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Identification of immune responsive biomarkers in saliva; Proteomics analysis

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ABSTRACT
The saliva samples were examined for its effectin immune responses to animals. Saliva samples were collected on day 7, 15, 45 and 90 days, starting by birth. Investigations were conducted within twenty-five buffalo calves at an early age to identify immune responsive salivary proteins by 2 Dimensional gel electrophoresis (2-DE) procedures with a collaboration with quantification, one dimension i.e. sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and protein identification by Mass Spectrometry (MALDI-TOF-TOF). Mascot was used for database search against SIB based ExPASy basis searches of 2D gel spots at day 45 and 90 days and revealed two proteins namely polymeric immunoglobulin receptor and serum albumin, respectively. These proteins were differentially expressed in saliva and obtained a significant score ($p<0.05$) of matched proteins to select protein entry.These proteins were appeared to provide a clue to better immunity of oral mucosa and animal health. The purpose of the study was to identify the biomarkers related to immunity and to give a novel area into prevention of disease, the health status of growing buffaloes.
Key words-*Buffalo calves, Immunity, Salivary proteomics, 2-DE.*

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INTRODUCTION

Buffaloes are the backbone of rural economy, dairy and meat industry. Traditional approaches to buffalo rearing, in contrast of functional endpoint from growth and reproductive performance to animal health and welfare, have shifted toward feeding on health and well-being, designer food, metabolic modifiers, regulation of gene expression, environmental quality, food safety and public health etc. To sustain the animal productivity we should have to ensure the health of the animal.

In the present study, saliva was access to diagnose and observe such conditions related tovarious diseases that accurately reflect normal and abnormal status in animals.The saliva sampling benefited as compared with other body fluids and it provides an attractive alternative to blood, serum or plasma because it can be non-invasively collected.This also explains the expanding research field in assay developments and technological advancements for the detection of various salivary biomarkers to improve clinical diagnosis, management, and treatment.

Saliva is secret mainly by paired salivary glands i.e. parotid, submandibular and sublingual and various minor glands (3). Saliva is watery fluidcomprises 99.5% water, electrolytes, glycoproteins, enzymes (7) andantimicrobial compounds like immunoglobulin, lysozymes (4). Earlier examinations suggested that saliva contains a wide-ranging of proteins giving information about immunity. In recent years some studies on salivary proteomics have performed in animals as well as in human beings, those having diagnostic utilities (6). Immunological proteins like defensin etc. were also found in saliva. Defensinplays a key role in defense against microorganism infections caused by food or drinks (1). An early diagnosis of some immune responsive diseases by means of salivary biomarkers may indicate about the pathogenicity of micro-organism and resistant power of animals.

MATERIALS AND METHODS

Sample Collection :Salivary samples were collected from twenty-five female buffalo calves with the help of pasture pipette at scheduled days 7, 15, 45 and 90 days, starting by birth. Collected samples were immediately kept at deep freezing (-80˚C), after centrifugation at 12000 rpm. The protein concentrations of individual saliva were measured against standard of bovine serum albumin in the concentrations of 0.125, 0.25, 0.50, 0.75, 1.0, 1.5 and 2.0 µg/µl.A small sample volume (5µl) and Bradford reagent (250µl) were used.However, saliva with known concentration was used for the further analysis.

1D gel electrophoresis : To minimize individual errors, pooling of samples from individual calves was practiced in proteomics investigations. Onwards, 1D SDS-PAGE was performed to determine the quality of proteins through standard technique (Bio-rad, USA), where proteins were separated on the molecular weight basis and visualized as protein bands.

2D gel electrophoresis : Saliva samples (100µg protein) were rehydrated by 2D Clean-Up Kit™ to remove contaminating substances such as nucleic acids, salts, lipids or detergents. After solubilizing, proteins were loaded into IPG strips (pH 3-10 linear). The first dimension isoelectric focusing was performed to separate the proteins on the basis of their isoelectric point at definite pH in an electric field of 15000 volt-hours for five hours and kept immediately at -80˚C for further analysis. After performing isoelectric focusing, second dimension separation wasperformed; IPG strips were equilibrated in equilibration buffer. IPG strips loaded on 12% SDS polyacrylamide gel at 125 volts to separate proteins on the basis of their molecular weight. After electrophoresis of SDS PAGE, silver staining was performed to visualize the protein spots on polyacrylamide gel. Protein spots were scanned and analyzed by densitometric software (PD Quest 8.0.1.Bio-Rad USA) to identify differentially expressed proteins. The investigated ratio in percent volume of each spot gives an idea of the change that occurred in differentially expressed protein. Selected spots were fragmented by peptide mass finger printing, onwards mass spectrometry was performed using a MALDI-TOF-TOFUltraflex III mass spectrometer. Protein identified as peptide masses against SIB based ExPASy and NCBI data basis. Finally obtained a significant score ($p<0.05$) of matched proteins to select protein entry.

RESULTS AND DISCUSSION

In the present studytwo proteins (significant $p<0.05$)*viz.* serum albumin and polymeric immunoglobulin receptor (plgR)have mascot score 83 and 80 respectively, wereexpressed. These proteins were matched with species *Bubalus bubalis* (Domestic water buffalo) proteins from SIB based ExPASy and NCBI databases (Tables1). Many researchers have found the immune-specific role of these proteins. In the study,serum albumin protein was expressed in saliva at 90 days of age, indicating to be an important role in immunological as well as a pathological condition at anearly age. (2) observed that low protein, caloric diet and some chronic diseases lead to inflammation which, ultimately affects the serum albumin level. Hence, the study may beconcluded as, serum albumin like other immunogenic protein is responsible for the defensive mechanism in the body during the early phase of life.

Polymeric immunoglobulin receptor (plgR) proteins expressed in mucosal lining and secreted into the saliva. In present study this protein was expressed significantly ($p<0.05$) at age of 45 days in buffalo calves. Some studiesdemonstrated that plgR having role in immunoglobulin (IgA) transport and provoke the immune response to various diseases. However, the level of IgA level was found greater in the sublingual gland than in the submandibular (8). On the other hand role of IgA in the defensive deeds on the mucosal layer of epithelial cells of the gut and urinary tract, which prevent pathogen adhesion. In future prospectives, these proteins may have greater potential for disease diagnosis.(5) found the defensive effect of plgR in the intestine and gut via secretion of intestinal secretory immunoglobulins. These plgR and SIgA are responsible for the regulation and activity of the microbiota in the gut. If a deficiency inplgR expression occurs, it can lead to an imbalance in the gut microbiota, which is inflammatory. Meanwhile, this salivary polymeric immunoglobulin receptor has been suggested in the diagnosis for the disease of thegut.

CONCLUSIONS

The present study of saliva proteome suggested a significant ($p<0.05$) correlation between expressions of salivary proteins and immune status. The early expression of these two immunogenicproteins namely, Serum albumin and polymeric immunoglobulin receptor can be used as part of an assessment to diagnose pathophysiological condition during early age. Therefore, these proteins may be used as important tools for disease forecasting and earlier strategy for disease management.

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Table- 1 : Identified salivary proteins with their accession no. and mascot score

Proteins	Accession No.	Mascot Score	Related species
Serum albumin	gi594045062	80	*Bubalusbubalis*
polymeric immunoglobulin receptor	gi594038508	83	*Bubalusbubalis*

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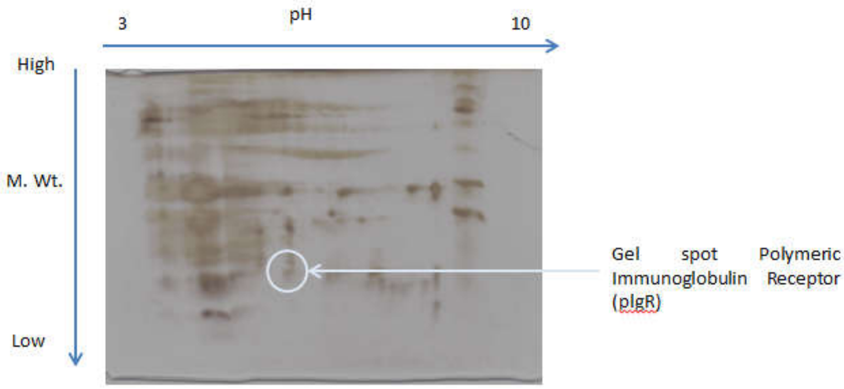

Fig-1 : spot of gel image of polymeric immunoglobulin receptor (pIgR) at 45 days of age

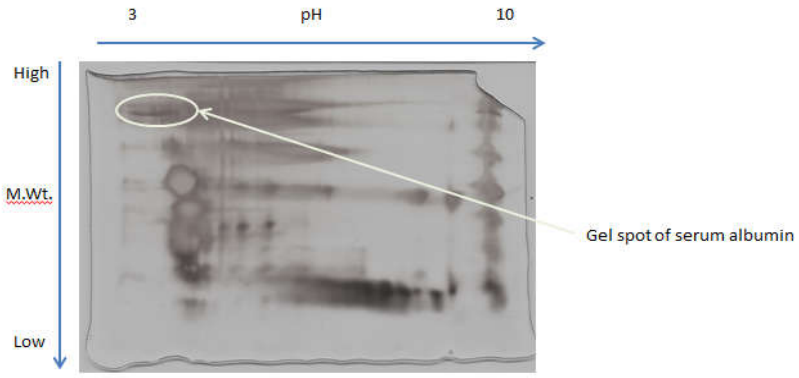

Fig-1 : spot of gel image of serum albumin at 90 days of age

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