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# Efficacy of Black Quarter Vaccine on Cattle: A Spectroscopic Approach

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In this work, normal healthy pre vaccinated blood samples (zero day) and post vaccinated (7th, 14th, 21st day after vaccination) blood samples are analyzed by employing FTIR and UV-Vis spectroscopic techniques. The internal standards among the application peaks are calculated in both the methods. Variation is expected due to the production of antibodies in animal. Among the various techniques to study the antibody production, ELISA (enzyme-linked immuno sorbent assay) is considered a better one for the present, but it can be done only in sophisticated laboratories. Hence there is a need to try for other techniques to know the immune status of vaccinated animals. The present work can be extended and compared to the ELISA technique.

**Key Words: Black quarter vaccine, UV-visible and FTIR spectra, Internal standard.**

## INTRODUCTION

Vaccinology as a scientific field and vaccine production as a commercial activity are areas of rapid growth. Vaccines are generally considered the most-efficient tool of public health, research and development in vaccinology may be considered a major public concern. Vaccination is the best and cheapest method to protect the body against bacterial and viral diseases. Among the various bacterial diseases affect the animal, Blackleg is one of the fatal disease of young cattle and sheep and occasionally in other animal species. *Clostridium chauvoei* is the causative organism in most cases. Some affected animals have also been found to be affected with *Clostridium feseri*. These two microbes are gas-producing bacteria. They also form spores, which can live in soils for many years. The microbes cannot spread the disease Blackleg from one animal to another simply by contact. The common symptoms are cattle not being able to walk properly, loses interest in food, high temperature, swelling that develops in shoulder, back, neck and makes a cracking sound under pressure. Gas is formed inside the enlarged swellings. Once affected with Blackleg, the animal can die within 48 h. Without proper treatment, cattle can be found dead with no time to react. After the initial symptoms, the bacterium causes a gradual

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poisoning reaction in the body. In sheep, Blackleg is mostly found after instances of physical wounds. The spores of the bacterium might be present in the gut of the cattle for sometime. These spores do not immediately result in the disease. Medical research is ongoing to find what conditions cause these ingested spores to give rise to the disease. Naz *et al.*[1] have taken thigh muscle samples from 6 cows and 2 buffalo died in outbreaks of Black Quarter (BQ) at different districts of Punjab. *Clostridium chauvoei* was isolated from all the samples, caused death in guinea pigs within 24 to 78 h with rapid development of symptoms identical to the symptoms of (BQ) in cattle. It is evident from their study that *Clostridium chauvoei* alone is isolated from the affected muscle samples of cattle and buffalo which causes Blackleg disease. Sudarsanam *et al.*[2] assessed the result of cell mediated immune response and humoral immune response combinedly in production against Black Quarter. They recorded the result of monitoring those responses in guinea pigs vaccinated with black quarter vaccine and correlating the responses with protection to virulent challenge afforded by these animals along with unvaccinated controls. Srinivasan *et al.*[3] assessed the serological response of bovines to combined vaccine containing foot and mouth disease virus, rabies virus, *Pasteurella multocida* and *Clostridium chauvoei* antigens and individual component vaccines also. Serological response of the calves was assayed on days 21 and 90 post vaccination. There was no significant variation in the serological response elicited by individual component vaccines and combined vaccine containing all four antigens. 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 a defense mechanism[4,5]. In this work, normal healthy pre vaccinated blood samples (zero day) and post vaccinated (7th, 14th, 21st day after vaccination) blood samples are analyzed by employing FTIR and UV-Vis spectroscopic techniques.

## EXPERIMENTAL

The experiments were carried out in a village Kaveripakkam, Vellore District, Tamilnadu. Ten healthy cattle were used. Blood samples were collected from each of them from jugular vein. They were housed in a clean shed with good ventilation. No antibiotics were given during the experimental period. Pre-vaccinated blood samples were taken. Then they were immunized with 0.5 mL of black quarter vaccine. Subsequent blood samples were collected on 7th day, 14th day and 21st day from the same vaccinated animal. After collecting the blood, the serum was 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.*[6] reported that the IR absorption spectrum of thiocyanate ion 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 1 mL of serum was diluted with an equal volume of 4mg/L aqueous potassium thiocyanate solution 20 µL of each diluted sample was spread evenly over the surface of a circular KBr window (9 mm diameter and 2 mm thickness). Infrared spectra in the region 4000-500 $cm^{-1}$ were recorded on an ABB BOMEM MB SERIES-one 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 in the same, the serum samples are air dried to form a thin uniform film on the KBr pellet[6,7]. IR transparent KBr material without the samples was scanned as background 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. For UV-vis spectroscopic measurements, the whole blood was used. The whole blood was diluted with normal saline at a concentration of 0.9 % and the spectra were recorded using Shimadzu UV 1601 spectrometer in the region 200 to 700 nm. Both the spectra were taken at Dr. Ceeal Analytical Lab., Chennai, India.

## RESULTS AND DISCUSSION

**FTIR Spectral analysis:** The spectra of pre and post-vaccinated sera samples were all distinct from one another but were dominated mainly by the absorption of the protein constituents which provides the selectivity in infrared based serum analysis. Table-1 presents the vibration band assignment of serum. The vibration band at 3300 $cm^{-1}$ is due to 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 2956 and 2896 $cm^{-1}$, respectively. The other two vibration bands in C-H stretching region are found to be present near 2922 and 2851 $cm^{-1}$, which are due to the asymmetric and symmetric stretching vibration of the methylene group. The strong absorption band present at 1655 $cm^{-1}$ is attributed to C=O stretching of amide-I of the proteins. In the same way the presence of the band at 1548 $cm^{-1}$ is due to the amide-II or N-H bending vibration that are strongly coupled to the C-N stretching vibrations of the protein amide groups. The peaks at 1456, 1400 and 1315 $cm^{-1}$ are considered to be asymmetric and symmetric deformations of the methyl group of proteins. The peak at 1400 $cm^{-1}$ may also considered due to $COO^-$ stretch of ionized amino acid chains, suggesting an increased contribution

TABLE-1
INFRARED VIBRATIONAL BAND FREQUENCY ASSIGNMENT OF SERUM

| Vibration band ($cm^{-1}$) | Assignment |
|---|---|
| 3296 | N-H stretching of secondary amides of protein: amide A |
| 2960 | $CH_3$ asymmetric stretching of proteins and lipids |
| 2936 | $CH_2$/CH stretching |
| 2874 | $CH_3$ symmetric stretching of proteins and lipids |
| 2851 | $CH_2$/CH stretching |
| 1660 | 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 |
| 1398 | $CH_3$ 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 |

from carboxalate. The lipid phosphate band due to the asymmetric P-O stretching vibration is found to occur at 1240 $cm^{-1}$. The spectral region 1250-925 $cm^{-1}$ 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 955 $cm^{-1}$ is considered to be due to P-O symmetric stretching of the phosphate bond of proteins. The medium strong vibration bond present at 702 $cm^{-1}$ is assigned as N-H out-of-plane bending with the contribution of C-N torsional vibrations.

The FTIR spectra of all the sera sample both pre and post vaccinated show the corresponding absorption bands in their specific regions qualitatively. But quantitatively there is a considerable difference in the spectra between the pre and post-vaccinated sera. The absorbance was directly proportional to the concentration. Hence the different sera samples were analyzed quantitatively by calculating the intensity ratio among the absorption peaks. In order to quantify the spectral difference, three intensity ratio parameters have been introduced. They were $R_1 = I_{3296}/I_{2960}$ due to the N-H stretching of secondary amides of protein, amide A and $CH_3$ asymmetric stretching of proteins and lipids, $R_2 = I_{1398}/I_{1457}$ due to $CH_3$ asymmetric deformation $COO^-$ stretching of amino acids and $CH_3$ asymmetric deformation and $R_3 = I_{1660}/I_{2874}$ due to the ratio of the intensities of amide-I and $CH_3$ symmetric stretching of proteins and lipids. Though the infrared spectra of all the sera samples were similar, considerable differences were found to be present in the internal standards among the absorption peaks in pre and post vaccinated samples.

Table-2 summarizes the internal standard calculations of the pre and post-vaccinated samples. The values of $R_1$ were found to be around 1.32 to 1.47 for pre-vaccinated serum. But the values of $R_1$, during the period of 7th and 14th day changed due to the production of antibodies. On 21st day of vaccination the values increases to the range of 1.46 to 1.8. Similarly $R_2$ values were from 1.026 to 1.078 in the pre-vaccinated state and it changes to 1.137 to 1.179 in the post vaccinated state. The values of $R_3$ were found to be around 1.516 to 2.375 for pre-vaccinated serum. But the values of $R_3$ during the period of 7th and 14th day changed due to the production of antibodies. On 21st day of vaccination the values increased to the range of 2.126 to 2.473. These variations occur due to the antibodies produced by the way of vaccination.

The UV-Vis spectra have been recorded for the pre and post vaccinated blood samples. The UV-Vis spectra of all the blood samples exhibit the presence of two strong absorption peaks at 417 and 576 nm. But there was a marked difference in the absorption levels of pre and post vaccinated blood samples. Table-3 summarizes the internal standard representation among the absorption peaks for the pre- and post-vaccinated blood samples. The internal standard among the absorption peaks was found to be around 6.555 to 8.324 in pre-vaccinated blood sample. But it decreased in the 7th day and increased in 14th and 21st day after vaccination. But its value was less than the pre vaccinated state. These variations occur due to the production of antibodies between zero day and 21st day. The antibodies were produced at the maximum level on the 21st day. The immunity following vaccination was established in about 10 days and is expected to confer protection against natural infection for a period of 6 to 9 months.

## Conclusion

Animal diseases cause enormous economic loss through mortality, inefficient production and increase in the stock replacement rates, which all require additional resources. Control and treatment of the diseases also contribute to the losses[8]. Control measures in present-day programme include quarantine of imported animals; cooperation of agencies in the study and control of animal diseases; inspection of red meat and poultry to minimize the danger of spread of animal disease to human beings; inspection and evaluation of vaccines and other pharmaceutical and biological products as to purity, efficacy and safety; inspection of the mass slaughter of animals and the destruction of carcasses. Universities and other research institutions conduct studies on the many disease problems that arise.

Spectroscopy has been employed as a diagnostic tool in the study of blood. FTIR and UV-Visible spectroscopic methods have been employed to study the pre and post vaccinated blood samples. The internal standards among the absorption peaks were calculated. By studying this, the potency of the vaccine can be justified. Present work is a serological analysis in infrared spectroscopy. In future this study can be compared with the ELISA serological procedure.

TABLE-2
INTERNAL STANDARD CALCULATIONS OF THE PRE AND POST-VACCINATED SAMPLES OF CATTLE

| Category of sample | Days | $R_1=I_{3296}/I_{2960}$ | $R_2=I_{1398}/I_{1457}$ | $R_3=I_{1660}/I_{2874}$ |
|---|---|---|---|---|
| Cattle 1 | Pre | 1.493 | 1.026 | 2.224 |
| | Post 1 | 1.328 | 1.012 | 1.638 |
| | Post 2 | 1.297 | 1.018 | 1.368 |
| | Post 3 | 1.657 | 1.137 | 2.392 |
| Cattle 2 | Pre | 1.603 | 1.136 | 2.375 |
| | Post 1 | 1.325 | 1.006 | 1.712 |
| | Post 2 | 1.604 | 1.120 | 2.274 |
| | Post 3 | 1.611 | 1.136 | 2.473 |
| Cattle 3 | Pre | 1.320 | 1.038 | 1.677 |
| | Post 1 | 1.847 | 1.096 | 3.360 |
| | Post 2 | 1.646 | 1.138 | 2.447 |
| | Post 3 | 1.823 | 1.087 | 3.344 |
| Cattle 4 | Pre | 1.453 | 1.078 | 2.080 |
| | Post 1 | 1.578 | 1.136 | 2.360 |
| | Post 2 | 1.661 | 1.130 | 2.292 |
| | Post 3 | 1.442 | 1.179 | 2.179 |
| Cattle 5 | Pre | 1.475 | 1.062 | 2.048 |
| | Post 1 | 1.576 | 1.040 | 2.524 |
| | Post 2 | 1.274 | 1.018 | 1.268 |
| | Post 3 | 1.320 | 1.038 | 1.677 |
| Cattle 6 | Pre | 1.322 | 1.039 | 1.582 |
| | Post 1 | 1.577 | 1.042 | 2.451 |
| | Post 2 | 1.921 | 1.143 | 2.661 |
| | Post 3 | 1.466 | 1.256 | 2.023 |
| Cattle 7 | Pre | 1.471 | 1.039 | 2.058 |
| | Post 1 | 1.442 | 1.035 | 2.063 |
| | Post 2 | 1.223 | 1.028 | 1.476 |
| | Post 3 | 1.502 | 1.028 | 2.282 |
| Cattle 8 | Pre | 1.279 | 1.032 | 1.516 |
| | Post 1 | 1.474 | 1.053 | 2.126 |
| | Post 2 | 1.415 | 1.011 | 2.009 |
| | Post 3 | 1.569 | 1.04 | 2.491 |
| Cattle 9 | Pre | 1.748 | 1.024 | 2.671 |
| | Post 1 | 1.491 | 1.053 | 2.116 |
| | Post 2 | 1.408 | 1.966 | 1.830 |
| | Post 3 | 1.571 | 1.040 | 2.598 |
| Cattle 10 | Pre | 1.446 | 1.106 | 1.841 |
| | Post 1 | 1.583 | 1.040 | 2.530 |
| | Post 2 | 1.334 | 1.012 | 1.601 |
| | Post 3 | * | * | * |

TABLE-3
RATIO OF ABSORBANCE AMONG THE PEAKS USING UV-VIS SPECTRA

| Category of sample | Days | Wavelength (nm) and absorbance value | | |
|---|---|---|---|---|
| | Pre | 417 | 576 | $A_{417}/A_{576}$ |
| Cattle 1 | Post 1 | 0.7662 | 0.1018 | 7.526 |
| | Post 2 | 0.3642 | 0.0976 | 3.732 |
| | Post 3 | 0.3517 | 0.0931 | 3.778 |
| Cattle 2 | Pre | 0.3187 | 0.0902 | 3.533 |
| | Post 1 | 0.8382 | 0.1007 | 8.324 |
| | Post 2 | 0.9728 | 0.1810 | 5.374 |
| | Post 3 | 0.9646 | 0.1627 | 5.929 |
| Cattle 3 | Pre | 0.9562 | 0.1542 | 6.201 |
| | Post 1 | 0.9052 | 0.1381 | 6.555 |
| | Post 2 | 0.9637 | 0.1722 | 5.596 |
| | Post 3 | 0.9528 | 0.1658 | 5.747 |
| Cattle 4 | Pre | 0.9372 | 0.1582 | 5.924 |
| | Post 1 | 1.0675 | 0.1310 | 8.149 |
| | Post 2 | 0.8704 | 0.1310 | 5.212 |
| | Post 3 | 0.8610 | 0.1670 | 5.595 |
| Cattle 5 | Pre | 0.8541 | 0.1539 | 6.308 |
| | Post 1 | 0.9129 | 0.1354 | 7.717 |
| | Post 2 | 1.0942 | 0.1183 | 5.744 |
| | Post 3 | 1.0812 | 0.1905 | 5.788 |
| Cattle 6 | Pre | 1.0524 | 0.1868 | 6.261 |
| | Post 1 | 0.7362 | 0.1067 | 6.900 |
| | Post 2 | 1.1101 | 0.1987 | 5.587 |
| | Post 3 | 1.1091 | 0.1798 | 6.168 |
| Cattle 7 | Pre | 1.1016 | 0.1683 | 6.545 |
| | Post 1 | 0.8768 | 0.1082 | 8.104 |
| | Post 2 | 0.6717 | 0.1415 | 4.747 |
| | Post 3 | 0.6661 | 0.1355 | 4.916 |
| Cattle 8 | Pre | 0.6546 | 0.1215 | 5.388 |
| | Post 1 | 0.9092 | 0.1314 | 6.919 |
| | Post 2 | 1.2489 | 0.2113 | 5.911 |
| | Post 3 | 1.2391 | 0.2031 | 6.101 |
| Cattle 9 | Pre | 1.2185 | 0.1925 | 6.330 |
| | Post 1 | 1.0342 | 0.1409 | 7.340 |
| | Post 2 | 0.8619 | 0.1574 | 5.476 |
| | Post 3 | 0.8540 | 0.1498 | 5.701 |
| Cattle 10 | Pre | 0.8361 | 0.1245 | 6.716 |
| | Post 1 | 0.9371 | 0.1285 | 7.293 |
| | Post 2 | 1.0572 | 0.1672 | 6.323 |
| | Post 2 | 0.9192 | 0.1551 | 5.777 |
| | Post 3 | 0.9032 | 0.1342 | 6.730 |

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