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ISOLATION AND CHARACTERIZATION OF CLOSTRIDIUM CHAUVOEI STRAINS ISOLATED FROM CASES OF BLACKLEG IN CATTLE IN IRAN

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INTRODUCTION

Blackleg in cattle was first diagnosed in Iran in 1938 (1,2). The disease is found in many parts of the country especially on wet bottom lands, low hilly and sandy areas. Several sporadic outbreaks have so far been reported from different parts of the country, however the most severe one on record occurred in August 1968 among herds of cattle in southern Iran (3). Fifteen strains of Clostridium chauvoei have been isolated from the specimens received for laboratory diagnosis at this Department.

This paper deals with identification and characterization of the isolated strains of Cl. chauvoei in Iran.

MATERIALS AND METHODS

Isolation of causative organism

Pieces of infected muscles and bone marrow were used for isolation of the causative organisms. Muscle lesions were removed and inoculated immediately on to the fresh blood agar plates (4) and incubated anaerobically at 37°C for 48 hours. Three colonies which resembled Cl. chauvoei were picked into fresh liver broth and were incubated anaerobically for 24 hours. Bones of blackleg suspected cattle had also been sent to the laboratory for diagnosis. The bone marrow was inoculated into fresh liver broth and incubated anaerobically at 37°C for 24–48 hours. Up to the present time ten strains of Cl. chauvoei have been isolated from bone marrow in pure cultures.

The identification of Cl. chauvoei in culture and tissue smears by using fluorescent labelled antibodies has been applied as described by Batty and Walker (5). (Fig. 1).

Smaers were prepared from the colonies and the cultures were stained with Cl. chauvoei, Cl. septicum and Cl. oedematiens specific fluorescent labelled andtisera (*). Smears stained only with Cl. chauvoei labelled antiserum fluoresced brightly under fluorecent microscope (**).

Smears were also taken from the gelatinous oedema of the guinea-pigs that had previously been injected with a 24 hour culture from each isolated strain and on staining with Cl. chauvoei labelled antiserum, they flouresced brightly under fluorescent miccroscope (Fig. 2).

Strain

Fifteen isolated strains of Cl. chauvoei were used in this study.

Medium

Each strain was inoculated in a tube containing 20 ml. thioglycollate medium (Difco) for 24 hours at 37°C. The cultures were centrifuged at 3000 r.p.m. for ten minutes and the supernatant was removed for haemolytic tests.

Red cell suspension

Bloods of horse, cattle, sheep and rabbit were collected in Alsever's solution and washed three times in five volumes of 0.85% NaCl and used as a one per cent solution.

Demonstration of haemolysis.

Toxin and saline were put in ten sterile tubes on the four rows to get final dilutions of 1, 1/2 1/5, 1/10, 1/30, 1/50, 1/100, 1/200, 1/500 and 1/1000 in a total volume of 1 ml. To each row of tubes one ml. of one per cent suspension of the washed red blood cell from horse, cattle, sheep and rabbit was added respectively. The contents of all tubes were well mixed up by inverting them over a piece of non absorbent paper. At the same time one ml. of the toxin was mixed with 0.1 ml. of the specific Cl. chauvoei antiserum. The tubes were kept at room temperature for 30 minutes and then were added 0.5 ml. of red blood cells suspension of cattle, sheep, rabbit and horse. The haemolysin produced by Cl. chauvoei strains in the tubes was observed at the end of sixteen hours at 37°C.

Antibiotics sensitivity tests

Sixteen sensitivity disks in medium concentration (Difco) were used to determine the susceptibility of the isolated strain to antibiotics. The technique used in this study has been described in detail by Prévot (6). The paper disks contained the following concentration: tetracycline 10 ug; penicillin 2 units; aureomycin

(*) – Fluorescent Antibodies, Clostridium species, Wellcome, Research Laboratories, Beckenham ENGLAND.

(**) – Fluorescence Microscope, Carl Zeiss, West Germany.

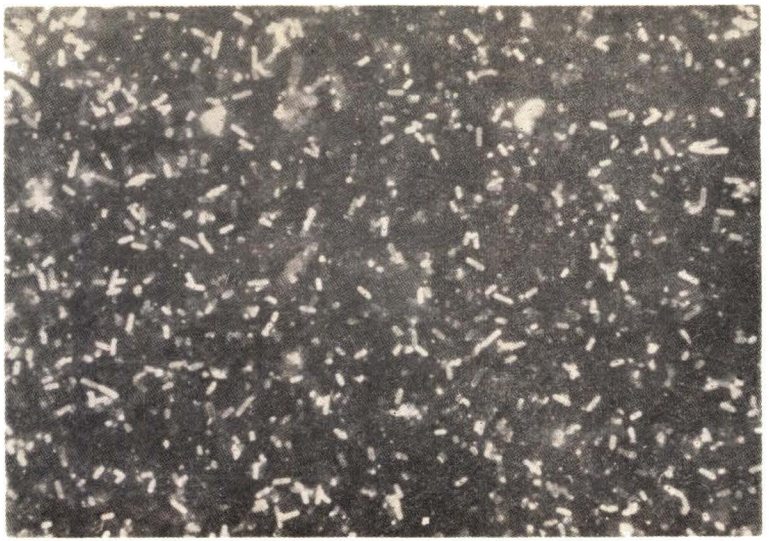

Fig. 1- Tissue smear from infected muscle of a case of blackleg in cattle stained with fluorescein-labelled Clostridium chauvoei antiserum. X500

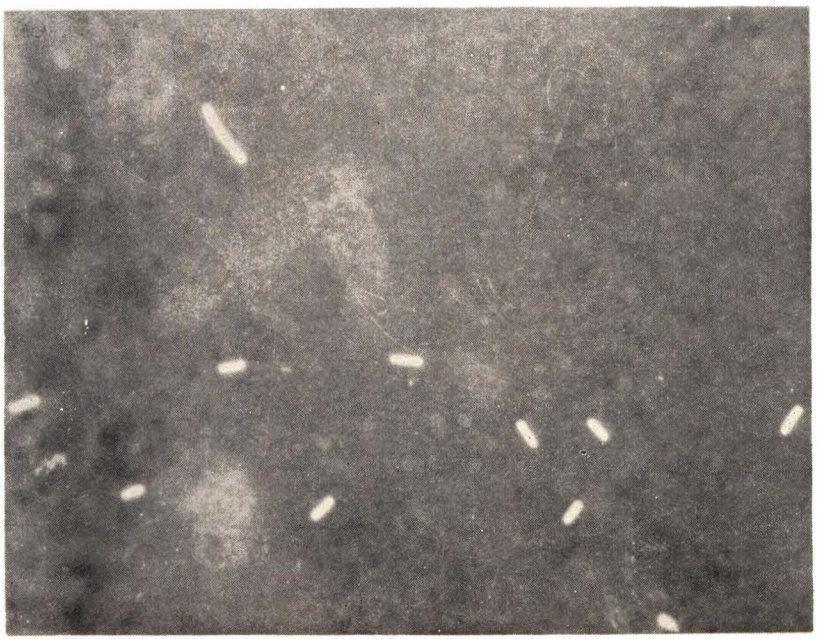

Fig. 2 – Tissue smear from muscle of guinea-pig infected with Cl. chauvoei stained with fluorescein-labelled Cl. chauvoei antiserum.X 1000

10ug; ampicillin 10ug; doxycycline hydrochloride 30ug; erythromycin 10ug; novobiocin 30ug; neomycin 10ug; kanamycin 30ug; chloramphenicol 10ug; streptomycin 10ug; colistin sulphate 10ug; oleandomycin 10ug; elkosin 15ug; dihydro-streptomycin 10ug; polymixin B 300 units.

Toxicity determination.

A 48–hour culture of the isolates in thioglycollate broth was used to determine lethal dose of the toxin produced by the isolates. It was centrifuged at 3000 r.p.m. for ten minutes and then dilutions were made from the supernatant in borate buffer saline. Each toxin dilution was immediately injected intravenously into white mice (each weighing 18 to 20 g.) using three mice per dilution. The inoculated mice were observed for 72 hours and the titre was recorded.

RESULTS

Fifteen strains of Cl. chauvoei were isolated from infecetd muscles and bone marrows of cases of blackleg in cattle from different parts of Iran. Fluorescent-labelled antibodies were used to confirm the identification.

The haemolytic activity of the isolates were studied by using red blood cells of horse, cattle, sheep and rabbit. The results of this experiment as summarized in Table (I) indicated that the toxin of all strains was more active on red blood cells of cattle, sheep, rabbit and horse respectively.

Table 1 – Haemolysis of different types of erythrocytes of animals by Clostridium chauvoei toxin

No. of Strains tested	Red cell type	Titre of haemolytic activity							
		I	I/2	I/5	I/10	I/50	1/100	I/200	I/500
15	cattle	XX	XX	XX	XX	XX	XX	X	X
15	sheep	XX	XX	XX	XX	XX	X	—	—
15	rabbit	XX	XX	XX	XX	X	—	—	—
15	horse	XX	XX	XX	XX	—	—	—	—

XX – Complete haemolysis
X – Partial haemolysis
\- – No haemolysis

Sixteen different antibiotic sensitivity disks in medium concenteration were selected for testing the sensitivity of the isolated strains of Cl. chauvoei. It was found out that the isolated strains were more sensitive to tetracycline, penicillin, aureomycin and ampicillin. The organisms showed resistence or a slight sensitivity to neomycin, kanamycin, elkosin, polymyxin B, dihydro-streptomycin, doxycycline hydrochloride, colistin sulphate, oleandomycin, elkosin, novobiocin, chloramphenicol and erythromycin.

The titre of the toxin produced by all the fifteen strains under the testing condition varied between 5 to 20 minimum lethal dose per mililitre.

SUMMARY

Fifteen strains of Clostridium chauvoei were isolated from cases of blackleg in cattle in Iran. The technique of isolation and identification by using fluore scent labelled antibodies is described. The haemolytic activity, toxicity and sensitivity of the isolates to several antibiotics were studied.

RESUME

Quinze souches de Clostridium chauvoei ont été isolées dans la maladie de charbon symptomatique chez les bovins en Iran. La technique de l'immunofluorescenée a été utilisée pour l'isolation et l'identification de Clostridium chauvoei. Les caracteres hemolytique toxicologiques et sensibilité des antibiotiques ont été etudies.

Acknowledgment

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REFERENCES

1- KAVEH, Personal communication, 1975

2- RAFYI,A., ARDEHALI, M. - Les maladies causées par les Anaérobies pathogènes sporulés chez les animaux domestique, Bull, Off, int, Epiz., 59, (9–10) 1283–1312, 1963.

3- ARDEHALI, M., KHALILI, KH. and DOWRAN, H.-Characterization of Clostridium chauvoei strains isolated from an outbreak of blackleg in Iran. Arch. Inst. Razi, 23,119–123,1971.

4- WALKER, P.D., HARRIS, S and MOORE, W.B- Isolation of anaerobes, The Society for applied Bacteriology, No, 5 Academic press. London, 1971.

5- BATTY, I and WALKER, P.D.- Differentiation of Clostridium septicum and Clostridium chauvoei by the use of fluorescent labelled antibodies. J. Path & Bact. Vol. 85, No. 2,517–521, 1963

6- PREVOT, A. Techniques pour le diagnostic des Bactéries Anaérobies, Paris, 1960.