**CHANGES OF BLOOD AND BIOCHEMICAL PARAMETERS IN SHEEP BEFORE AND AFTER VACCINATION**

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**Clinical Report Submitted as per approved style and content**

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**March, 2014**

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| **Abbreviations** | **Elaborations** |
| **BLRI** | Bangladesh Livestock Research Institute |
| **CBC** | Complete Blood Count |
| **DLC** | Differential Leucocyte count |
| **TLC** | Total Leucocyte Count |
| **WBC** | White Blood Cell |
| **PCV** | Packed Cell Volume |
| **TSP** | Total Seram Protein |
| **Hb** | Hemoglobin |
| **MCHC** | Mean Corpuscular Hemoglobin Concentration |
| **RBC** | Red Blood Cell |
| **EDTA** | Ethylene di Amine Tetra Acetic-acid |

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ABSTRACT

The changes of blood parameter and total blood protein at before and after vaccination of Peste Des Petits Ruminants (PPR) in non-descriptive indigenous sheep were carried out in the district of Chittagong during the period from August to November 2013. The study against PPR with a commercial PPR VAC® was conducted on 20 sheep by inoculating @ 1.0 ml vaccine / animal subcutaneously. The haematological and biochemical levels were determined at before vaccination and at 7th day of after vaccination. The mean blood parameter and total blood protein value at before vaccination TEC 6.07± 0.36, TLC 9.23±0.52, Lymphocytes 60.5±1.97, Neutrophils 26.4±1.18, Eosinophils 8.25±1.17, Monocytes 4.6±0.49, Basophiles 0.7±0.13, PCV 27.85±1.66, Hb 7.13±0.34, TBP 6.48±0.15 and 7th day of vaccination in sheep TEC 6.08± 0.23, TLC 8.65±0.35, Lymphocytes 65.05±1.43, Neutrophils 22.95±1.10, Eosinophils 6.25±0.95, Monocytes 5±0.93, Basophiles 0.7±0.13, PCV 27.2±1.26, Hb 7.42±0.33, TBP 6.27±0.11 were insignificant (p > 0.05). Results of haematological examination showed that the PPR VAC® has no effects on TEC, TLC, Lymphocytes, Neutrophils, Eosinophils, Monocytes, Basophils, PCV, Hb and TBP at the day of 7th after vaccination. The results indicate that there was no significant variance in hematological and biochemical parameters in before and after vaccination.

KEYS WORDS: Sheep, PPR, Vaccination, hematology, biochemical analysis

CHAPTER I

INTRODUCTION

The domestic sheep is a multi-purpose animal used for wool and mutton production. Average population of sheep in Bangladesh is 3.07 million (DLS, 2010-11). Sheep are concentrated mainly in the northern and southern regions of the country. However, smaller populations are found all over the country. In the south they are kept mainly in the low lying areas, where their ability to survive under wet conditions gives them certain advantages over goats. Even though their economic value is lower than goats, they are kept for their meat and wool. Sheep and Goats are the natural host of PPR virus where as goats are more susceptible than sheep.

Haematological and biochemical indices of animals may give some insight as to their production performance of the West African Dwarf goats reports (Tambuwal et al., 2002) have documented. Peste des Petits Ruminants (PPR) is an acute, highly contagious, infectious and notifiable transboundary viral disease of domestic and wild small ruminants (Abubakar et al., 2008; Balamurugan et al., 2010; Khalafalla et al., 2010; Luka et al., 2011). Peste des Petits Ruminants virus (PPRV), the causative agent, belongs to the genus Morbillivirus of the family Paramyxoviridae (Olivier et al., 2011). Peste des petits ruminants (PPR), an office international des epizooties list a disease of sheep and goats caused by morbillivirus is characterized by high morbidity and high mortality rates resulting into heavy economical losses. The disease has been regularly reported from various parts of the world but mainly from Africa, Arabia, the Middle and Near East and the Indian subcontinent (Kataria et al., 2007). In Bangladesh, Dr Taylor identified the first PPR outbreak during 1993.Then it spread throughout the country and had divesting effects in organized Goat Farm .In Bangladesh it is thought that the diseases might have come from India. The outbreaks of PPR caused 74.13% morbidity and 54.83% mortality in Black Bengal goats in Bangladesh (Islam et al., 2001; Das et al., 2007). The seroprevalence of PPR has been reported to be 36.0% in sheep, 49.17% in goats and 19.05% in cattle from Bangladesh (Razzaque et al., 2004)

The disease is transmitted by direct contact involving secretions or excretions from infected animals to healthy animals in close proximity. Clinically, PPR is characterized by sudden onset of depression, fever, lacrimation, sores in the mouth, dyspnoea and coughing, foul smelling diarrhoea and death. Post-mortem findings, normally restricted to the alimentary tract, consist of extensive erosive stomatitis and heamorrhagic gastro-enteritis, and often include streaks of congestion along the folds of the mucosa resulting in the characteristic ’zebra-striped‘ appearance (Chauhan et al., 2009). There are several diseases of goat and sheep especially Pests des petits ruminants (PPR), which cause higher mortality and great economic losses, was one of the major constraints for not having a fully successful implementation of the said program. PPR is now endemic and epidemic in Bangladesh (Islam et al., 2001). These risk factors are often quite simple attributes of the sub-population such as the amount of movement, exchange of animals between households and flocks as a result of social practices and changes in economic conditions that exhibit seasonal patterns, distance from services, lack of large scale vaccination campaigns, altitude, season, and inter-species contact or interaction with wildlife (Radostits et al., 2007; Waret-Szkuta et al., 2008; Elsawalhy et al., 2010). PPR can cause serious economic losses due to its high morbidity rates that range from 50 to 90% and casefatality rates that reach 55 to 85% in goats, 10% in sheep, and 50% in camels (Radostits et al., 2007; Abubakar et al., 2008; Khalafalla et al., 2010; Luka et al., 2011). It is an acute highly contagious and fatal disease of small ruminants, caused by Morbillivirus close to Rinderpest virus and characterized by fever, necrotic stomatitis, gastro-enteritis and pneumonia.In unprotected animals the morbidity can be up to 100% and mortality may be 20 to 90% and in severe outbreaks with 100% case fatality particularly in goats (Samad, 2008). The evaluation of the levels of blood parameters and its fraction supply the information required to interpret the occurrence of dehydration, infectious, immune diseases and inflammatory responses. The disease is transmitted by aerosols between animals living in close contact. The determination of blood parametric values using laboratory procedure to aid the diagnosis of several diseases and dysfunction, as they provide reliable results and also inputs for research studies on nutrition, physiology and pathology (Bounous et al., 2000). The morbidity and mortality rates from PPR can be up to 100% in severe outbreaks. In milder outbreaks, morbidity is still high but the mortality rate may be closer to 50%. Severity depends upon the susceptibility of the population with young animals (4-8 months) usually having more severe cases. Poor nutritional status, stress of movement and concurrent parasitic and bacterial infections enhance the severity of clinical signs. The incidence of PPR in an endemic area is similar to that of rinderpest in that a low rate of infection exists continuously. When the susceptible population builds up, periodic epidemics occur with almost 100% mortality (Zewdie et al., 2009).

To overcome the problem, a homologous PPR live vaccine was developed, but the main disadvantage of this vaccine, like other Morbillivirus vaccine, is its poor thermal stability (Diallo, 2002). Although, lyophilization and stabilizing excipients have conferred marginal stability on many vaccines, the development of adequately thermostable vaccine has historically been hindered by the inherent lability and complex nature of the vaccine entities (Rexroad et al., 2002). Sheep vaccinated with escaped mutant virus were 100% sera converted following 15 days post vaccination and developed protective immunity against the field strains of PPR for more than 2 years. Thermostable PPR vaccine has been developed experimentally by scientists of BLRI and DLS (Chowdhury et al., 2004). Vaccination very few were on goats and hardly any on the effect of PPR vaccine that is widely used for the prevention and treatment of the PPR disease on these indices. This study therefore has as its objective to determine the effect of PPR vaccine on the haematological and blood biochemical values of WAD goats’ population under the extensive system of production. (Aikhuomobhogbe et al., 2008). Thermostable PPR vaccine has been developed experimentally by scientists of BLRI and DLS (Chowdhury et al., 2004)

Limited works have been done on this study. Keeping these things in mind present experiment was designed to achieve the following objectives:

* To study the changes of hematological and total blood protein of the PPR-vaccinated sheep.
* To have basic information on some blood parameters in PPR-vaccinated sheep.

CHAPTER II

REVIEW OF LITERATURE

The available literature related to the field of vaccination of PPR in sheep or goat is rather scarce, and only a few authors have looked into this topic. Some of them tested the concentration of blood biochemical indicators exclusively in Healthy goats and sheep and did not observe any significant changes from the physiological Values (Banik et al., 2008). This work is designed to contribute to the understanding of potential Hematological and Biochemical changes concentration of PCV, Hb, ESR, TLC, DLC, total proteins in the blood of sheep.

2.1. PPR: THE DISEASE

Geerts, (2009) reported Peste des petits ruminant (PPR) is an acute viral disease of small ruminants caused by a Morbillivirus and characterized by fever, oculonasal discharges, stomatitis, diarrhoea and pneumonia. The symptoms of PPR are very similar to those of rinderpest: fever, anorexia, depression, nasal and ocular discharges, difficult respiration, necrotic lesions on gum, lips and tongue resulting in salivation, erosions on the nasal mucosa and finally diarrhoea. The formation of small nodular skin lesions on the outside of the lips around the muzzle and the development of pneumonia during the later stages of the disease are frequently seen in PPR but not in rinderpest. Mild cases also occur with less marked clinical symptoms and absence of one or more of the cardinal features. Morbidity up to 100 % and mortality rates between 20 and 90 % are common, except in endemic areas or when mild disease occurs.

Samad, (2008) stated PPR virus was considered a variant of Rinderpest virus, specially adapted for goats and sheep that had lost its virulence for cattle. It is now known that the two viruses are distinct though closely related antigenically. Goats and sheep are the natural hosts of PPR, but goats appear to be more susceptible and suffer a more severe clinical disease than sheep. In endemic areas, goats more than 4 months up to 24 months age are affected.

Peste des petits ruminants (PPR) or goat plague is highly contagious viral disease of small ruminants such as sheep and goats (Dhar et al., 2002; Asim et al., 2009).

Raghavendra et al., (2000) stated the natural disease affects mainly goats and sheep, but it is usually more severe in goats where, it causes severe morbidity and mortality and is only occasionally severe in sheep.

Roger et al., (2001) reported that PPRV was also suspected to be involved in the epizootic disease that affected single humped camels in Ethiopia in 1995–1996.

Pawaiya et al., (2004) stated the clinical disease resembles Rinderpest in ruminants, which is acute, and after an incubation period of 3-6 days, the clinical symptoms become apparent, which include high rise of temperature, oral and ocular discharges, necrotic stomatitis, severe pneumonia, dyspnoea, coughing, enteritis, severe diarrhoea followed by death and substantial amount of virus is known to be present in the ocular and nasal secretions, as well as faeces of the infected animals.

Banik et al., (2008) reported that Peste des Petits Ruminants (PPR) is the French name of a Rinderpest-like disease in sheep and goats first described in Ivory Coast, West Africa in 1942. Many others prefer the appellation of stomatitis-pneumonia-enteritis complex disease, pseudo-rinderpest of small ruminants and kata. But official instances like FAO and OIE use the French name PPR. It is an acute highly contagious and fatal disease of small ruminants, caused by Morbillivirus close to Rinderpest virus and characterized by fever, necrotic stomatitis, gastro-enteritis and pneumonia. In unprotected animals the morbidity can be up to 100% and mortality may be 20 to 90% and in severe outbreaks with 100% case fatality particularly in goats.

2.2. MORPHOLOGY: THE VIRUS

Rashid et al., (2008) said that Pestes des Petits Ruminants (PPR) is an acute, contagious disease caused by a morbillivirus in the family Paramyxoviridae.

Barrett et al., (2005) reported that PPR viruses are enveloped, pleomorphic particles containing single stranded RNA, approximately 16 kb long with negative polarity as a genome and are classified under Paramyxovirus genus Morbillivirus.

Bailey et al., (2005) stated that PPR viruses are pleomorphic in shape and are enveloped. The genome is single stranded RNA, and is enclosed in a ribonucleoprotein core together with nucleocapsid protein. The genome is composed of 15,948 nucleotides, which is the longest of all the Morbillivirus members.

Mahapatra et al., (2006) reported the PPRV genome encodes six genes, each responsible for transcription of a single protein in the order N, phosphoprotein (P), matrix (M), F, hemagglutinin (H) and the large RNA polymerase (L).The P gene encodes for two additional non-structural proteins, C and V. The three viral proteins (M, F and H) are associated with the host-derived envelope. The matrix (M) protein is linked to the nucleocapsid and surface proteins (F and H).

Dhar et al., (2002) reported although, there is only one serotype of the virus PPRV isolation the basis of partial sequence analysis of the fusion (F) protein gene, can be grouped in to four distinct lineages. Lineage 1 and 2 are found exclusively in West Africa, whereas lineage 3 has been found in eastern Africa and Arabia. The fourth lineage is confined exclusively in the Middle East Arabia and Indian subcontinent. Except one isolate (TN92/1) from southern India, which belonged to lineage 3, all Indian PPRV isolates identified so far belonged to lineage 4 only.

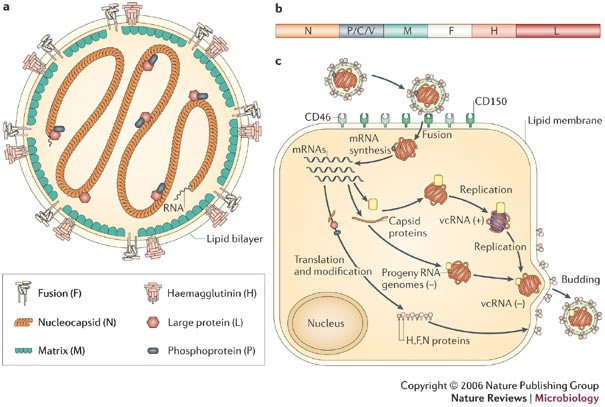


Fig: Morphology of Morbillivirus

2.3. DEFINITION OF BLOOD:

The Franklin Institute Inc., (2009) stated Bloods is fluid specialized connective tissue propelled through the closed channel popularly known as blood vessels having cellular and non cellular components, usually bright red in color and slights alkaline in chemical reaction (ph7.4) and carry oxygen as well as other nutrients to tissue and expel carbon dioxide with other tissue metabolic toxic products, remaining in circulation constantly. Blood is the fluid of life, transporting oxygen from the lungs to body tissue and carbon dioxide from body tissue to the lungs. Blood is the fluid of growth, transporting nourishment from digestion and hormones from glands throughout the body. Blood is the fluid of health, transporting disease fighting substances to the tissue and waste to the kidneys.

The American Heritage Medical Dictionary, (2004) stated the fluid consisting plasma, red blood cells, white blood cells, and platelets that’s is circulated by the heart through the arteries and veins, carrying oxygen and nutrients to and wastes materials away from all body tissues. One of the four humors of ancient and medieval physiology, identified with the blood found in the vessels and believed to cause cheerfulness, decent from the common ancestor, parental linkage”.

2.4. IMPORTANCE OF BLOOD PARAMETER

Tariq et al., (2014) reported the hematological feature has attracted many workers to look at these features in order to make clinical predictions of ht e health status of a particular animal, the blood picture changes with the advancement of the animal age and also varies with certain condition as stress .bacteria, viral intoxication. The blood of the domestic animals contains erythrocyte, non granular and granular leucocytes as well as platelets suspended in plasma. This case study signifies the diagnosis of Peste des Petits Ruminants (PPR) in a buck of discrete breed on the basis of clinical signs and hematology. Clinical examination revealed high fever, increase respiration, pulse rate and capillary refill time. Blood oozing lesions on gums and swollen lymph nodes were seen. Lymphopenia based on complete blood count (CBC) provided another clue to diagnose the disease as PPR on clinical basis. Treatment was done symptomatically for 7 days with amoxicillin, lactated ringer and somogel. Along with, a multivitamin powder was also advised to add in the feed to tone up the animal. Animal recovered completely on 10th day after visit.

Fasuy, A.O. (2007) stated that Blood with its myriad of constituents provides a valuable medium both for clinical investigation and nutritional evaluation of the organism. The ingestion of numerous dietary components has measurable effects on blood constituents. Nutrients levels in blood and body fluid might not be valid indication of nutrients function at cellular levels. They are considered to be the proximate measure of long term nutritional status. Consequently blood sampling for the assay of biochemical constituents and hematological traits are frequently employed in nutritional and clinical studies. Changes in the constituent’s components of blood when compared to normal value could be used to interpret the metabolic state of the animal as well as quality of feed.

Manoharan et al., (2005) reported that Haemagglutination activity of PPR virus was the highest using buffer pH 6.8-7.0 and lowest at pH 8.0 for optimal result of the HA test for PPR virus. HA titer of tissue suspensions at optimum buffer pH was 16 which fall in the titer range (16 to 64).

Aikhuomobhogbe and Orheruata, (2006) showed in his study the increase in the value of total blood protein (TBP), albumin (ALB) and globulin (GLO) concentrations at pre and post vaccination were not consistent. The correlation between PCV and Hb concentration was high, positive and highly (P<0.001) significant. Low (r = -0.345), negative and significant (P>0.05) correlation was obtained between post-vaccination packed cell volume (PCV).

Aytekın et al., (2011) reported Peste des petits ruminants virus infection occurs most commonly in Asia and African countries however it may spread through other parts of the world 1-7. Attention should be given to this disease because it has very high morbidity and mortality rate and causes heavy economic loss. For these reasons, this severe disease must be investigated carefully with multiple parameters. To describe the disease better, to make an accurate and fast diagnosis and for appropriate treatment (symptomatic) and protection against PPRV infection; clinical, hematologic, biochemical and histopathologic findings should be examined carefully.

2.5. SEROLOGICAL TEST OF PPR

O.I.E. Manual, (2013) confirms the virus serologically by detecting their specific antibodies, monoclonal antibody based ELISAs have been developed. Alternatively, a polymerase chain reaction (PCR) technique using F-gene primers has been developed. Among the various techniques developed for the detection of PPRV, this PCR technique developed using F-gene primers has been the most popular and highly sensitive tool so far for diagnosis of PPR. A list including all the types of tests available for peste des petits ruminants is given below Competitive ELISA, Virus neutralization, RT-PCR, Real time RT-PCR, Virus isolation in cell culture, Immuno-capture ELISA, Agar gel immunodiffusion and counter immune electrophoresis.

2.6. PPR VACCINE

Chowdhury et al., (2004) reported both conventional and thermostable PPR vaccines induced a partial seroconversion and in both cases the sero-positivity also declined with time. Normally, homologous PPR vaccine attenuated after 63 passages in Vero cell has been reported to have produced a solid immunity in 98% of the vaccinated animals for the whole economic life around 3 years. The PPRV homologous vaccine was found to be safe under field conditions even for pregnant animals. Thermostable PPR live homologous vaccine was developed by Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, in collaboration with and Livestock Research Institute (LRI), Mohakhali, Dhaka.

Sil, (2000) said two candidates of homologous Vaccine have already been developed are in Ethiopia of Africa & another in Bangladesh.

Hegde et al., (2009) reported that vero cells grown in Minimum Essential Medium (Gibco) with six percent fetal calf serum (Biological Industries, Israel) were infected with vaccine virus strains at a multiplicity of infection (m.o.i) of 0.01 per cell at a cell density of 1 X 105 cells/ml of the medium by co-cultivation method. The coverslip cultures were also infected. The infected cultures were stained with haematoxylin & eosin and May & Grunwald’s Giemsa stain at periodic intervals and cytopathic effect of PPR virus was observed.

Banik et al., (2008) reported that the clinical, haematological, biochemical and antibody levels were determined at pre-immunization and at 7th, 14th and 21st day of postimmunization. The mean antibody titre at 7th, 14th and 21st day of post-immunization in sheep (38.28 ± 4.34, 58.63 ± 3.60 & 68.27 ± 3.09) and goats (49.84 ± 4.37, 63.23 ± 3.64 & 76.60 ± 4.07) were significantly (p < 0.01) increased in comparison to the respective pre-immunization mean titre of sheep (20.0 ± 6.78) and goats (14.00 ± 6.96). Results of haematological examination showed that the PPR VAC® has no effects on haemoglobin (Hb), erythrocyte sedimentation rate (ESR), packed celled volume (PCV) and total erythrocyte count (TEC). The TLC was markedly increased at all the stages of postimmunization in both sheep and goats but significantly (p < 0.05) increased at 21st day (10.08 ± 1.55 103 / mm3) in sheep and at 14th day (14.76 ± 0.84 103 / mm3) in goats in comparison to pre-immunization values of sheep (7.95 ± 0.97 103 / mm3) and goats (9.00 ± 1.28 103 / mm3). No distinct difference was observed on rectal temperature, pulse and respiratory rate between the pre- and post immunization values which indicates PPR-VAC® has no clinical effects in vaccinated animals. No significant differences on calcium, albumin, total serum protein (TSP) and glucose levels were observed between the pre- and post-immunization values in both the sheep and goats. But the TSP in both the sheep 8.43 ± 0.69 g/dl) and goats (8.26 ± 0.50 g / dl) at 21st day of post-immunization increased insignificantly (p > 0.05) in comparison to the pre-immunization values of sheep (7.66 ± 0.79 g / dl) and goats (7.89 ± 0.92 g / dl).

Rahman et al., (2011) stated that two types of PPR vaccine, conventional PPR vaccine developed by BLRI and produced by Livestock Research Institute (LRI), Mohakhali, Dhaka and thermostable vaccine experimentally prepared by BLRI, Savar, Dhaka were used in this study. Conventional PPR vaccine was collected from LRI from a freshly prepared batch. Proper cold chain was maintained during transportation of the vaccine.

CHAPTER III

MATERIALS AND METHODS

3.1. STUDY AREA

The study was conducted at the Khulshi Thana in Chittagong District.

3.2. DURATION OF STUDY

The study was conducted from August 2013 to November 2013.

3.3. DATA COLLECTION

20 Sheep were selected from Chittagong city. The sheep grazed mostly on natural pasture of mainly grasses, herbaceous forage legumes and consuming kitchen and food processing waste in the locality. No conscious efforts were made by the farmers to provide supplementary feed. The sheep were identified with permanent marking in ear prior to blood collection. Data were collected on only non-pregnant sheep to reduce error that may be introduced as a result of pregnancy status.

3.4. IMMUNIZATION STUDIES

Twenty non-descriptive indigenous sheep were used for study. Those were immunized with PPR-VAC® (LRI, Mohakhali, Dhaka) @ 1.0 ml / animal subcutaneously

3.5. LABORATORY TESTING

3.5.1. COLLECTION OF SAMPLE

Approximately 5ml of blood was collected aseptically from the jugular vein of each 20 PPR infected goats. 3ml blood was kept in a vial containing Ethyline diamine tetra acetic acid (EDTA, 1mg/ ml) & 2ml was kept in another vial for smooth coagulation. Blood samples were transported to the Physiology laboratory of Department of Physiology, Biochemistry and Pharmacology, Chittagong Veterinary and Animal Sciences University (CVASU) within half an hour and kept in the chiller part of refrigerator for Total Leukocyte Count (TLC), Differential Leukocyte Count (DLC), Erythocyte Sedimentation Rate (ESR), Packed Cell Volume (PCV) and for serum preparation.

3.5.2. SERUM SAMPLE PRESERVATION: 2ml of coagulated blood was kept overnight in refrigerator for collecting the serum. The serum samples obtained after centrifugation and stored at 2ºC in refrigerator for further biochemical test.

3.5.3. HEMATOLOGICAL EXAMINATION:

Different hematological parameters were studied according to the methods described by Alcon (2000). The detail of the parameters studied as follows:

3.5.4. TOTAL LEUCOCYTE LOUNT (TLC)

PROCEDURE: A dry and clean counting chamber was placed under the microscope. There after the chamber was examined under low power objective without the cover slip in order to understand the ruling. Blood mixed properly with EDTA was sucked into the WBC pipette upto the mark 0.5 keeping the pipette nearly horizontal. The diluting fluid was sucked into the pipette until the mixture of blood and the fluid reaches 11 marks above the bulb. The pipette was rotated and inverted several times to ensure thorough mixture blood with diluting fluid. A few drop of mixture was discarded from the pipette and wiped out its tip. A small drop of diluting fluid mixture was allowed to form at the tip of the pipette. The tip was placed on the surface of the chamber touching the edge of the cover slip at an angle of 45 to the horizontal. The dilute blood was allowed to flow evenly and slowly under the cover slip by capillary action. There should be no overflow into the central or side trenches as the excess fluid will lift up the cover slip and gives false high count. A few minutes were spent before the count to allow the corpuscles to settle.

3.5.5. WBC COUNTING: The leucocytes were counted in four large squares (each having 1 sq. mm is divided into 16 small squares) at the four corners of the counting chamber. This means that the white blood corpuscles in 64 small squares were counted. The white blood cells were appeared as round faintly dark and refractive dots under low power where as high power the stained nucleus were visible with clear granular cytoplasm around it. In counting the cells which touched the left hand line or upper line of the square were taken to be within that square and those which touched the lower and right lines were omitted as outside the square. This technique was followed to avoid the duplicate counting and counting was started from upper left chamber.

CALCULATION FACTOR

1. Area of chamber: 4 sq. mm
2. Depth of chamber: 1/10 mm
3. Dilution obtained: 1:20 =1/20

TOTAL VOLUME: 4\*1/10\*1/20 cu. mm

=1/50 cu. mm

Suppose 1/50 cu. mm contains x number of cells

So, 1 cu. mm contains x\*50 number of cells.

The total numbers of cells were multiplied by the calculation factor. i.e. 50 and expressed the result in thousand/cu.mm by dividing the total number of cell by 1000.

3.5.6. DIFFERENTIAL LEUKOCYTE COUNT (DLC)

PROCEDURES: Several clean grease free slide with smooth unbroken end were selected, then a drop of blood was placed at right end of the slide. The slide was taken on a piece of paper on the table holding firmly by the force or thumb finger on left hand. The even edge of the second slide was placed near the drop of blood towards the middle of slide. The second slide was drawn towards the drop of the blood at an angle of 450 and the blood was spread along the edge. Keeping the second slide in the same angle a quick even push was given with a uniform force toward the other end of slide. When the blood film dried, it was placed in the staining rack. 8-10 drops of Wright’s stain was poured over the slide to cover the blood smear and allowed to stain for 2 minutes. Distilled water above double quantity of stain was added and mixed by blowing pipe and allowed to stand for 5 minutes. Then the film was washed with distilled water without much distribution the slide till the slide film become pinkish. The slide was placed against the support and allowed to dry in air.

3.5.7. WBC COUNTING: The slide was placed on a fixed place and a drop of immersion oil on the slide. Then the cells were identified by using high power objective 100x following parallel stripe method. The counting was continued till 100 cells were counted. Different types of cells were counted by tally method.

3.5.8. Erythrocyte Sedimentation Rate (ESR) by Wintrobe’s Method

PROCEDURE: With the help of special loading pipette the Wintrobes tube was filled with blood starting at its bottom and withdrawing the pipette as the tube was filled from below up-wards. The tube was filled up to the mark 10 air bubble removed from the top of the column of blood so that it stood exactly at 10. The tube was placed in the rack in vertical portion. The reading was taken after one hour by noting down the level of lower end of the clear plasma.

3.5.9. DETERMINATION OF PACKED CELL VOLUME (PCV)

PROCEDURE: With the help of special loading pipette the Wintrobes tube was filled with blood starting at the bottom and withdrawing the pipette as the tube was filled from below upwards. The tube was filled up to the mark 10; air bubble was removed from the top of the column of blood. So that it would be stood exactly at 10. The Wintrobes tube was centrifuged at a rate of 3000 rpm for about 30 minutes. After 30 minutes the tube were taken out of centrifuged machine and PCV was read directly from the calibrated on the right side of the tube. The result was expressed in percentage.

3.5.10. BIOCHEMICAL EXAMINATION:

Total Protein (TP) was determined by automated biochemical analyzer (Humalizer 3000) established in Physiology laboratory of CVASU

Photometric Colorimetric Test for Total Proteins (Biuret Method)

Contents

|  |  |  |
| --- | --- | --- |
| Reagents: | 4×100 ml or 1×1000 ml Color reagent | |
|  | Sodium hydroxide | 200mmol/l |
|  | Potassium sodium tartrate | 200mmol/l |
|  | Copper sulfate | 12 mmol/l |
|  | Potassium iodide | 30 mmol/l |
|  | Irritant R 36/38 |  |

|  |  |  |
| --- | --- | --- |
| Standard | 1×3 ml Standard | |
|  | Protein | 8g/dl |
|  | Or | 80g/l |
|  | Sodium azaid | 0.095% |

Assay

Wavelength: Hg 546 nm, 520-580 nm

Optical path: 1 cm

Temperature: 20---250C

Measurement: Against reagent blank

Only one reagent blank per series was required.

PIPETTING SCHEME: 1000 μl of reagent was taken in previously marked three eppendorf tubes for reagent blank, sample and standard separately by micropipette. 20μl of sample and standard were added in the previously marked eppendorf tube, respectively. Reagent blank eppendorf tube only contain reagent. Sample and standard for total protein were mixed separately in each eppendorf tube and incubated for 10 minutes at 250C. Absorbance of the sample and standard were measured against the reagent blank within 30 min (ΔA) λmax in 546 nm by spectrophotometer of the Humalyzer 3000®.

|  |  |  |
| --- | --- | --- |
| Pipette into cuvettes | Reagent blank | Sample/Standard |
| Sample/Standard | ------ | 20μl |
| Reagent | 1000μl | 1000μl |
| Mixed and incubated for 10 min. at 20---250C. Absorbance of the sample and standard were measured against the reagent blank within 30 min(ΔA) | | |

CALCULATION OF THE PROTEIN CONCENTRATION

WITH STANDARD-

C=80× ΔA sample/ ΔA standard [g/l]

3.8. STATISTICAL ANALYSIS

After collection of data, the collected data were edited to summarize them meaningfully and were processed by using Microsoft excel 2007. The data were imported to STATA.11 statistical software for analysis. T-test was done to compare each variable. The results were compared at 5% level of significance.

CHAPTER IV

RESULTS AND DISCUSSION

PPR is very contagious and fatal disease of small ruminants specially goat and sheep. PPR disease can be prevented by vaccination. The present study is done to know the changes of blood and biochemical parameter of sheep.

Table: different blood parameters of PPR vaccinated and non-vaccinated sheep

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Variable | Category | Mean | 95% confidence interval | P-value |
| TEC (106 /mm3) | Before vaccination | 6.07± 0.36 | 5.31- 6.82 | 0.98 |
| After vaccination | 6.08± 0.23 | 5.59- 6.56 |
| TLC (103 /mm3) | Before vaccination | 9.23±0.52 | 8.14-10.32 | 0.34 |
| After Vaccination | 8.65±0.35 | 7.92-9.37 |
| Lymphocyte % | Before vaccination | 60.5±1.97 | 56.37-64.63 | 0.096 |
| After vaccination | 65.05±1.43 | 62.05-68.05 |
| Neutrphil % | Before vaccination | 26.4±1.18 | 23.94-28.86 | 0.3 |
| After vaccination | 22.95±1.10 | 20.65-25.25 |
| Eosinophil % | Before vaccination | 8.25±1.17 | 5.81-10.69 | 0.22 |
| After Vaccination | 6.25±0.95 | 4.25-8.25 |
| Monocyte % | Before vaccination | 4.6±0.49 | 3.57-5.63 | 0.73 |
| After Vaccination | 5±0.93 | 3.05-6.94 |
| Basophil % | Before vaccination | 0.45±0.15 | 0.13-0.77 | 0.24 |
| After Vaccination | 0.7±0.13 | 0.43-0.97 |
| TSP (g / dl ) | Before vaccination | 6.48±0.15 | 6.16-6.8 | 0.29 |
| After Vaccination | 6.27±0.11 | 6.04-6.49 |
| PCV ( % ) | Before vaccination | 27.85±1.66 | 24.38-31.32 | 0.72 |
| After Vaccination | 27.2±1.26 | 24.56-29.84 |
| Hb (gm %) | Before vaccination | 7.13±0.34 | 6.43-7.83 | 0.60 |
| After Vaccination | 7.42±0.33 | 6.73-8.10 |

Hematological studies

Table shows that Total Erythrocyte Count (TEC) was found 6.07± 0.36 million / ml before vaccination and which was found 6.08± 0.23 million / ml after vaccination of sheep blood. There was no significant (p>0.05) variation in the TEC content of blood of sheep at before and after PPR vaccination. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observe insignificant (p>0.05) values in West African Dwarf Goat.

Total Leucocytes Count (TLC) was found 9.23±0.52 million / ml before vaccination and which was found 8.65±0.35 million / ml after vaccination of sheep blood. The result was insignificant (p>0.05) variation in the TLC content of blood of sheep at before and after PPR vaccination. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observe insignificant (p>0.05) values in West African Dwarf Goat.

Total lymphocytes were found 60.5±1.97 million / ml before vaccination and which was found 65.05±1.43 million / ml after vaccination of sheep blood. The result was no significant (p>0.05) variation. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. But (Aikhuomobhogbe and Orheruata, 2006) showed significant (P<0.05) influence on post-vaccination at 14th day of vaccination. It may be occurred due to locality and environmental effect.

Neutrophils were found 26.4±1.18 million / ml before vaccination and which was found 22.95±1.10 million / ml after vaccination of sheep blood. Insignificant (p>0.05) variation was found. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observed insignificant (p>0.05) values in West African Dwarf Goat.

Eosinophils were found 8.25±1.17 million / ml before vaccination and which was found 6.25±0.95 million / ml after vaccination of sheep blood. There was no significant (p>0.05) variation in the Eosinophils of blood of sheep at before and after PPR vaccination. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observed insignificant (p>0.05) values in West African Dwarf Goat.

Monocytes were found 4.6±0.49 million / ml before vaccination and which was found 5±0.93 million / ml after vaccination of sheep blood. The result was insignificant (p>0.05) variation in the Monocytes of blood of sheep at before and after PPR vaccination. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observed insignificant (p>0.05) values in West African Dwarf Goat.

Basophils were found 0.45±0.15 million / ml before vaccination and which was found 0.7±0.13 million / ml after vaccination of sheep blood. Insignificant (p>0.05) variation was found. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observed insignificant (p>0.05) values in West African Dwarf Goat.

Packed Cell Volume was found 27.85±1.66 gm / dl before vaccination and which was found 27.2±1.26 gm / dl after vaccination of sheep blood. Insignificant (p>0.05) variation was found. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observed insignificant (p>0.05) values in West African Dwarf Goat.

Hemoglobin was found 7.13±0.34 gm / dl before vaccination and which was found 7.42±0.33 gm / dl after vaccination of sheep blood. The result was insignificant (p>0.05) variation in the Hemoglobin of blood of sheep at before and after PPR vaccination. . Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observed insignificant (p>0.05) values in West African Dwarf Goat.

Biochemical studies

Total Blood Protein was found 6.48±0.15 gm / dl before vaccination and which was found 6.27±0.11 gm / dl after vaccination of sheep blood. The result was insignificant (p>0.05) variation in the Total Blood Protein of sheep at before and after PPR vaccination. Results obtained compared favorably with reports in literature (Banik et al., 2008). These authors reported similar values for Sheep as was obtained in this study on sheep. (Aikhuomobhogbe and Orheruata, 2006) also observed insignificant (p>0.05) values in West African Dwarf Goat.

CHAPTER V

CONCLUSION

The study indicates that the hematological and biochemical profile of sheep before and after vaccination of PPR. There are some changes occur in parameter before and after vaccination of PPR. But statistically these values are not significant (p>0.05). There was some limitation in my study such as small sample size and time limitation. I recommend further study on changes in blood and biochemical parameter in sheep before and after PPR vaccination.

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