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Effect of bacterial and viral vaccines on bovine - A spectral analysis

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Abstract

Livestock are to be regularly vaccinated to protect them from many infectious and contagious diseases. Anthrax is a disease of mammals, including human, is caused by a spore-forming bacterium called Bacillus anthracis. Anthrax Spore Vaccine (ASV) is a glycerinated suspension of live spores of uncapsulated avirluent strain of Bacillus anthracis. ASV can be used to protect all species of animals viz, cattle, sheep, goat, horse, ass, elephant, pigs and camel. Foot and Mouth Disease (FMD) is an acute, highly contagious picornavirus infection of cloven hoofed animal. The virus exists as seven serotypes: A, O, C, Asia1, SAT I, SAT II, and SAT III. Cattle are mainly infected by inhalation, often from pigs, which excrete large amounts of virus by respiratory aerosols and are considered highly important in disease spread. FMD monovalent vaccine is a liquid preparation containing any one type (O, A, C, ASIA-I) of FMD virus inactivated with formalin and adsorbed on buffered aluminium hydroxide gel. Two groups of five animals were vaccinated with anthrax spore vaccine (bacterial) and foot and mouth disease vaccine (viral). Pre and post vaccinated sera samples of cattle were collected and tested using Fourier Transform Infra Red (FTIR) spectrometer. Spectroscopic method of blood analysis is an alternate technique to the clinical method since they require fewer samples and provide more information. The variation in peaks was due to the change in protein and lipid levels in the animals due to introduce of antigens. The present work can be extended and compared with the other immunology tests in future.

Key Words: Fourier Transform Infra Red (FTIR) spectrometer, anthrax spore vaccine, foot and mouth disease vaccine, uncapsulated , avirulent, antigen.

INTRODUCTION

Among the various diseases affecting the animal, anthrax is a bacterial disease and foot and mouth disease is a viral disease. Animal diseases can cause enormous economic loss through morbidity and mortality. Vaccination is one of the routine procedures adopted to control specific diseases. It is considered to be the most effective method of preventing infectious disease. The material administrated (vaccine) can either be live, but attenuated forms of pathogens such as bacteria or virus, killed or inactivated forms of these pathogens, or purified material such as proteins. The immune system recognizes vaccine agents as foreign, destroys them, and 'remembers' them. When the virulent version of an agent enters a body, the immune system recognizes the protein coat on the infective agent, and thus is prepared to respond, by (i) neutralizing the target agent before it can enter cells and (ii) by recognizing and destroying infected cells before that agent can proliferate.

Blood is the chief circulatory medium in human and in animal body and participates in every functional activity by virtue of its circulation through every organ. The study of blood is one of the fascinating branches of clinical medicine. The application of spectroscopy for the study of biomedical compounds has increased tremendously in recent years. Keeping this in mind the work was undertaken to study the effect of Anthrax Spore Vaccine (ASV) and Foot and Mouth Disease Vaccine (FMDV) on cattle.

A high-affinity monoclonal antibody to anthrax protective antigen passively protects rabbits before and after aerosolized *B.anthracis* spore challenge was studied by Mohamed *et al* [1]. Krishna P. Shakya *et al* studied the evaluation of immune response to orally administered Sterne strain $34F_2$ anthrax vaccine [2].

Singanallur Balasubramanian Nagendrakumar *et al* studied the "Molecular Characterization of Foot-and-Mouth Disease Virus Type C of Indian Origin with those of European, South American, and Southeast Asian viruses revealed that the Indian viruses form a distinct genotype" [3]. Chitravel.V et *al* studied the "Antibody response in Danish Jersey heifers to food-and-mouth-disease vaccine" [4]. Pregnant Jersey heifers imported from Denmark to India were vaccinated subcutaneously with foot and mouth disease virus polyvalent (0, A22, C, Asia-I) vaccine during quarantine. A booster dose was given on day 21. Primary and secondary antibody responses were assessed by micro-serum neutralization test. The animal did not have detectable levels of antibodies before vaccination. An antibody response was observed on day 7 post-vaccination (PV). After the booster dose, the titres reached maximum levels within a week. At the end of the quarantine period (day 40 PV or day 19 after booster vaccination), the percentage of animal with protective titres was 96, 98, 98 and 98% for types 0, A22, C and Asia-I, respectively. Alonso *et al* studied the foot-and-mouth disease virus infection-associated antigen antibodies, comparison of the enzyme-linked immunosorbent assay and agar gel immunodiffusion tests [5]. The results indicate that the ELISA has the same specificity as the AGID test, but is more efficient in detecting cattle exposed to FMD virus. Though many studies have already been carried out on the disease and on the vaccines, no work has been performed using spectroscopic method and the present work aims to employ FTIR spectroscopic technique to analyze the effect of vaccines on cattle.

In serological study, the sample used is very less and hence it is a best method. The fluid portion plasma contains a large number of organic and inorganic substances such as proteins, vitamins, minerals, lipids, etc. Although the main function of blood is to transport various minerals to all cells of the body, blood also provides the temperature regulating defense mechanism. In this work normal healthy pre vaccinated blood samples (zero day) and post vaccinated (21^{st} day after vaccination) blood samples were analyzed by employing FTIR spectroscopic techniques.

MATERIALS AND METHODS

The experiments were carried out in a village Kaveripakkam, Vellore Dt, Tamilnadu. Blood samples were collected from jugular vein of the healthy cattle. The cattle were housed in a clean shed with good ventilation. No antibiotics were given during the experiment period. Pre-vaccinated blood samples were taken. One group of five animals was vaccinated with 5 ml of anthrax spore vaccine and the other group of five animals was immunized with 5 ml of foot and disease vaccine. Subsequent blood samples were collected on 21^{st} day from the same vaccinated animal. After collecting the blood, the serum was separated. Using the conventional method, the samples were prepared by spreading a small volume of serum on an infra red transparent material, allowing drying and measuring the absorption spectrum of the film. Infrared spectroscopy is a powerful method for the study of various biomedical tissue or biofluid samples [6]. Shaw *et al* reported that the IR absorption spectrum of thiocyanate ion (SCN) includes absorption at 2060 cm^{-1} in a spectral region where sera samples and subsequently normalizing all of the spectra to equal intensities therefore compensated for the imprecision in the film preparation [7]. A volume of 1ml of serum was diluted with an equal volume of 4mg/l aqueous potassium thiocyanate (KSCN) solution 20µl of each diluted sample was spread evenly over the surface of a circular potassium bromide (KBr) window (9mm diameter and 2 mm thickness). Infrared spectra in the region 4000-500 cm^{-1} were recorded on an ABB BOMEM MB SERIES FTIR spectrometer equipped with an air-cooled DTGS (Deuterated triglycine sulphate) detector. The strong absorption band of water in the mid IR region is hindered and to eliminate in the same, the serum samples were air dried to form a thin uniform film on the KBr pellet. Infra red transparent KBr material without the samples was scanned as back-ground for each spectrum and 23 scans were co added at a spectra resolution of 4 cm^{-1}. The collected signal was transferred to the PC. The data were processed by windows based data program–spectrum software. The spectra were base line corrected and they were normalized to acquire identical area under the curves and the maximum absorbance values of the corresponding characteristics bands were noted.

RESULTS AND DISCUSSION

The FTIR spectra of all the sera samples, both pre and post vaccinated showed the corresponding absorption bands in their specific regions. Table 1 and 2 present the absorbance values of pre and post sera samples of cattle vaccinated with ASV and FMDV for various wavenumbers.

Table 1 - the absorbance values for various wave numbers of the pre and post vaccinated sera samples of cattle vaccinated with ASV

Category	days	3296	2960	1660	2874	1398	1457
Cattle 1	Pre	1.259	0.841	1.371	0.689	0.743	0.713
	Post	0.904	0.583	1.174	0.479	0.468	0.451
Cattle 2	Pre	0.942	0.567	1.021	0.445	0.469	0.415
	Post	1.226	0.826	1.242	0.653	0.813	0.768
Cattle 3	Pre	0.923	0.583	1.174	0.464	0.468	0.451
	Post	1.253	0.849	1.426	0.696	0.743	0.713
Cattle 4	Pre	0.911	0.553	1.032	0.431	0.461	0.405
	Post	1.261	0.992	1.345	0.885	0.935	0.906
Cattle 5	Pre	0.911	0.552	1.021	0.431	0.461	0.405
	Post	0.738	0.412	1.019	0.304	0.311	0.284

Table 2 - The absorbance values for various wave numbers of the pre and post vaccinated sera samples of cattle vaccinated with FMDV

Category	days	3296	2960	1660	2874	1398	1457
Cattle 1	Pre	0.923	0.583	1.174	0.476	0.468	0.451
	Post	0.955	0.721	1.015	0.601	0.643	0.615
Cattle 2	Pre	1.261	0.831	1.432	0.681	0.744	0.714
	Post	1.291	0.845	1.377	0.692	0.743	0.713
Cattle 3	Pre	0.721	0.412	0.884	0.325	0.294	0.287
	Post	0.947	0.651	1.168	0.541	0.654	0.556
Cattle 4	Pre	0.918	0.552	1.021	0.421	0.461	0.405
	Post	1.261	0.849	1.372	0.696	0.743	0.713
Cattle 5	Pre	1.253	0.834	1.369	0.696	0.746	0.718
	Post	1.259	0.845	1.377	0.692	0.743	0.713

From the above two tables we came to a conclusion that on the 21st day of vaccination (post) the absorbance values increased compared to the pre vaccinated state for almost all the cattle according to the pre immune status of the animals. These changes were expected due to the increased level of proteins, lipids and amino acids in the animal body due to vaccination. It can be compared with other immunity tests in future. The immunity following vaccination is established in about 10 days and is expected to confer protection against natural infection for a period of six to nine months. Vaccine is advised every year. Vaccination has to be done only under the direct supervision of a veterinarian.

The mean absorbance values for various wave numbers for the pre and post vaccinated sera samples of cattle vaccinated with anthrax spore vaccine (ASV) and foot and mouth disease vaccine (FMDV) were tabulated in table 3 and 4 respectively.

Table 3 - The mean absorbance values for various wave numbers of the pre and post vaccinated sera samples of cattle vaccinated with ASV

wavenumber cm-1	mean absorbance value	
	pre	post
3296	0.989	1.076
2960	0.619	0.732
1660	1.124	1.241
2874	0.492	0.603
1398	0.521	0.654
1457	0.478	0.624

Table 4 - the mean absorbance values for various wave numbers of the pre and post vaccinated sera samples of cattle vaccinated with FMDV

wavenumber cm-1	mean absorbance value	
	pre	post
3296	1.015	1.143
2960	0.595	0.766
1660	1.176	1.262
2874	0.521	0.644
1398	0.543	0.705
1457	0.515	0.662

From the above table 3 and 4, the mean absorbance values for all wave numbers of the post vaccinated state was found to be greater than the pre state. These variations were expected due to the changes took place in the animal body because of the vaccination.

The following figures 1 and 2 represent the overlaid spectral graph of cattle 1 to 5 vaccinated with ASV (pre and post vaccinated states). The spectra of pre and post-vaccinated sera samples were all distinct from one another, but are dominated mainly by the absorption of the protein constituent which provides the selectivity in infrared based serum analysis [8]. The infrared spectrum provides various useful information of a biomolecule like structure, functional groups, types of bonds and its interactions [9]. The variations in the peaks were expected due to the changes took place in the animal body because of the vaccination.

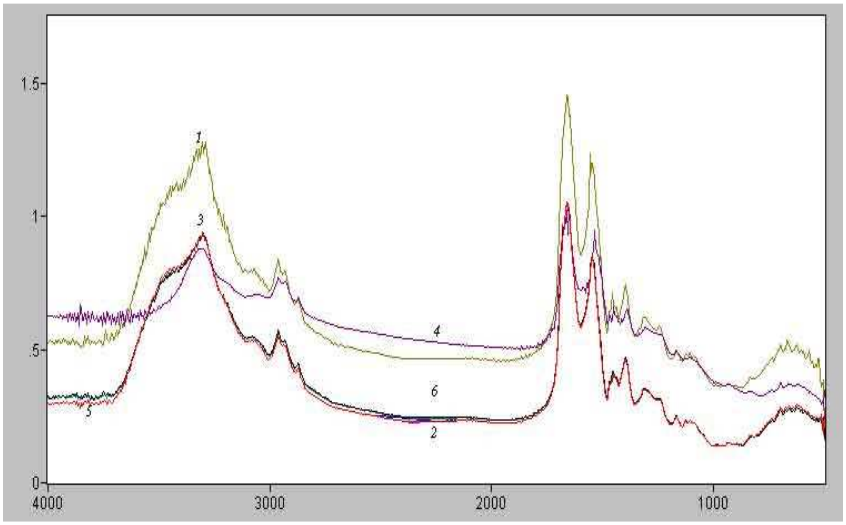

Fig 1 – FTIR overlaid spectra of cattle vaccinated with ASV – Zero day of vaccination

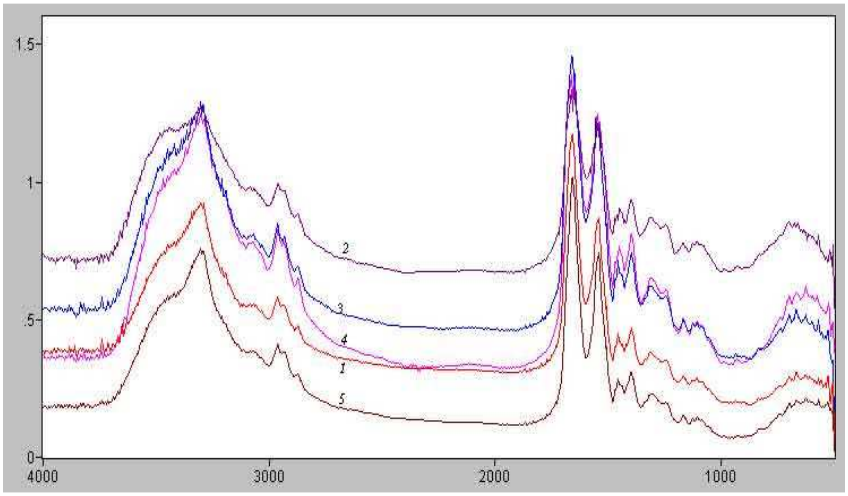

Fig 2 – FTIR overlaid spectra of cattle vaccinated with ASV – 21stday of vaccination

The following figures 3 (a) and 3 (b) represent the spectral graph of pre and post states of cattle 1 vaccinated with FMDV. Figures 4 (a) & 4 (b) to 7 (a) & 7 (b) represent the spectral graph of pre and post states of cattle 2 to 5 vaccinated with FMDV. The variation observed in peaks indicating the change in protein and lipids levels in the animals was due to introduce of antigens. Spectroscopy has been employed as a diagnostic tool in the study of blood [10].

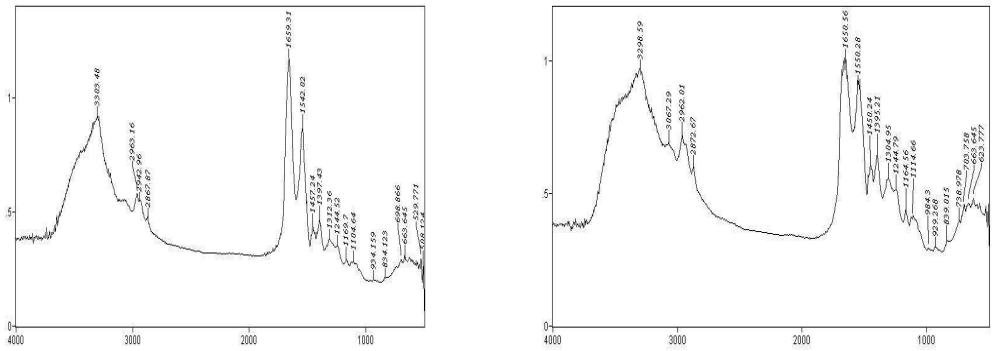

Fig 3 (a) & 3 (b) FTIR spectral graphs of pre and post states of Cattle 1 vaccinated with FMDV

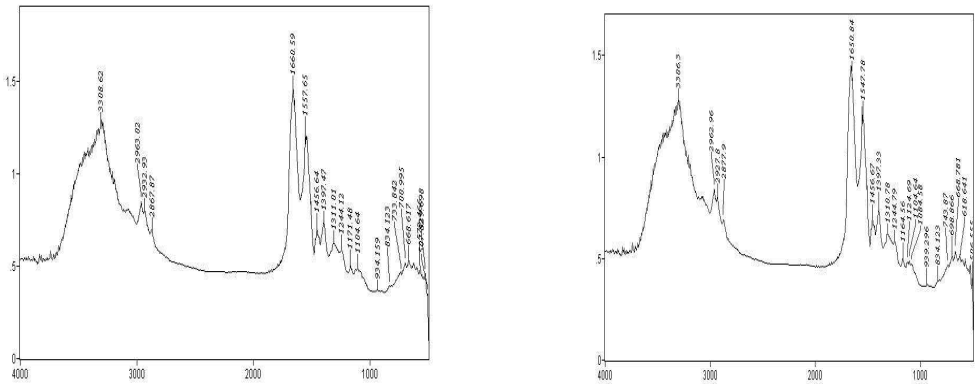

Fig 4 (a) & 4 (b) - FTIR spectral graphs of pre and post states of Cattle 2 vaccinated with FMDV

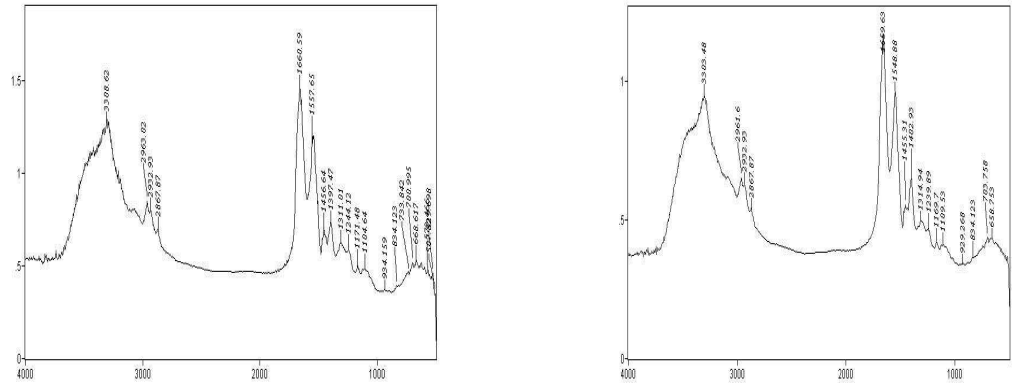

Fig 5 (a) & 5 (b) - FTIR spectral graphs of pre and post states of Cattle 3 vaccinated with FMDV

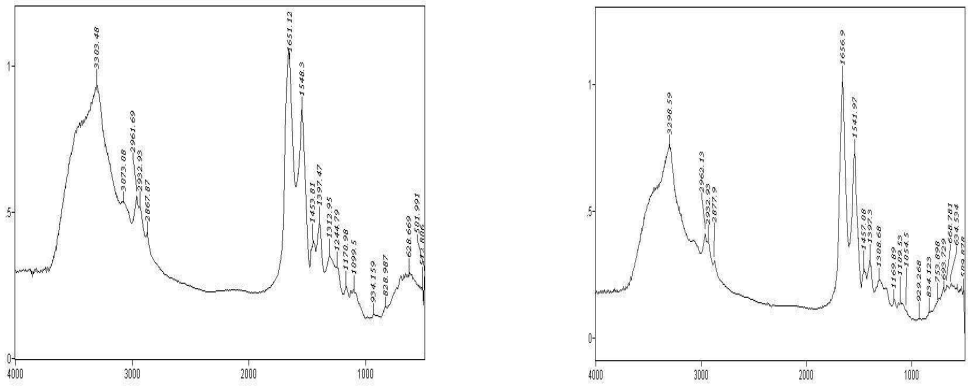

Fig 6 (a) & 6 (b) - FTIR spectral graphs of pre and post states of Cattle 4 vaccinated with FMDV

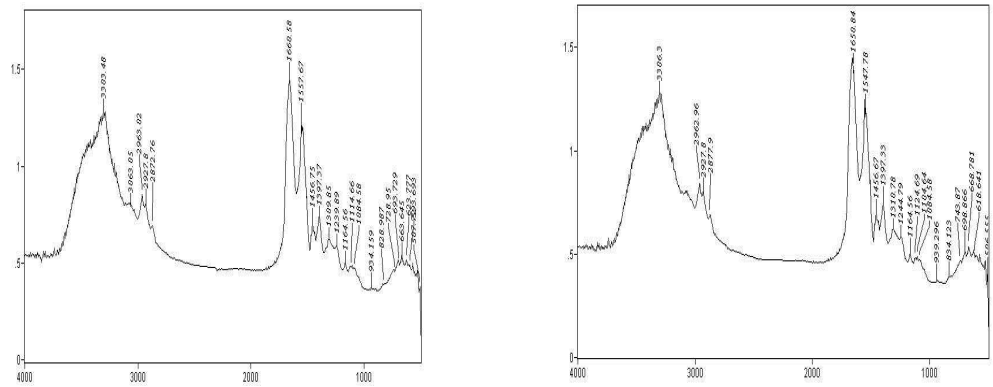

Fig 7 (a) and 7 (b) - FTIR spectral graphs of pre and post states of Cattle 5 vaccinated with FMDV

CONCLUSION

This method can be used as a screening test on animals to be vaccinated as well as to assess the potency of vaccine in vaccine production laboratories. This spectral analysis can be effectively used as an in vitro test to screen the animal and also assessing potency of vaccine. In vivo challenge test can be replaced once this procedure is standardized which can satisfy the CPCSEA-"Committee for the Purpose of Control and Supervision on Experiments on Animal"- which imposes stringent regulations to use animal for experiments.

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