EFFECT OF ANTHRAX SPORE VACCINE ON CATTLE – A SPECTROSCOPIC APPROACH

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ABSTRACT

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) on cattle. Anthrax is a disease of mammals, including human, is caused by a spore-forming bacterium called *Bacillus anthracis*. ASV is a glycerinated suspension of live spores of uncapsulated avirluent strain of "*Bacillus anthracis" (B.anthracis).* ASV can be used to protect all species of animal viz, cattle, sheep, goat, and elephant. Vaccination is the only best and cheapest method to protect the body against bacterial and viral diseases. In this work normal healthy pre vaccinated (zero day) and post vaccinated (7^{th}, 14^{th}, and 21^{st} day after vaccination) blood samples were analyzed by employing Fourier Transform Infra Red Spectroscopic (FTIR) techniques. Among the various techniques to study the antibody production, ELISA (Enzyme-Linked Immuno Sorbent Assay) is considered to be a better one, which can be done only in sophisticated laboratories. Spectral study can be taken as an alternate method and it can be compared with ELISA in future. Spectroscopic methods of blood analysis have an alternate technique to the clinical methods since they require fewer samples and provide more information.

Keywords: Anthrax spores vaccine, FTIR, Bio analysis.

INTRODUCTION

Anthrax is an infectious disease caused by the bacterium called *Bacillus anthracis.* Anthrax occurs naturally around the world in wild and domestic hoofed animal, especially cattle, sheep, goats, camels and antelopes. Anthrax is a disease of herbivorous animals caused by *Bacillus anthracis*, and human incidentally acquire the disease by handling infected dead animals and their products[1]. Anthrax is usually spread in the form of a spore. Disease occurs when spores enter the body, multiply and release toxins. The incubation period of natural infection in animals is typically 3 to 7 days with a range of 1 to 14 days. In cattle and sheep, the per-acute course of illness may last only 1 to 2 hours. The very first indication of problems may be sudden death of the animal. The inhalation of anthrax spores can lead to infection and disease. The possibility of creating aerosols containing anthrax spores has made *B.anthracis* a chosen weapon of bioterrorism. Several powers may have the ability to load spores of *B. anthracis* into weapons. Domestic terrorists may develop means to distribute spores via mass attacks or small scale attacks at local level. As an agent of biological warfare it is expected that a cloud of anthrax spore would be released at a strategic location to be inhaled by the individuals under attack. Spores of *B.anthracis* can be produced and stored in a dry form and remain viable for decades in storage or after release. Spores of *B.anthracis* can survive extremely long period of time in the environment. Survival time in soil, carcasses, textiles, water, sewage sludge and surfaces under open air conditions are of special interest in epidemiology. Survival times depend on the amount of exposed spores, temperature and a lot of other factors. Evaluation of serological tests for diagnosis of anthrax after an outbreak was made by Harrison *et al* (1989)[2]. Johnson-Winegar (1984) compared enzyme-linked immunosorbent with indirect hemagglutination assays for determining anthrax antibodies[3]. A high-affinity monoclonal antibody to anthrax protective antigen passively protects rabbits before and after aerosolized *B.anthracis* spore challenge was studied[4]. Krishna P. Shakya *et al* (2007) studied the evaluation of immune response to orally administered Sterne strain $34F_2$ anthrax vaccine[5]. 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 Anthrax Spore Vaccine on cattle.

MATERIALS AND METHODS

Five healthy cattle were under test in the village Kaveripakkam, Vellore District. Blood samples were collected from jugular vein of the cattle. After collecting the blood samples (pre vaccinated or zero day), the animals (cattle 1 to 5) were vaccinated with ASV. Blood samples were collected from the same animals on, 7^{th}, 14^{th} & 21^{st} day after vaccination. After collecting the blood, the sera samples were separated. Using the conventional method, the samples could be prepared by spreading a small volume of serum on an IR-transparent material, allowing drying and measuring the absorption spectrum of the film. The accuracy of the method may be compromised by any variation in the amount of serum successfully deposited on the KBr window, particularly with the manual sample preparation. In order to make up for this variation and to assess its impact on the overall accuracy of the method, a standard solution is added to each serum sample. The solution is chosen in such a way that it respond to IR radiation at the point where serum sample contains no absorption peak. Shaw *et al* (1998) 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. A volume of 1ml of serum was diluted with an equal volume of 4 mg/l aqueous potassium thiocyanate (KSCN) solution and 20µl of each diluted sample was spread evenly over the surface of a circular KBr window (9mm diameter and 2mm thickness). Mid Infrared spectra in the region 4000-500 cm^{-1} were recorded on an "ABB BOMEM MB SERIES"–a FTIR spectrometer equipped with an air-cooled DTGS (Deuterated triglycine sulphate) detector. It has already mentioned that the strong absorption band of water in the mid IR region is hindered and to eliminate the same, the serum samples are air dried to form a thin uniform film on the KBr pellet. IR 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[6].

RESULTS AND DISCUSSION

A satisfactory vibrational band assignment of the absorption bands of the spectra was done with the idea of the group frequency of the various constituents of the sera samples[7]. Table-1 presents the vibration band assignment of serum. The vibration band at 3304 cm^{-1} is due top the N-H stretching vibration of the secondary amides of protein. The asymmetric and symmetric stretching vibrations of the methyl group of proteins and lipids are found to be present at 2955 and 2869 cm^{-1} respectively. The other two vibration bands in C-H stretching region are found to be present near 2936 and 2851cm^{-1}, which are due to the asymmetric and symmetric stretching vibration of the methylene group. The strong absorption band present at 1656 cm^{-1} is attributed to C=O stretching of amide –I of the proteins. In the same way the presence of the band at 1545 cm^{-1} is due to the amide-II or NH bonding vibration that are strongly coupled to the C-N stretching vibrations of the protein amide groups. The peaks at 1456, 1315 cm^{-1} are considered to be due to the asymmetric and symmetric deformations of the methyl group of proteins. The peak at 1403 cm^{-1} may also considered due to COO^- stretching of ionized amino acid chains, suggesting an increased contribution from carboxalate. The lipid phosphate band due to the asymmetric PO_2 stretching vibration is found to occur at 1240 cm^{-1}. The spectral region 1169 - 1081 cm^{-1} is predominantly occupied by the C-O stretching vibrations of glucose. The absorption peaks present at 1169, 1153, 1107, 1079 and 1035 cm^{-1} are considered to be due to the different C-O stretching vibrations of C-O-H and C-O-C bonds. The weak absorption band at 955cm^{-1} is considered to be due to PO_2 symmetric stretching of the phosphate bond of proteins. The medium strong vibration bond present at 625 cm^{-1} is assigned as N-H out-of-plane bending with the contribution of C-N torsional vibrations. The infrared spectrum provides various useful information of a biomolecule like structure, functional groups, types of bonds and its interactions. Many research works have been conducted to study the blood serum using FTIR spectrum[8, 9].

Table 1: Infrared vibrational band frequency assignment of serum.

Wave number (cm^{-1})	Assignment
3304	N-H stretching of secondary amides of protein: amide A
2955	CH_3 asymmetric stretching of proteins and lipids
2936	CH_2/CH stretching
2869	CH_3 symmetric stretching of proteins and lipids
2851	CH_2/CH Stretching
1656	C=O stretching (80%) weakly coupled with C-N stretching (10%) and NH deformation (10%)-amide I
1545	NH deformation (60%) strongly coupled with C-N stretching (40%) amide II
1457	CH_3 asymmetric deformation
1403	CH_3 asymmetric deformation COO^- stretching of amino acids
1315	CH_3 symmetric deformation
1240	asymmetric PO_2 stretching of lipid phosphates
1169	C-O stretching
1128	C-O stretching
1081	C-O stretching
955	PO_2 symmetric stretching of lipid phosphates
701	NH asymmetric deformation coupled with CH_2 rocking amide V
625	O=C-N deformation (40%) coupled with other ring deformation (60%) amide IV

The FTIR spectra of all the sera samples, both pre and post vaccinated show the corresponding absorption bands in their specific regions. The absorbance is directly proportional to the concentration. Hence the different sera samples were analyzed and the absorbance values were tabulated.

Table 2 presents the mean absorbance values of the zero day, 7th, 14th and 21st day of vaccination. The absorbance values corresponding to the wave number 3296 for cattle 2, 3, 4 & 5 were 0.94, 0.92, 0.91 & 0.91 respectively. The values increased to 1.25, 1.19, 1.28 & 1.37 respectively in the 7th day of vaccination. Between 7th to 14th day of vaccination also the values increased for the cattle 2, 3 & 5. For cattle 4 alone it decreased. Between 14th and 21st day, expect for cattle 4 the absorbance value decreased. The vaccine was expected to protect the animal for 6 to 9 months. Similar variations were observed in the absorbance values for the other wave numbers given in the table for cattle 2, 3, 4 & 5. For cattle 1 alone, the absorbance values for all the wave numbers were larger in the pre vaccinated state compared to the other cattle 2 to 5.

Figures 1-4 present the FTIR overlaid spectra of the pre vaccinated (zero day) and post vaccinated (7th, 14th and 21st day of vaccination) sera samples.

Table 2: Absorbance values for various wave numbers for cattle 1 to 5

Category	days	3296	2960	2874	1660	1545	1457	1398	1315	1240	1169
cattle1	Pre	1.26	0.84	0.69	1.37	1.20	0.71	0.74	0.62	0.59	0.50
	P 1	0.92	0.59	0.48	1.18	0.87	0.45	0.47	0.38	0.35	0.29
	P 2	0.92	0.58	0.48	1.17	0.87	0.45	0.47	0.38	0.35	0.29
	P 3	0.90	0.58	0.48	1.17	0.87	0.45	0.47	0.38	0.35	0.27
cattle 2	Pre	0.94	0.57	0.45	1.02	0.85	0.42	0.47	0.35	0.29	0.25
	P 1	1.25	0.85	0.70	1.37	1.21	0.71	0.74	0.62	0.59	0.50
	P 2	1.27	0.99	0.89	1.35	1.21	0.91	0.94	0.87	0.84	0.78
	P 3	1.23	0.83	0.65	1.24	1.25	0.77	0.81	0.65	0.61	0.50
cattle 3	Pre	0.92	0.58	0.46	1.17	0.87	0.45	0.47	0.38	0.35	0.29
	P 1	1.19	0.80	0.64	1.28	1.09	0.64	0.78	0.58	0.49	0.41
	P 2	1.29	0.97	0.84	1.32	1.27	0.86	0.89	0.80	0.76	0.67
	P 3	1.25	0.85	0.70	1.43	1.23	0.71	0.74	0.62	0.59	0.50
cattle 4	Pre	0.91	0.55	0.43	1.03	0.85	0.41	0.46	0.35	0.31	0.25
	P 1	1.28	0.97	0.83	1.40	1.28	0.85	0.89	0.80	0.76	0.67
	P 2	1.12	0.70	0.56	1.38	1.10	0.57	0.64	0.50	0.44	0.37
	P 3	1.26	0.99	0.89	1.35	1.21	0.91	0.94	0.87	0.84	0.78
cattle 5	Pre	0.91	0.55	0.43	1.02	0.85	0.41	0.46	0.35	0.30	0.25
	P 1	1.37	1.07	0.89	1.50	1.34	0.97	0.00	0.86	0.82	0.69
	P 2	1.45	1.03	0.89	1.53	1.36	0.91	0.96	0.85	0.80	0.71
	P 3	0.74	0.41	0.30	1.02	0.74	0.28	0.31	0.23	0.20	0.15

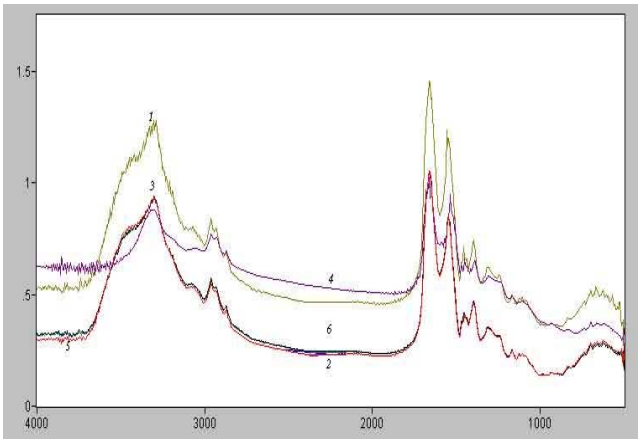

Figure 1: FTIR overlaid spectra of pre vaccinated cattle – Zero day of vaccination

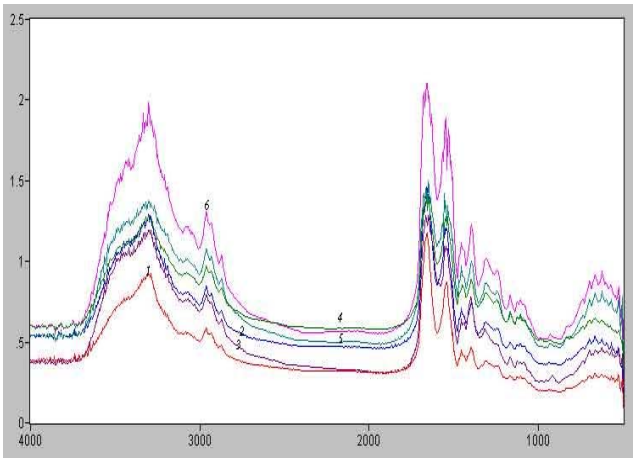

Figure 2: FTIR overlaid spectra of post vaccinated cattle – 7th day of vaccination

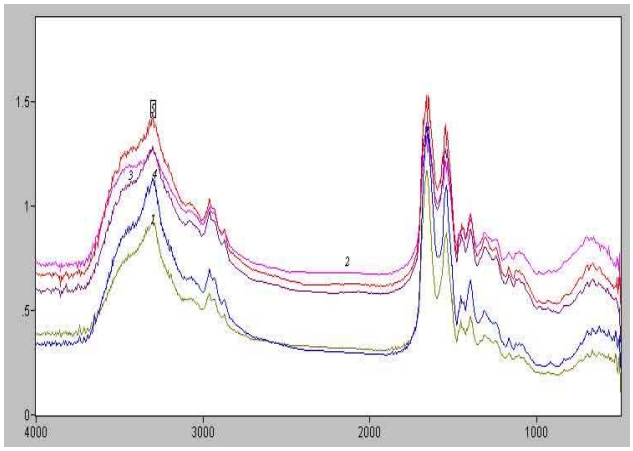

Figure 3: FTIR overlaid spectra of post vaccinated cattle – 14th day of vaccination

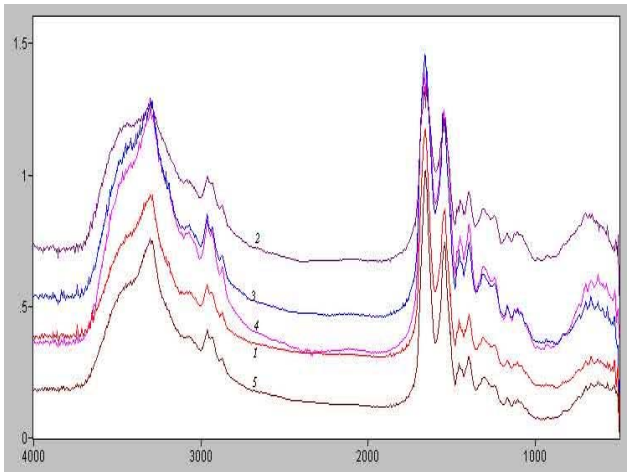

Figure 4: FTIR overlaid spectra of post vaccinated cattle – 21st day of vaccination

Table 3 summarizes the mean of the absorbance values for various wave numbers for the pre to post states. The mean values for the pre, post 1 and post 2 states continuously increased. Post 2 to post 3 (14^{th} to 21^{st} day of vaccination) the mean absorbance values decreased for all the wave numbers. These variations were expected due to vaccination.

Table 3: The mean of the absorbance values for various wave numbers

Wave number cm-1	pre	post 1	post 2	post 3
3296	0.989	1.202	1.207	1.076
2960	0.619	0.855	0.856	0.732
2874	0.492	0.708	0.729	0.603
1660	1.124	1.345	1.351	1.241
1545	0.927	1.157	1.161	1.061
1457	0.478	0.725	0.739	0.624
1398	0.521	0.575	0.781	0.654
1315	0.411	0.651	0.681	0.552
1240	0.369	0.601	0.639	0.518
1169	0.308	0.511	0.562	0.441

For the entire wave numbers given in the table 1 the standard deviation (S.D) was calculated and tabulated in table – 4. It was observed that the value of S.D on the 7^{th} day was less than the pre vaccinated state. Between 14^{th} to 21^{st} day, the values were increased gradually. The S.D value on 21^{st} day was greater than the pre vaccinated state. These variations were expected due to the changes took place in the animal body because of the vaccination.

Table 4: The standard deviation of the absorbance values for various wave numbers

Wave number cm-1	pre	post 1	post 2	post 3
3296	0.151	0.171	0.199	0.241
2960	0.125	0.185	0.201	0.232
2874	0.111	0.163	0.196	0.221
1660	0.153	0.121	0.129	0.157
1545	0.154	0.185	0.188	0.236
1457	0.133	0.199	0.215	0.252
1398	0.124	0.356	0.215	0.257
1315	0.119	0.191	0.224	0.249
1240	0.125	0.191	0.226	0.251
1169	0.108	0.168	0.221	0.242

CONCLUSION

In vaccine production centers or institutes, safety and potency test was conducted to test the quality of the vaccine. Animals are generally procured from approved contractors with unknown history. This test can serve to screen animal to be vaccinated and as well to asses the potency of vaccine in vaccine production laboratories. Compared to ELISA, spectral analysis is cost effective test besides it requires small amount of sample for analysis. One instrument can analyze infinite number of samples, since it is window based data program-spectrum software. The internal standards among the absorption peaks can be calculated. 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 Central and Supervision on Experiments on Animal"- which imposes regulations to use animal for experiments.

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