

BIOCHEMICAL CONFIRMATORY TEST ON SUSPECTED ISOLATES OF *CLOSTRIDIUM PERFRINGES* USING INDIRECT ACID PHOSPHATASE

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ABSTRACT

The effectiveness of indirect acid phosphatase method of confirmatory identification of suspected *Clostridium perfringens* (Bacteria) isolates from faecal samples of goats and sheep is described. *C. perfringens* infections has not been much reported in the Nigerian animal husbandry systems, thus this method could help for easy identification of this disease. The results proved acid phosphatase, a quick, reliable, simple and accurate method for the biochemical confirmatory test for *C. perfringens*. This test proved same to the reference strains obtained for comparison.

Keywords: Clostridium, acid phosphatase

INTRODUCTION

It is generally recognised that *C. perfringens* occurs in a variety of infections and its enterotoxaemia is fatal in both animals and human beings, although it has been known as a normal intestinal flora. Its enterotoxaemia has been reported in feed-lots where dietary changes create favourable conditions for the disease outbreaks. Sheep normally receive a series of vaccination when they are newly brought into a feedlot (Odendel, Visser, Botha and Frinslob, 1988). Because *C. perfringens* is an important cause of human food poisoning, various media and methods have been employed for its isolation and identification from faecal samples and foods. However, there has been no information on the use of acid phosphatase in the confirmatory identification scheme of anaerobic bacteria infections in the animal husbandry systems in Nigeria. In this study, attempts were only being made to assess phosphatase production and possibly as an indicator of virulence (Porscehen and Spanldling, 1974). A similar method was also reported for the identification of *Staphylococcus aureus* (Schollehn and

Brandis., 1973.

Despite all the improved ability to recover *C. perfringens*, selective isolation media in current use have not been able to eliminate the need for confirmatory test, thus acid phosphatase method was used to ensure that the isolates belonged to the desired species (Mead, Leon, and Adams, 1981).

MATERIALS AND METHODS

Test organisms used in this study were suspected isolates of *C. perfringens* from stock cultures of recent faecal samples. Two reference strains of ATCC type D8504 and strain NCTC type C3180 were obtained from the Department of Veterinary Microbiology of the University of Hoehenheim, Stuttgart. The one hundred suspected *C. perfringens* isolates and two reference strains were subcultured in blood glucose agar plates and incubated in anaerobic glove box for 24hr at 37°C. A colony each was obtained from the blood glucose agar plates of both the reference strains and the Nigeria strains and gram stain procedures were performed. A drop of already prepared acid phosphatase was applied directly on each *C. perfringens* colony on the plates.

Composition of acid phosphatase solution was as follows:

Na-Citrate buffer solution at pH 4.5: made from 9.8g Trinatrium citrate + 40g NaOH in 1 litre of H₂O. 68ml of the above solution was then titrated with 1N HCL till it was pH 4.5. From this solution, 10ml was taken and added: 0.3g - Naphtyl - 1 - phosphatase + 0.5g "Echtblausalz" - di - azonium - 0 - dianisidium.

This solution was thoroughly dissolved by shaking and left for about 1 hour at 4°C. The solution was filtered and portioned into test tubes. It could be kept at - 20°C and when required, it could be warmed at 37°C (UENO, 1970).

RESULTS AND DISCUSSION

Clostridium perfringens colony on blood glucose agar plates incubated in anaerobic glove box at 37°C for 24 hours showed a flat olive greyish butyrous colouration with a double zone of haemolysis and under a microscope showed a large "car-box" shape. It is gram positive. However, the morphology of the Nigerian strains of *C. perfringens* were not significantly different from the typical morphology of the reference strain ATCC type D8504 and strain NCTC type C3180 obtained for comparison. At the time of this study no pictures were taken to show the colouration and morphology of the Nigerian strains. A strong enzymatic reaction was observed within 2 - 5 minutes after a drop of the acid phosphatase solution on the colony. The olive greyish colour changed to dark brown violet. In negative case there was no colour development, rather it turned white. A colony picked with a wire loop and spread onto a filter paper petri-dish showed a positive reaction of brown purple colour within 2 - 5 minutes when a drop of acid phosphatase was applied. Both the reference strains used and the Nigerian strains showed similar reactions. 98 of the tested organisms proved positive to this test compared with the two reference strains.

Suspected isolates of *C. perfringens* from faecal samples of sheep and goats and reference strain ATCC type D8504 and strain NCTC type C3180 were studied, using acid phosphatase. No single test like acid phosphatase has been reported to replace series of biochemical tests such as lactose fermentation, gelation liquification, nitrate reduction which are very cumbersome to perform. In this study, 0.3g alpha-naphtyl-l-phosphatase acid 0.5g "Echtblausalz" - di - azonium - O - dianisidine was used instead of 0.2 and 0.4g as reported by UENO, 1970. The difference in concentration of the solution did not however affect the results.

The Nigerian strains agreed generally with the reference strains, thus confirming this

biochemical test for distinguishing *C. perfringens* from other clostridia species growing on selective media in current use (Hauschild and Hilscheim, 1971). The advantages of this test especially on field diagnosis are its rapidity and simplicity in determining the results. The pH level of acid phosphatase for this study was 4.5. Thus more information is required on the phosphatase enzymes of *C. perfringens* and the pH optimum for differential purposes. This method can easily be introduced into field diagnosis of *C. perfringens* infections in the Nigerian animal husbandry systems. It reduces costs and saves time.

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