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Clinical, Hemato-Biochemical, and Diagnostic Studies of Peste Des Petits Ruminants (PPR) in Yearling Goats of Basrah, Iraq

Ali Jarad[1], Mohanad H. Lafta[2], Kamal M. Al Saad[3*]
[1,2,3] University of Basrah, College of Veterinary Medicine, Department of Internal Medicine, Iraq
kamalsad58@yahoo.com

Abstract

Peste Des Petits Ruminants was detected in yearling goats by Eliza serodiagnosis at Basrah province of Iraq. One hindered fifty four (154) animals of 10-12 months old and of both sexes was examined and 25-yearling goats were considered as controls. Results indicated that diseased yearling goats show signs of anorexia and depression, Dehydration with a rough coat and sunken eyes, Diarrhea that mixed either with mucous or blood, Erosive mouth (erosive stomatitis)with frothy salivation, Conjunctivitis with obvious congestion of eyes and serious ocular discharge, and Pneumonia with abnormal lung sound on auscultation. Moreover, diseased animals show a significant rise in body temperature, and increase heart and respiratory rate. Leukocytopenia with lower lymphocyte count is a significant feature of hematological changes in infected goats in addition to increasing PCV and ESR values. A difference in clotting factor indices was also indicated in disease animals than in controls. Histopathological changes of diseased goats revealed a massive inflammatory infiltration (mostly lymphocytes) in the lamina properia of the villi mass as well as sloughing of the epithelium in the superficial intestinal layers.

Keywords: PPR, Yearling goats. Basrah, Iraq, Elisa.

Introduction

One of the common viral diseases affected small ruminants is the Peste des petits ruminants (PPR) which considered as an contagious disease responsible for high morbidity and mortalities that might be reached more than 50% (Constable et al., 2017). Particularly sheep and goats are the primary host (sheep are less susceptible than goats) ,However, it may infect other domesticated animals in a mild or sub-clinical form, such as camels and cattle.(Aguilar et al., 2020).

It has been documented that the first description of Peste des petits ruminants in Côte d'Ivoire in 1942, Nevertheless, the disease has different other synonymies such as The Kata syndrome of stomatitis and/or pneumo-enteritis, Goats plague as well as Ovine rinderpest, (Albina et al., 2013).

The virus (PPR virus) belongs to the subfamily morbillivirus of the family Paramyxoviridae and is described as a single-stranded negative-sense RNA virus (Amarasinghe et al., 2017), As, It was shown that, this virus encodes specific eight (8) types of genome proteins, named, The phosphor-protein (P), The nucleocapsid protein (N), The fusion protein (F), The matrix protein (M), The haemagglutinin protein (H), The polymerase protein (L), beside the more two nonstructural protein named V and C. (Couacy-Hymann, 2014), Moreover, The causative virus represent as one of specific serotype, However, is could be divided as four separated lines started from line I to line IV, mostly based on the protein (F) at a genetic grade (Anees et al ., 2013).

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It has been shown that the disease was distributed globally and spread among most Middle East countries, Africa, and Eurasia (Kozatm and Sepehrizadeh, 2017 ., Donduashvili et al., 2018).

Peste des petits ruminants appear in the form of per acute, acute, or maybe chronic and the most important clinical manifestations showed by diseased sheep and goats are pyrexia, ocular and nasal discharge (which mostly appears as purulent), erosive stomatitis which could be developed to necrotizing lesions, gastroenteritis manifested with severe diarrhea as well as bronchitis and pneumonitis (Diallo and Libeau, 2014). Further, The disease could lead to abortion in pregnant animals, terminated with neonatal death, triggering economic losses (Jones et al.,2016).

It was mentioned that Peste des petits ruminants infection could depend on different factors such as animal species, the severity and the virulence of the causative virus, the sex and age of the animals, the host immune status as well as the breed, Nevertheless, The previous exposure to the virus also played a good role,(Abubakar et al., 2016), On the other hand, The saliva of diseased animals, ocular and nasal discharges, as well as the fecal materials, was also have large quantities of the viral antigen which in turn increase the spreading of the causative virus in the environment (Lutomia, 2014; Constable et al., 2017).

Diagnosis of the causative PPR virus could be achieved by different laboratory methods, detection of the antigen, viral isolation, nucleic acid amplification as well as indirect detection and determination of the virus-specific antibodies, (Sakhare, 2019;Shahriari et al., 2019).

Information concerning Peste des petits ruminants in Basrah, Iraq is very scarce and little data and facts have been provided. Thereby, The current work was designed to study clinical, hematological, biochemical, and Eliza serodiagnosis of PPR In yearling goats.

Methods

This study was designed to examine (154) yearling goats whose ages range between 8-12 months, males and females, which are grazing outdoor in different areas during the day times and indoor feeding at night time at Basrah governorate, Iraq. Suspected goats show signs of anorexia, emaciation, Mouth erosive stomatitis with salivation, and diarrhea. Twenty-five (25) clinically healthy yearling goats served as a control group. Both groups are subjected to complete clinical and laboratory examinations according to standard techniques.

Sampling and examination of blood:-

Blood (12 ml)was drained from the jugular vein of each animal. Three milliliters (3ml) were mixed with Ethylene diamine tetra acetic acid (EDTA) for the determination of total RBC count, Hb, PCV, Total Thrombocytes count, Mean thrombocytes volume, thrombocytes distribution width, and Total leukocytes count using Hematological analyzer, from Genex / USA, Moreover, Differential leukocytes count was calculated according to Harvey, (2012) using Giemsa stain blood smears, Furthermore, Clotting time was estimated according to (Dayyal, 2016). Additionally, (3ml) of blood mixed with Trisodium citrated using plasma for evaluation of Prothrombin time and activated partial thromboplastin time according to

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(Biolabo / France). Moreover, ESR was estimated using the Wintrobe technique (Harvey, 2012).

Serological and biochemical examinations:-

Serum was extracted and used for confirmative diagnosis of PPR using Goat Peste des petits ruminants (PPR) ELISA Kit (Sandwich-ELISA/ SunLong Biotech Co.LTD).
According to manufacture intrusions, the result determine as follow:-
The effectiveness of the test:

- Value of **positive** control is ≥1.00, However, The value of **negative** control is ≤0.10.
- The critical value (cutoff) was calculated as follow :
  The critical value = The average value of the **negative** control + 0.15
- The negative judgment: If the OD value < cutoff, the sample is goat PPR **negative**
- The **Positive** judgment: if the OD value ≥ cutoff, the sample is goat PPR positive.

Moreover, Evaluation of ALT, AST, and CK. Was done via spectrophotometric biochemical analysis using a special kits from (BioAssay Systems/USA).

Histopathological Examination:

According to Maxie, (2016) specimens of 1cm³ were taken from the intestine and were kept in 10% buffered neutral formaldehyde solution immediately after removal, then after (72h) of fixation time, the specimens were washed with water (tap water), then, routine handling and processing were also done with a set of upgrading 70% alcohols to 100% of absolute alcohol for two hours to each concentration to eliminate the water from the processing tissues, Furthermore, the clearance process will be done using xylol, then, the specimens were embedded using liquid paraffin wax at 58°C on two stages, for manufacturing of specimens block which sectioned at 5 µm for all tissue. All the manufacturing tissues will be stained with the standard hematoxylin and eosin stain and examined under a light microscope.

Statistical analysis: -

Statistics were done according to (Leech et al., 2015). By applying (SPSS program) student *t*-test between diseased and control group, However, $P<0.05$ with Means and S.E of means was also adopted for statistical comparison.

Results

Results indicated that out of (154) suspected yearling goats with PPR (121) animals give positive results with the Eliza test with an infection rate of 78.5%. Moreover, diseased yearling goats show significant high values ($P<0.05$) of body temperature as well as respiratory and heart rate than the control group. table.1. In addition, The diseased animals showed different clinical signs represented by, Anorexia and depression (97.5%), Dehydration with a rough coat and sunken eyes (87.6%), Diarrhea that mixed either with mucous or blood (83.4%), Erosive mouth (erosive stomatitis)with frothy salivation (81.8%), Conjunctivitis with obvious congestion of eyes and serious ocular discharge (62.8%), and pneumonia with abnormal lung sound on auscultation (36.3%). table 2.

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Table 1: Vital signs of diseased yearling goats with PPR and the control group.

Vital signs	Control group n=25	Infected yearling goats n=121
Body Temperature / C °	38.11± 0.12	41.6± 1.56*
Respiratory Rate / min.	21.23 ±3.65	74.4 ±6.2*
Heart Rate/ min.	81.71± 3.54	115.6 ±12.2*

*** (P<0.05).**

Table 2: The clinical manifestations of infected yearling goats with PPR

Clinical manifestations	Infected yearling goats n=121	%
Anorexia and depression	118	97.5
Dehydration with a rough coat and sunken eyes	106	87.6
Diarrhea that mixed either with mucous or blood	101	83.4
Erosive mouth (erosive stomatitis)with frothy salivation	99	81.8
Conjunctivitis with obvious congestion of the eyes and serious ocular discharge	76	62.8
Pneumonia with abnormal lung sound on auscultation	44	36.3

Changes in the hematological analysis of diseased yearling goats and the control group revels Increase in (P<0.05) in PCV and ESR in diseased animals than that of controls group, Furthermore, Leukocytopenia as well as Lymphopenia (P<0.05) are also registered in infected yearling goats compared with the controls. table 3.

Table 3: Alterations in the hematological analysis of infected yearling goats and the controls

Parameters	Control group n=25	Infected yearling goats n=121
RBC $\times 10^6$	7.23± 1.11	7.67± 1.21
Hb g/dl	11.34 ± 1.22	11.37± 1.76
PCV %	32.2±1.44	40.23± 4.78*
ESR mm/24hr	5.34± 1.2	23.12± 5.54 *
TLC $\times 10^3$	12.14 ± 1.54	9.34 ± 3.67*
Nutrophiles /absolute	6875±634.11	6190.34 ± 364.22
Lymphocytes /absolute	5321 ± 122.23	3201.13± 135.41*
Monocytes /absolute	170 ± 43.13	172 ± 11.44
Esinophiles /absolute	201± 21.33	202.11± 44.18
Basophiles /absolute	30± 2.17	32.22± 14.55

*** (P<0.05).**

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Yearling goats infected with PPR disease show a significant difference (P<0.05) in their indices of the clotting factors, As, Total thrombocytes count was significantly lowered in diseased animals than the controls, Moreover, thrombocytes distribution width, mean thrombocytes volume, clotting time, the prothrombin time and the activated partial thromboplastin time was higher, Where, the increase in these values was significant(P<0.05) compared with the control group. table 4.

Table 4:Changes in clotting factor indices of yearling goats with PPR and the control group

Parameters	Control group n=25	infected yearling goats n=121
Thrombocytes count g/ L	488.51 ± 14.55	321.39± 44.11*
The thrombocytes distribution width %	15.23 ± 2.21	22.76 ±5.76*
The mean thrombocytes volume /fL	10.41 ±1.23	16.23 ±7.23*
Clotting time/ min.	3.22 ± 1.25	4.89 ± 1.35*
Prothrombin time/ sec.	13.23 ±1.77	19.42 ±3.11*
The activated partial thromboplastin time/sec.	52.34 ±3.12	67.22 ±6.54*

*** (P<0.05).**

The biochemical changes of diseased yearling goats and the control group indicate a significant difference, Where, values of ALT, AST, and CK rise (P<0.05) in infected animals than that of the controls . table. 5.

Table 5: Biochemical changes of infected yearling goats with PPR and the control group

Parameters	Controls n=25	Infected yearling goats n=121
ALT (U/L)	35.45± 3.78	101.66±15.67*
AST (U/L)	93.13± 4.46	135.11±28.25*
CK (U/L)	118.56± 5.21	175.34±18.71*

*** (P<0.05).**

A pathological autopsy was performed to the dead yearling goats
and the results revels an obvious mouth lesion in the form of erosive stomatitis, In addition, that, dead animals show signs of dehydration, emaciation with severe congestion of the intestine reflecting hemorrhagic enteritis, especially of the small intestine, Fig. 1, Moreover, Both lungs show severe congestion. On the other hand, Histopathological changes show a massive inflammatory infiltration (mostly lymphocytes) in the lamina properia of the villi mass as well as sloughing of the epithelium in the superficial intestinal layers, Figures (2 & 3).

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Figure 1: Dead yearling goat show signs of emaciation with severe congestion of intestine reflecting hemorrhagic enteritis

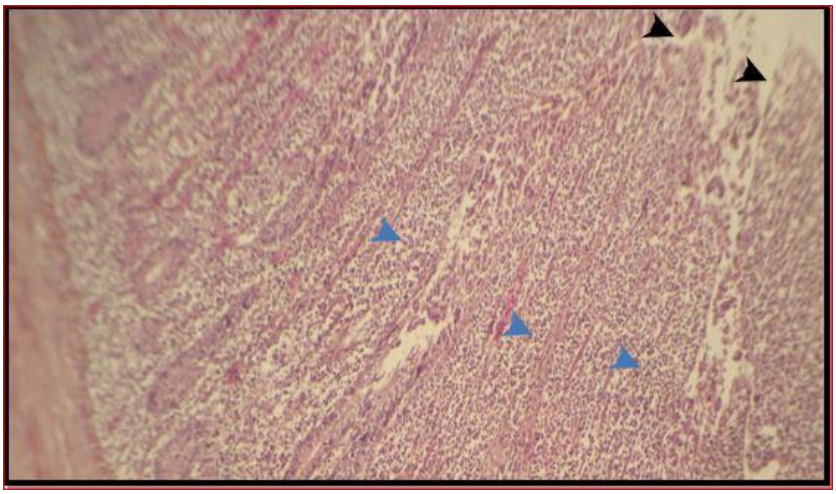

Fig (2) Section of yearling goat intestine with PPR showed massive inflammatory infiltration in the lamina properia of the villi (black arrowhead), sloughing of the epithelium in the superficial layer (red arrowhead) H&E 125X

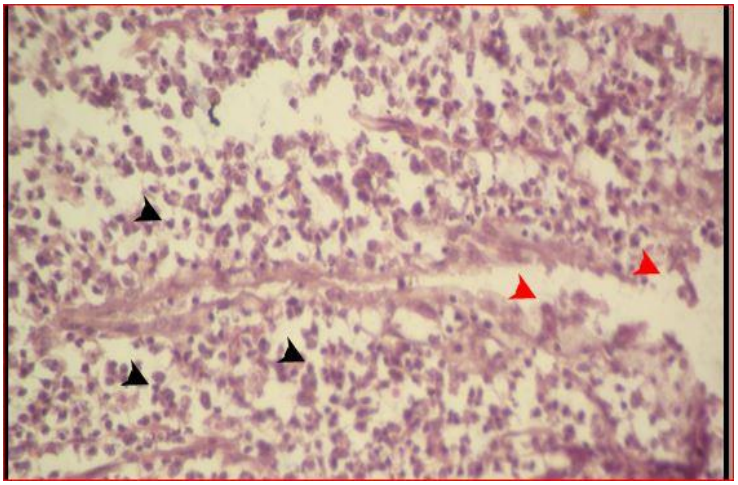

Fig (3) Section of yearling goat intestine with PPR showed that most of the inflammatory cells are lymphocytes in the lamina properia of the villi (black arrowhead), sloughing of the epithelium in the superficial layer (red arrowhead) H&E 125X

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Discussion

It has been shown that Peste des petits ruminants (PPR) are an acute highly contagious viral disease caused by a virus which belong to Morbillivirus with RNA and a single-stranded negative-sense characters. Moreover, goats are more affected than sheep (Abubakar etal., 2016). The disease causes a high infection rate which mostly terminated with death of diseased animals (Constable et al ., 2017).

Diseased yearling goats show various signs which , similar signs was also described by (Abdollahpour et al, 2006., Aytekin, 2008., Constable etal., 2017), However, (Baazizi etal, 2017) added that The clinical signs of this disease could be variable sometimes due to the genetic mutation tendency in the viral strains themselves, Therefore, different forms of the disease was mentioned by scientific workers, The peracute, acute, and the mild form depending on the severity of the disease, Further, it was indicated that The intensity of PPR in small ruminants might depend on several factors such as the species and animal breeds, the causative agent virulence, immune status of diseased animals, as well as animal age(Lutomia, 2014).

It was shown that the disease could have good relations with bovine rinderpest causative virus, reflecting a severely harmful effect on small ruminants(especially goats and sheep), Thereby, it will an accompanied by high morbidities and mortalities, (Hoffmann et al., 2012). Moreover, the Transmission of Peste des petits ruminants virus to clinical normal animals could be created through direct contact with the diseased animals as well as its infected materials mostly through the ocular, nasal, and oral discharges, However, It should also be remembered that the fecal materials of the diseased animals could carry a major amount of the causative virus, Furthermore, animal movements will have a big role in increase disease transmission (Abubakar et al., 2012).

In the current study, the Diagnosis of Peste des petits ruminants confirmed by the use of Eliza test. Where, it has been mentioned that this type of Sandwich Eliza can determine and figured the antigen in between the both antibodies layers (antibodies strata), Moreover, the targeted antigen should have at least two binding sites in order to engage with the specific antibodies. Furthermore, The Monoclonal and / or the polyclonal antibodies can be utilized for capturing and determine the targeting antibodies in this type of ELISA mechanism. Furthermore, The monoclonal antibodies will acknowledge a single epitope that permits little quantity of small differences in antigen, Further, A polyclonal is utilized as the hunt antibody to pull down the causative antigen as much as possible. This test can remove the sample purification step before analysis and it is considered more sensitive than the direct or indirect Elisa (Elgert, 2009., Rakhi et al, 2010).

It was documented that, The disease in Iraq was registered and diagnosed based on clinical symptoms and serological tests (Hoffmann, et al, 2012), However, An outbreak of the disease in sheep in sulaimania, Kurdistan region during 2012- 2013 was detected and diagnosed, where, It has been found that there is a close relationship between the causative virus in Iraq with the occurrence of disease outbreaks throughout the countries of the Middle East, Moreover, In Iraq, the vaccination trials are applied as one of control measure trials at more specific infected places, Nevertheless, using of the vaccine is based on Nig.75/1 in

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lineage II, and the identified virus isolates in Iraq depending on the phylogenetic tree and the analysis of sequence which are classified in lineage IV (Barhoom et al.,.,2013).

The Results of the current study indicated a significant increase in Packed cell volume parameters of infected yearling goats. This was in agreement with that indicated by (Aikhuomobhogbe and Orheruata, 2006., Constable et al., 2017). Who refer to the increase of body fluid losses which reflected by increase haemo-concentration state ended by dehydration, in diarrheic goats. In addition, ESR also rises significantly in disease yearling goats compared with the control group. The significantly high levels of ESR indicated in the present work of infected yearling goats might reflect the severe inflammatory processes that are caused by PPR, practically for the intestinal tissues of diseased goats, Hereby, if an inflammatory reaction started, Hyper-fibrinogenemia will be elevated in the blood circulation causes red blood cells to clump with fast sedimentation (Aziz et al, 2019).

Results of the present work found out a leukocytopenia which occurred because of a an clear decrease in the absolute number of lymphocytes(Lymphocytopenia) in diseased yearling goats, This results could be confirmed a lymphoid tissue lesion, reflected damage, and lowering lymphocytes which commonly indicated during the acute stages of the disease, However, previous studies assure that the PPR virus localizes in Peyer's patches, enterocytes, spleen, thymus, lymph nodes, tonsils as well as the liver (Aikhuomobhogbe and Orheruata, 2006., Kozatm and Sepehrizadeh, 2017). Moreover, (Jagtap et al.,2012) added that Leucopoenia in PPR will be noted mostly from the day four (4^{th}) after the infection and might back again depending on the disease progression. In addition, it was shown that CD4+ cells were lowered and depressed started from The fourth day 4 (4^{th}) post-infection and, remarkably, the ratio of CD8+luckocytes, although unchanged initially, rises within seven days post-infection (Baron et al., 2014., Herbert et al., 2014). On the other hand, it was mentioned that Immunosuppression is a common finding following morbillivirus infections in animals because a direct correlation between leukocytes infection rate and Immunosuppression has been indicated, Whereby, the depressed immune response might be due to inhibition of interferon production, change in cytokine response, inflammatory response suppression, leukocyte destruction, B cell destruction, and cell cycle disruption(Kumar et al., 2004., Lutomia, 2014).

Peste des petits ruminants could change the coagulation system of diseased animals which will enhance the development of disseminated intravascular coagulopathy, This could be supported by the haemoconcentration of the blood and the rises in the activators of coagulation mechanism together with a depression of coagulation inhibitor activities (Bick, 2003). It was shown that the common coagulation state in diseased yearling diseased goats with PPR is a hypercoagulable condition related to disseminated intravascular coagulation, However, the severity of this coagulopathy will depend on the intensity and duration of the intestinal lesions and the severity of inflammation(Constable et al., 2017).

In the current study, diseased yearling goats show a significant difference in the indices of clotting factors compared with the control group. Where decrease in total thrombocytes count and an increase in both Prothrombin time and The activated partial thromboplastin time could indicate the petechial hemorrhages seen on the mucus membranes of diseased

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goats, Further, This could refer to the libration of some endogenous mediators like a platelet-activating factor in inflammatory disorders(Pantanowitz, 2003).

Values of Alanine and Aspartate amino transferees and Creatine kinase were increased in diseased yearling goats compared with the controls, Similar results have also been obtained by(Abdollahpour et al, 2006., Aikhuomobhogbe and Orheruata, 2006., Aziz et al, 2019). (Kaneko, 2014), was mentioned that, any harmful effect to the skeletal or heart muscles as well hepatic tissues might result in a significant rise in the level of Aspartate and Alanine amino transferees due to the presence of those enzymes in different body tissues which are considered as abundant storage of enzymes liable to be liberated and rises during pathological damaged tissues, Moreover,(Teixeira and Borges, 2012) added that Creatine kinase (CK) will be liberated into the blood when muscle damage started, leading to increase it level in both serum and plasma. On the other hand, any changes in serum biochemical concentrations among PPR infection might be due to replication of the virus and damage to vital organs owing to inflammatory reactions in the liver, kidney, and lungs (Aziz et al., 2019).

An obvious pathological lesion was detected on postmortem and histopathological examinations of the dead yearling goats due to PPR in the current study, Similar results were mentioned by by (Kumar et al, 2002.,Constable., 2017), who described large necrotic and hemorrhagic lesions which were found in the digestive tracts, lymph nodes and spleen of both diseased domestic and wild small ruminants, As, oro-pharygeal, tonsilar, mandibular, and spleen ymph nodes considered as a good site for the viral replications, Moreover, Abdollahpour, (2006) added that, The intestine showed stunting and blunting of villi, with villous necrosis at the tips and erosion and infiltration of inflammatory cells in the lamina propria, However, congestion and obvious hemorrhages were also evident. It has been shown that dominant histopathological findings in PPR infection are the results of localization of the causative virus in the epithelial cells of the gastrointestinal tract causing blunting and stunted intestinal villi with congestion and desquamation, goblet cell hyperplasia, infiltration of inflammatory mononuclear cells in lamina propria as well as submucosal edema (Gitao et al., 2016), In addition, Squamous epithelial syncytia might also indicate in the digestive tract epithelium following PPR infection(Kumar etal., 2002).

Conclusion

It was concluded that the PPR of yearling goats reflected an important harmful disease-causing substantial losses, Therefore, early detection and complete control measure strategies are advised.

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