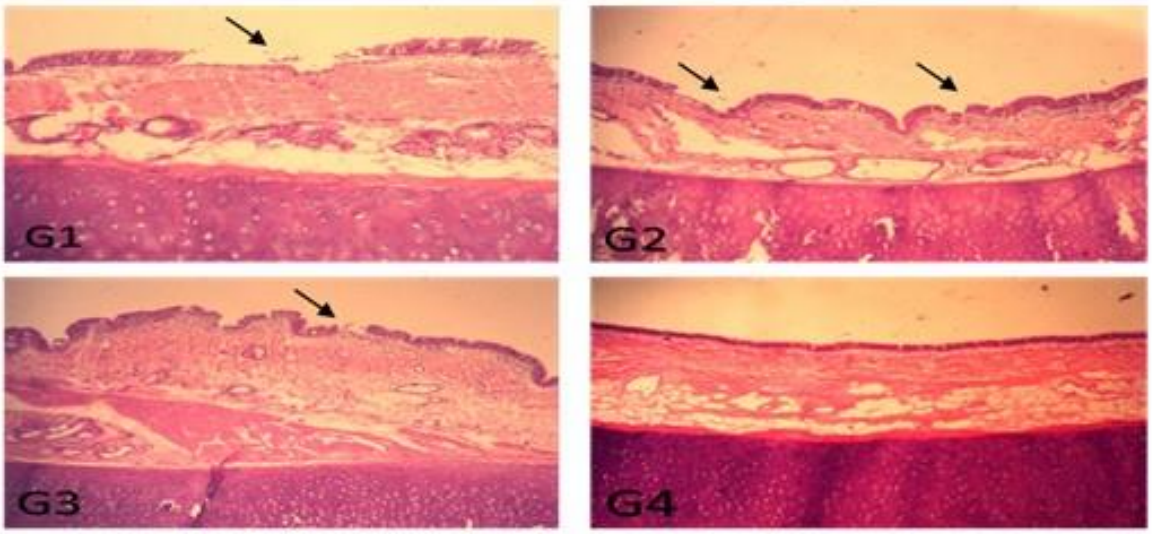

Figure 2. Histopathology of trachea of goats infected with CCPP at 40X, shows severe erosion ciliated columnar epithelial lining in G1 compared to G2 and G3 groups while G4 control group showed normal structure of trachea.

Lungs

Lungs showed the emphysema and atelectasis in all groups except G4 group, chronic serofibrinous bronchopneumonia with infiltration of neutrophils and serous fluid were seen in alveoli, bronchioles, and interstitial septa. In all infected lungs, the epithelial lining of alveoli and

bronchioles were thickened and interrupted severe in group G1 but seen moderate in group G2 and G3 compared to control group G4 as shown in figure 3.

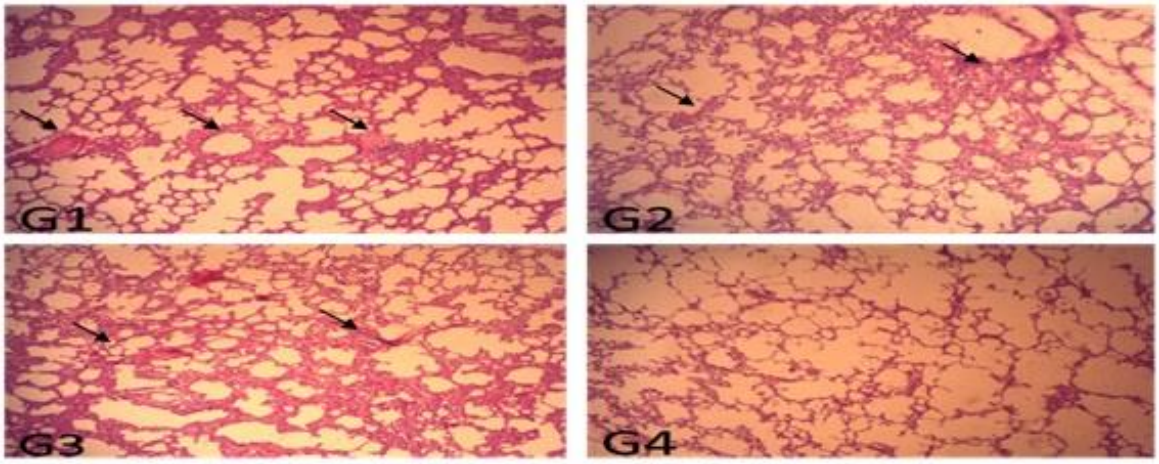

Figure 3. Histopathology of lungs of goats infected with *Mycoplasma mycoides subspecies capri* at 40X, shows the emphysema and thickening of bronchioles which were severe in G1 compared to G2 and G3 groups while G4 control group showed normal structure of lungs.

Kidneys

Kidneys of all infected groups (G1, G2 and G3), were particularly affected at various regions along with the glomerulonephritis. The renal capsule showed the glomerulus disturbance, and their collecting duct tubules were distended with the hyperemia which indicate inflammatory reaction as shown in figure 4.

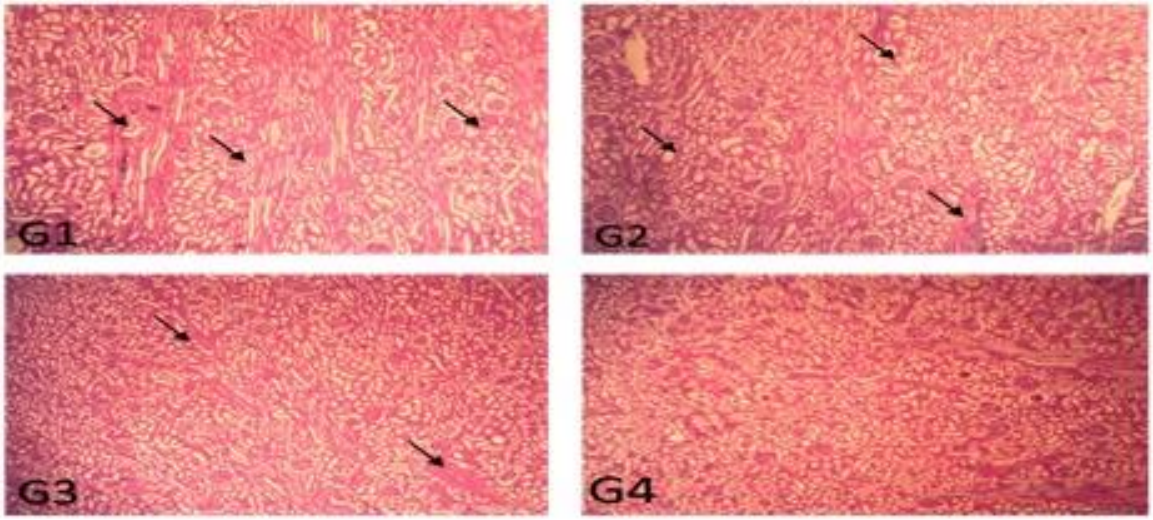

Figure 4. Histopathology of kidneys of goats infected with *Mycoplasma mycoides subspecies capri* at 40X, shows the glomerulitis and enlargement of collecting tubules (arrow) which was severe in G1 compared to G2 and G3 groups while G4 control group showed normal structure of kidneys.

Liver

Liver sections showed the congestion and hyperemia around the central portal vein was markedly seen in group G1 then group G2 and G3. Inflammatory reactions were seen at the interlobular sites and portal vein, which cause expand and interrupted the portal vein as shown in figure 5.

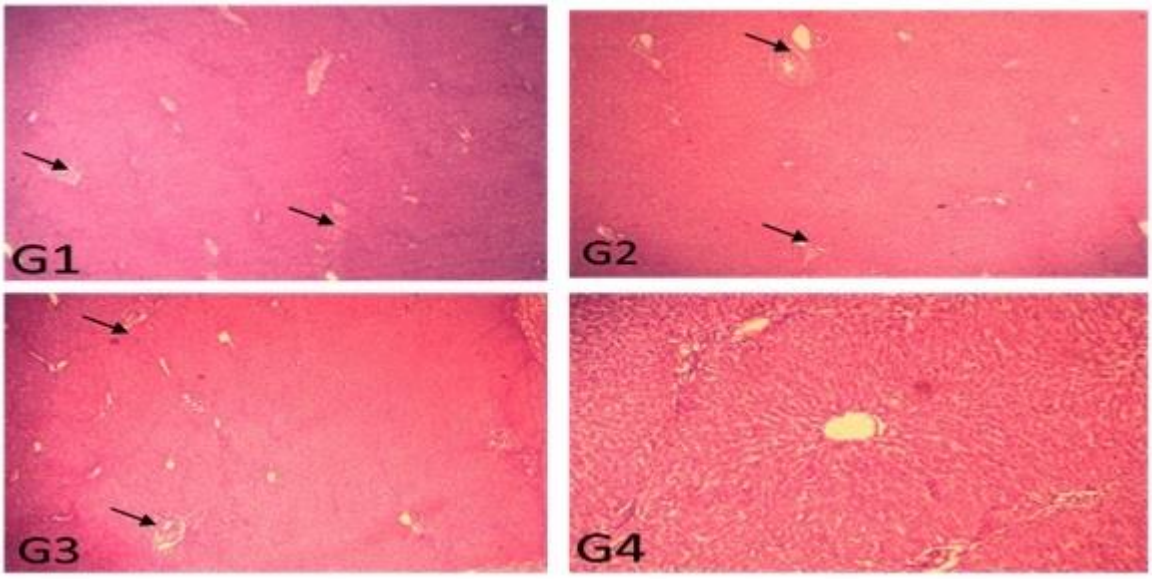

Figure 5. Histopathology of liver of goats infected with *Mycoplasma mycoides subspecies capri* at 40X, shows the congestion and hyperemia (arrow) at central portal vein which was prominent in G1 compared to G2 and G3 groups while G4 control group showed normal structure of liver.

Serological Findings:

The cELISA result shows the antibodies induced by CCPP infection and the positive samples which have the OD values more than 55, whereas the OD values between 15-30 are negative to CCPP infection. However, the positive control OD values were more than 90 OD values. The result shown the OD values after the infection were higher during the first week of the post infection (OD values in group G1 72, G2 76 and G3 80), which persistent during first week of infection and then gradually decrease after 2nd week of infection and lower OD values were seen in the 6th weeks of post infection (OD values decrease in groups G1 57, G2 60 and G3 66). These results showed the antibodies against *Mycoplasma mycoides subspecies capri* were recorded higher at first week and then gradually decreased to overcome the disease. However, the control group G4 (C) showed the OD values between 20 and 30 throughout the experimental period and it indicate the negative to the CCPP infection as shown in figure 6.

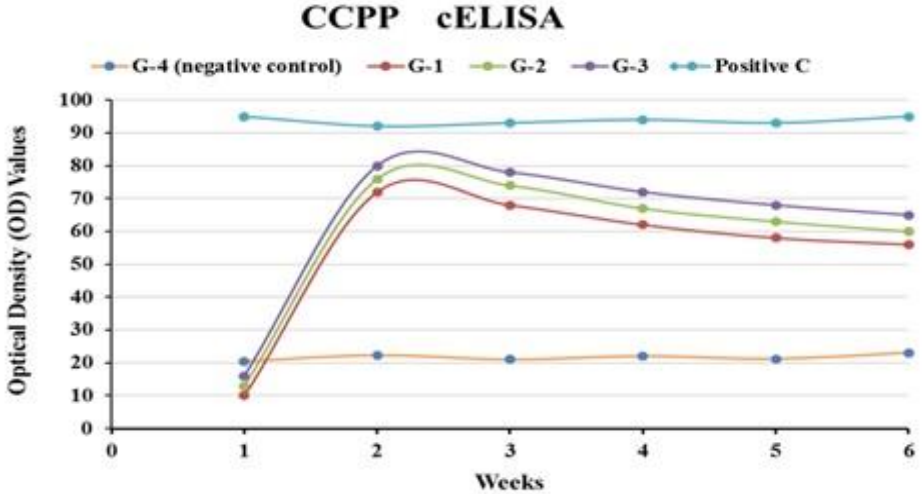

Figure 6. cELISA antibodies titer in group (G1, G2, G3 and G4) of goats during inoculation of the CCPP infection

Discussion

Contagious caprine pleuropneumonia (CCPP) is a major bacterial disease of caprine family; caused by *Mycoplasma mycoides subspecies capri*. CCPP is restricted to the pleural cavity, cause pleuropneumonia and significantly harm the goats either by reducing production or by death of animals. Present study revealed that the inoculation of disease in goats via the experiment caused high morbidity rate with no mortality. The animals of 1year old showed the severe symptoms such as lacrimation, lethargy and nasal discharge as compared to the animals of age 2 and 3 years. Similarly, clinical signs in goat such as cough and animal tend to lie down and the disease moves further, difficult breathing and progressively, the respiratory symptoms appeared prominent one with the nasal discharge continue with coughing [4, 5].

Gross pathological lesions with mild to moderate hemorrhages and purulent exudates accumulated in lumen of the trachea, in present study the lesions were seen on the mucosal surface of the rings of hyaline cartilages were striped with numerous streaks of hemorrhages,

Similar finding such as trachea was affected and kidneys swelling occurred in pyramids appeared [4, 11]. Correspondingly, it has been found that the CCPP produced by *Mmc* infected different organs including liver, kidneys. Kidney was seen normal in size but when incised kidney showed slightly congested and necrotic foci present at cortex regions [12]. However, medullary regions were slightly congested and showed edematous swelling [13]. In present study liver was found normal in size but the gall bladder showed enlargement in its size in all experimental groups. Liver was thickened and external surface was uneven and showed the strips line all over the liver which agrees with the [11]. Both of these reported enlargements of gallbladder which may be due to low temperature, low metabolic rate and GIT motility, which were only seen in this study.

In the current study, histopathological lesions were observed in lungs of all experimentally infected animals. The observed histopathological lesions in lungs were emphysema and atelectasis moreover, fibrin materials were present in the lumen of alveoli because of pneumonia and disperse at epithelium layer of alveoli and bronchi to cause sloughing off and disrupted together with polymorphic nucleated cells which were totally agreed with my results [12, 13].

The lesions observed in trachea were characterized by erosive and disrupted the lining of epithelial layer due inflammation. Moreover, leukocytic infiltration with hyperactive mucous secreting cell and haemorrhages in sub-mucosal layer were observed with in this study. Similar findings such as disrupted the lining of epithelial layer of trachea in CCPP were investigated previously [13, 14]. In existing study, glomerulonephritis, polymorphic nucleated cells at bowman capsules and collecting tubules were visualized in all experimental infected animals. Similarly, Abrasion at central portal vein of liver and hyperemia due to inflammation cause the rush up of polymorphic nucleated cells and focal necrosis were observed [14].

Present study represents that the antibody titer against *Mycoplasma mycoides subspecies capri* infected goats of different age groups were comparatively increased compared to control groups in three weeks of infection afterwards reduced gradually. However, young goats developed less immunity and associated with severe illness. The antibody titers rose from first week of post infection, similar to previous findings [15, 16], result state that the rapid development of clinical signs antibody titers are corelated with each other. Correspondingly, seroprevalence of CCPP was found higher in older goats of age 2 years (23.7%) than young goats of age 1 and less than years (10 and 8 %) [17]. Additionally, the less seroprevalence of about 4% CCPP was recorded in goats through cELISA [18]. However, CCPP infection in natural condition can spread rapidly and cause high morbidity rate. The animal infected with *Mycoplasma mycoides subspecies capri* infection triggers the antibody production; which were released in blood streams. The antibodies were found higher in old goats which represents the better immunity whereas young goads were associated with severe course of disease

Conclusion:
It was concluded that *Mycoplasma mycoides* subspecies capri (PG3) infects goats and cause CCPP infection. Gross pathological and histopathological findings revealed that the prominent changes were seen in younger animals than older ones. Nevertheless, cELISA revealed that CCPP antibody titers were higher in older animals as compared to young ones.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE
Not applicable.

HUMAN AND ANIMAL RIGHTS
No human subject was used in his study. All animal handlings were in accordance with the Institutional Animal Care guidelines of Sindh Agriculture University Tandojam.

CONSENT FOR PUBLICATION
Not applicable.

AVAILABILITY OF DATA AND MATERIALS
None.

FUNDING
None.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

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