Engineering and Technology Journal ISSN: 2456-3358

Fluorescence Analysis of Sample of Sheep to Establish the Presence of Bacterium Pasteurella Multocida the Cause of Dangerous Disease in Large And Small Ruminants

Vanya Plachkova[1], Chavdar Zemyarski[2], Petar Petrov[3], Marin Nenchev[4], Margarita Deneva[5]

Phd Student[1], Research Scholar[2,3], Professor[4], Assoc. Professor[5]

Dept. Optoelectronics & Lasers, Techn. Univ.-Sofia, Branch Plovdiv

ARTICLE INFO

corresponding Author:
Vanya Plachkova
Phd Student Department. Opto- electronics & Lasers, Techn. Univ.-Sofia, Branch Plovdiv

ABSTRACT

Field method for the study of herd animals was developed. This method allows the analyzing of samples from animals where the farm is. It does not require any special conditions. Therefore, it is widely applicable to the study of both large and small ruminants. The field method does not harm the animal or the operator who is working with the appliance. The experimental set up was used to characterize the samples of ruminants in the field test samples by means of a UV light source. What is needed is just a small sample from the body of the animal that fluoresces and depending on the emission wavelengths bacteria or other illegal substances can be detected. Samples taken from a sheep that suffered from Pasteurellosis disease show the presence of the bacterium Pasteurella multocida. Using UV and fluorescence spectroscopy we have studied spectral wavelength, except for Pasteurella multocida and for β-defensin, lipopigment lipid, infusoria, pepsin, P-glycoprotein and peptides. The device is applicable to both large and small herd animals. It will help farmers with fast and accurate diagnosis of ruminants to solve global problems for both livestock breeding and their products for the food industry.

KEYWORDS: ***Fluorescence analysis, Pasteurella multocida, peaks of fluorescence***

1. INTRODUCTION

Trend in recent years is the increasing interest of scientists in the area of Biophotonics. It provides efficient methods of investigation of biological samples with minimal risk to the test component. With the development of optoelectronics increased interest of engineering construction equipment for spectral analysis is towards optimizing the dimensions of devices. The small size of the experimental setup also ensure compactness and an ease of portability. Another advantage after fine setting of this modern optoelectronic technique is the extremely high accuracy, which offer its components.

This report presents a prototype device constructed of precision optoelectronic components. It enables field analysis of samples from large and small ruminants. The experimental setup described in this report analyzes the samples to the principle of fluorescence spectroscopy, detectable emission

Published Vol. 1 Issue 4 2016
DOI: 10.18535/etj/v1i4.03
ETJ 2016, 1, 101-106

wavelength of bacterial viruses, other regulated substances and ingredients useful in the liquid samples from animals. Field method allows mobile application of Biophotonics in veterinary medicine. This mobile experimental setup will enable the possibility to test liquid samples from large and small ruminants where the farm is. This main advantage will increase the quality of the analysis because the sample will be stored and transported to the research center, and will be analyzed immediately after being taken from the animal.

Pasteurella multocida cause of Pneumonia disease in small ruminants. It is located in the body of healthy animals and unfamiliar conditions of rearing and feeding (humidity, cold, overcrowding, frequent change of feed) causes the manifestation of the disease. Most often the disease takes a chronic pasteurellosis in the form of contagious fever. Animals usually show exacerbation of the disease process, loss of appetite, emaciation and sometimes they die. In other cases, pasteurellosis exhibits cold with fever and cough. Pasteurellosis is inherent disease to humans. The infection is transmitted to humans by scratches or bites from an animal. The lack of methods for early diagnosis of animals is a major problem for farmers, which results in a reduction in population of animals. In order to prevent a serious disease spread early diagnosis of the animal is required. Most drugs of the veterinary pharmaceutical industry are expensive for small and medium companies. As a very important factor for the choice of early diagnosis and it turns out that despite the expensive drugs used to treat the animals they do not guarantee their full recovery and livestock will have to be "scrapped". Early diagnosis of the animal diseases will allow increasing the benefits of livestock and reducing morbidity.

2. MATERIAL AND METHODS

2.1 Fluorescence measurements.

The basic scheme for observing and measuring the fluorescence signal is shown in Figure 1. Since fluorescence is often very weak, and in addition, in all directions, then in order not to saturate the receiver. The useful fluorescence signal is measured in a direction which is less than 45 ° relative to the excitation radiation. For measurement of the fluorescence is preferably used as acting source a laser diode (LD) as his spectral width is very small. The LED has a relatively wide spectral width of radiation from 30-40 nm and usually angular distribution of the radiation is in their large angular range of +/- 30□. It was selected to work with LD with a wavelength of 245 nm, since in preliminary studies it was found that bacteria and nutrients in the body of small ruminants have low emission wavelength. The source irradiates the sample and its emission wavelength is transmitted through the optical fiber to a CMOS detector. The sensitivity of the CMOS detector is in the range of 200 nm to 1100 nm. Its resolution is about □□= 5 nm.

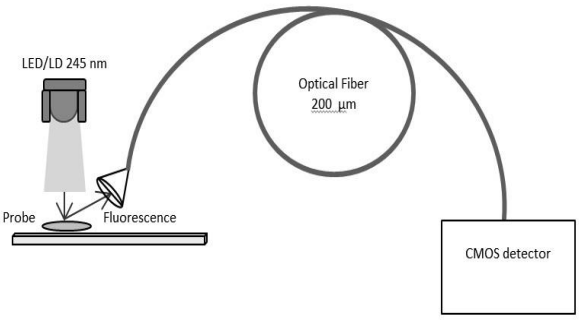

Fig.1. Scheme of the experimental setup.

The components contained within the experimental setup which is shown on Figure 1 have a relatively small size. This advantage allowed a fluorescence analysis to be carried out at a sheep farm. It was chosen to make measurements in the field, in order to avoid damage to the samples in transit and thus to ensure the more reliable fluorescence assay. With the above described setup were received emission wavelengths of Pasteurella multocida, β-defensin, lipopigment lipid, infusoria, pepsin and P-glycoprotein.

Published Vol. 1 Issue 4 2016
DOI: 10.18535/etj/v1i4.03
ETJ 2016, 1, 101-106

Samples taken from sick sheep whose diagnosis was established by a specialist were tested using fluorescence spectroscopy. The study was conducted after establishing the diagnosis of veterinary medic. A key moment in the field of fluorescence spectroscopy proved the fact that it was found the presence of bacteria, which cause a lung infection. Such a diagnosis was established by a veterinary specialist afterwards resulting in match which gives ground to the team of specialists to further improve the method for rapid and early fluorescence spectroscopy.

2.2 Excitation and emission spectra by classical fluorescence spectral analysis.

The excitation spectrum for all studied samples of dead sheep (sample of the abscess open in a lung sample from the small intestine, a sample of the colon) is 245 nm. Bacteria and nutrients in the body of small ruminants have low emission wavelength. Recorded were results with the emission wavelengths set at: 337 nm for Pasteurella multocida, 357 nm for sheep milk, 452 nm for β-defensin, 510 nm for lipopigment lipid, 400 nm for infusoria, 342 nm for pepsin and 475 nm for P-glycoprotein. The aim of the study was the bacterium Pasteurella multocida, which is responsible for the death of sheep. The other substances which are registered characterize the composition in the body of small ruminants.

3. RESULTS AND DISCUSIONS

Samples of a dead sheep were analyzed, sick of pasteurellosis, which was carried out by a specialist in autopsies. On Figure 2. is clearly visible emission wavelength of Pasteurella multocida, which was recorded in a sample of abscess around the open areas of the lung of the animal.

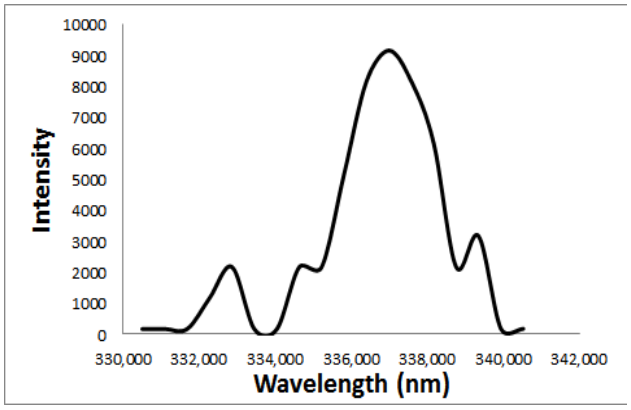

Fig.2. Fluorescence spectroscopy of Pasteurella multocida with the emission wavelength λ = 347nm with wavelength Excitation of λ = 245 nm.

Pasteurella multocida is usually found in the upper respiratory tract of sheep and goats. Healthy animal may also be a carrier of the bacteria, by contacting cause respiratory diseases and sepsis as well as in cattle, and in the other classes of pets. Pasteurellosis is part of the group of infectious and parasitic diseases that can be transmitted from animals to humans, and vice versa. It is a respiratory disease which is the result of an infection by bacteria of the genus Pasteurella. Pasteurella multocida is the most common causative bacterium of this disease. This bacterium is part of a well-known general harmful unregulated body uses (part of the normal bacterial flora) and the pathogen in the body of small dairy cattle. Infections in humans usually occur after contact with pets such as dogs and cats. The most common manifestation of pasteurellosis in humans is local infection of the wound, usually following the bite of an animal or other physical contact. This can become a serious infection of the soft tissue, and can also be complicated by abscesses, septic arthritis and osteomyelitis. Pasteurella multocida can also cause meningitis, ocular infections and respiratory tract infections, usually in patients susceptible to lung diseases.

Published Vol. 1 Issue 4 2016
DOI: 10.18535/etj/v1i4.03
ETJ 2016, 1, 101-106

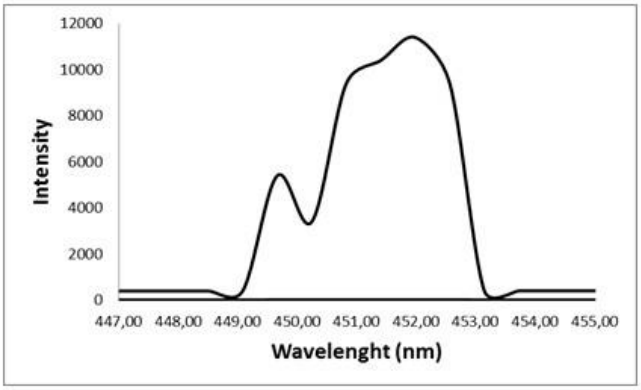

Fig. 3. Fluorescence spectroscopy of β-defense with the emission wavelength λ = 452nm with wavelength excitation of λ= 245 nm

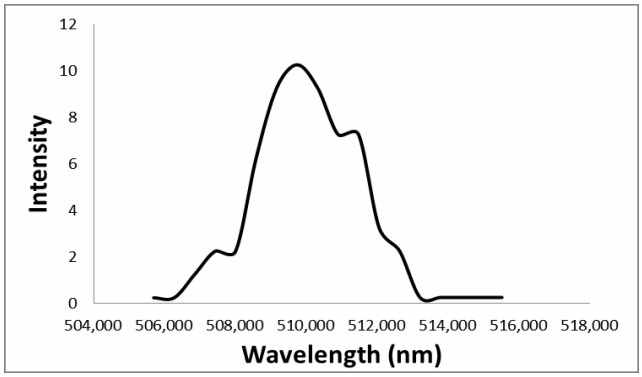

Fig.4. Fluorescence spectroscopy of lipopigment lipid with the emission wavelength λ = 510 nm with wavelength excitation of λ = 245 nm.

Besides samples of abscess fluorescent analysis was made of liquid samples taken from small and large intestines. On Figure 3 and Figure 4 is shown the emission wavelength of β-defensin and lipopigment lipid. These substances are characteristic of the composition of the large intestine in sheep, so that their presence does not mean anything alarming on Figure 5. On Figure 6 and Figure 7 is shown emission wavelength of infusoria, P- glycoprotein and pepsin. These substances are characteristic of the composition of the small intestine in sheep, so their presence means nothing alarming. The intensity of the signal is low for two reasons: test samples were quite turbid environments and studies have not been conducted in a laboratory where necessary create ideal conditions but on-site at a sheep farm. The experimental setup, which is the subject of this article is capable of single - channel measurement. The experimental protocol allows a change in the structure with the possibility of multi-channel measurement. If there is a much more powerful source than the current which will be used for the construction of the pilot invention multi-channel measurements can be achieved. The experimental setup allows it to be designed to test several samples of veterinary medicine divided into several optical channels.

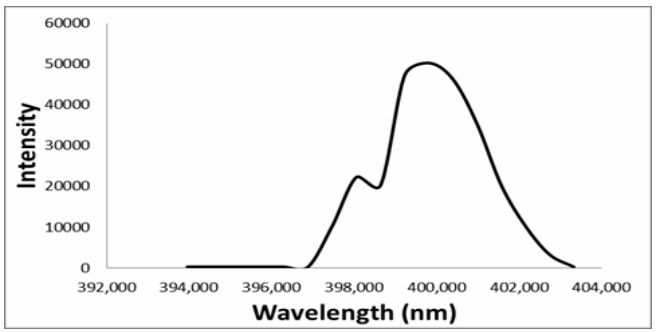

Fig.5. Fluorescence spectroscopy of infusoria with the emission wavelength 400nm - wavelength excitation of λ = 245 nm.

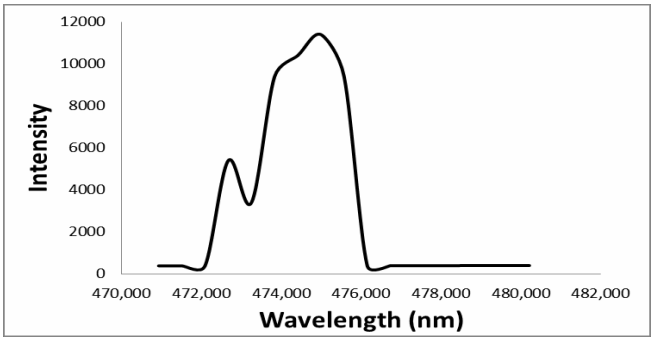

Fig.6. Fluorescence spectroscopy of P- glycoprotein with the emission wavelength λ = 475 nm- wavelength excitation of □ = 245 nm.

Thus the diagnosis was confirmed by a veterinary specialist for deadly fast growing pasteurellosis. Pasteurella multocida was opened by fast-acting fluorescent analysis as had previously studied its emission wavelength by reference in specialized sources. Other bacteria except Pasteurella multocida were not found, which means that the only disease in terms of fluorescence analysis is pasteurellosis.

Published Vol. 1 Issue 4 2016
DOI: 10.18535/etj/v1i4.03
ETJ 2016, 1, 101-106

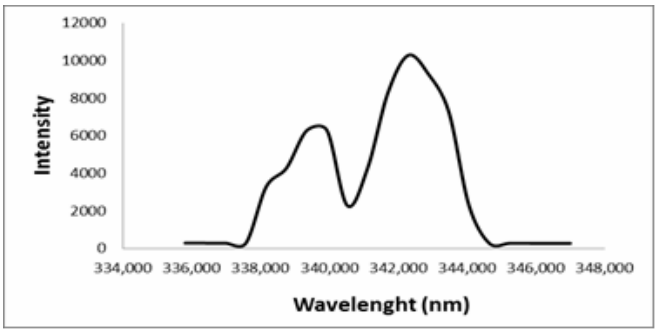

Fig.7. Fluorescence spectroscopy of pepsin with the emission wavelength λ = 342 nm with wavelength excitation of λ = 245 nm.

4. CONCLUSIONS

Applying fluorescence spectroscopy establishes accessible and rapid method for analysis of various biologic samples with one unit. Equally effective were tested as a blood sample, and samples of abscess, vaginal and nasal discharge. Fluorescence spectroscopy was performed on the spot, samples are not carried in the laboratory, it was possible because of the mobility of the test device. Bacterium Pasteurella multocida was discovered by fluorescence analysis in the body of sheep, which is dangerous for the human body. Easy and accessible both from a technological and financial point of view method for the detection of harmful bacteria to the human body, was discovered. Using fluorescence analysis a registration of the level of bacteria and nutrients can be made in the body of small ruminants. Via fluorescence analysis can be avoided current autopsies of sick animals with harmless to the human organism method as most autopsies of animals are harmful to humans. Fluorescence analysis can be applied well enough on the field or at a farm that has a diseased animal. Method can be applied to a mass testing because the pattern of spectral analyzer for Fluorescence spectroscopy described in the article is easy to operate and affordable. Fluorescence spectroscopy can safely replace labor- intensive and many consumables tests in trivial microbiology laboratories. The three main advantages of Fluorescence spectroscopy is that the method is fast, does not require consumables and can be performed on field. The fluorescence method is suitable for early diagnosis of liquid samples for the presence of bacteria in the body before the presence of symptoms of the disease causing bacteria relevant. The main objective of perfecting the experimental setup described in the report is the promotion of the method as a tool for diagnosis and prevention of medical centers, farms, factories and places of supply of finished products in them. The aim is regularly controlling the liquid samples from animals. This is because actually we humans depend on their health, and this will continue in the future, daily. Therefore care to enhance quality in veterinary health care is essential for all living organisms on our planet

5. REFERENCES

1. Michael R. Baldwin, Jeremy H. Lakey and Alistair J. Lax ” Identification and characterization of the Pasteurella Multocida toxin translocation domain” Molecular Microbiology 54 (1), 239–250 (2004)
2. M. Ballent, A. Lifschitz, G. Virkel, J. Sallovitz, and C. Lanusse, “Modulation of the P- glycoprotein-mediated intestinal secretion or ivermectin: In vitro and in vivo assessments drug metabolism and disposition” Vol. 34, No. 3 The American Society for Pharmacology and Experimental Therapeutics 457–463 (2006)
3. D. Dhanasekaran, S. Shanmugapriya, N. Thajuddin1 and A. Panneerselvam “ Aflatoxins and Aflatoxicosis in Human and Animals Aflatoxins “ Biochemistry and Molecular Biology 222-245 (2011)
4. Weidenborner, M.”Encyclopedia of food mycotoxins “ Spronger Publisher Berlin, New York, London 330 -451 (2001)
5. Tyden, E.; Olsen, L. Tallkvist, J. Tjalve, H.& Larsson, P. “Cytochrome P450 3A, NADPH cytochrome P450 reductase and

Published Vol. 1 Issue 4 2016
DOI: 10.18535/etj/v1i4.03
ETJ 2016, 1, 101-106

cytochrome b in the upper airways in horse" Research in Veterinary Science, Vol.85, pp.80–85 (2008)
6. D. Dhanasekaran1, S. Shanmugapriya1, N. Thajuddin1 and A. Panneerselvam, " 2 Aflatoxins and Aflatoxicosis in Human and Animals Aflatoxins" Biochemistry and Molecular Biology 222-254 (2011)
7. Michael R. Baldwin, Jeremy H. Lakey and Alistair J. " Lax Identification and characterization of the Pasteurella Multocida toxin translocation domain" Molecular Microbiology 54 (1), 239–250 (2004)
8. Marcelo NoËllmann, Robert Gilbert, Timothy Mitchell, Michele Sferrazza, and Olwyn Byron "The Role of Cholesterol in the Activity of Pneumolysin, a Bacterial Protein Toxin" Biophysical Journal Volume 86 May 3141–3151 (2004)
9. Timothy Mitchell, Michele Sferrazza and Olwyn Byron" The Role of Cholesterol in the Activity of Pneumolysin, a Bacterial Protein Toxin "Biophysical Journal Volume 86 3141–3151 (2004)
10. Lax, A. J., Chanter, N., Pullinger, G.D., Higgins, T.H., Staddon, J.M., and Rozengurt, E. , 1990 Sequence analysis of the potent mitogenic toxin of Pasteurella multocida". FEBS Lett 277: 59–64 (2003)

Published Vol. 1 Issue 4 2016
DOI: 10.18535/etj/v1i4.03
ETJ 2016, 1, 101-106