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NOTE

Characterization of Clostridium chauvoei strains isolated from an outbreak of blackleg in Iran

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Blackleg in cattle has been recognised in Iran since 1938. The disease is generally known to affect cattle and buffaloes in this country (1) and distributed in most of cultivated areas specially in plain rice field, low hills and sandy spots.

Isolated outbreaks have been reported from different parts of the country, and more than 13 strains of Clostridium chauvoei were isolated from specimens received from veterinary service for confirmation of provisional diagnosis.

In August 1968 a severe outbreak of blackleg occurred in epizootic form amongst cattle herds in Mamassani and Kouhkelooye, south part of Iran, in which 400 cattle died. The disease appeared in a vast area among cattle of 15 villages.

The present communication deals with this outbreak with emphasis on isolation and characterization of the causative organisms.

MATERIALS AND METHODS

Isolation of the causative organisms

Pieces of muscles from infected site of three calves died in the outbreak were examined.

Sheep blood agar plates we.e inoculated and then incubated anaerobically. The typical Cl. chauvoei colonies isolated by this procedure were picked up and transferred to a liver medium to obtain a pure culture, 1 ml. of a 10 per cent suspension of the infected muscles in sterile broth was also injected into the hind legs of three guinea pigs. The inoculated animals were observed and the dead ones were examined for typical lesions of the infection and reisolation of the organism.

Fermentation media containing glucose, glycerol, maltose, lactose sucrose and salicin were used for identification of the organisms.

Immunological tests

Protection tests were carried out in guinea pigs with culture of isolated organisms. Three animals were inoculated each with 1 ml. of Cl. oedematiens, Cl. septicum and Cl. chauvoei antiserum, respectively. 24 hours after the inoculation, all guinea pigs as well as a normal one were injected intramuscularly

with 1 ml. of an overnight culture of isolated strain. The animals were observed and the results were recorded.

Haemolytic activity test

The isolated strains were inoculated into thioglycollate broth (2) and after 24 hours' growth, cultures were centrifuged at 3000 r.p.m. for thirty minutes and the supernatant was removed for the tests. Red blood cells of sheep and rabbit, washed three times in five volumes of 0.85% NaCl, were used for haemolytic activity tests. In two rows of ten tubes, saline and toxin were mixed to give final dilutions of 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. Then 0.5 ml. of a 5 percent suspension of washed sheep red blood cells was added to each tube of the first row. The tube of the second row similarly received 0.5 ml. of a 5% suspension of washed rabbit red blood cells. The contents of all tubes were well mixed by inversion of tubes 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. It was allowed to stand at room temperature for 30 minutes and then mixed with 0.5 ml. of red blood cells suspension of sheep and rabbit as above. Titres of haemolytic activities were recorded 4 hours after mixing.

Neutralization test in the rabbit skin

Each strain was cultured into V.F. (viande-foie) broth and after 24 hours cultures were passed through a Seitz filter. 0.5 ml. filtrate of each strain was mixed with 0.2 ml. of nutrient broth and 0.1 ml. of specific antiserum of Cl. chauvoei. As a control 0.5 ml. of the filtrate was mixed with 0.3 ml. of nutrient broth. Mixtures were allowed to stand 30 minutes at room temperature. Serum-toxin mixture was injected intradermally on the right hand side and the control one on the left hand side of a depilated albino rabbit. The reactions were read after 24 and 48 hours.

Immunological differences between isolated strains

Each of three isolates was cultured in a medium consisted of bacto-casiton, yeast extraxt, cysteine hydrochloride, disodium hydrogen orthophosphate, glucose and normal horse serum, the pH being adjusted to 7.2. The culture was then converted to a killed vaccine. Each vaccine, prepared as above, was used to vaccinate a group of fifteen guinea-pigs by inoculating them twice at a 14 days interval with 2 ml. of the vaccine subcutaneously. Fourteen days after vaccination the vaccinated and two unvaccinated guinea pigs were challenged with 4 M.L.D. of isolated strains of Cl. chauvoei and the results were recorded.

Antibiotic and sulfonamide sensitivity tests

A 24 hour culture of the isolated strains were cultivated into fresh Bacto Brewer Anaerobic Agar (Difco) at 45°C. and 5% horse serum added to the mixture. The mixture delivered into plates, each plate containing four disks of

antibiotics or sulfonamides. The plates were placed into Brewer Anaerobic Jar and incubated for 24 hours at 37°C. The results were observed after 24 hours incubation (3).

RESULTS

Three strains of Cl. chauvoei were isolated from a severe outbreak of blackleg in southern part of Iran. The identification was based on the morphology, culture and biochemical properties of the organisms.

The pathogenicity, protection test, immunological test and several biological tests were used to confirm the identification.

The guinea pigs inoculated by the isolates died within 24 hours showing typical lesions of Cl. chauvoei infection at post mortem examination. Cl. chauvoei were recovered in pure culture from infected tissues.

In the immunological tests it was shown that guinea pigs immunized with Cl. oedematiens antiserum died with typical lesions characteristics of Cl chauvoei while those previously immunized with Cl. septicum antiserum survived. The animals inoculated with Cl. septicum antiserum resisted the challenge because the Cl. chauvoei infection was blocked by the septicum antiserum.

The results of neutralization tests in the skin of rabbit, showed no necrotic reaction on the site of injection.

The results of immunological differences between isolated strains summarized in Table No 1, indicated clearly that guinea pigs vaccinated with a formolized vaccine prepared from the isolates resisted the inoculation of virulent Cl. chauvoei. The findings indicated also that there is no immunological differences between the isolated strains.

Table No. 1

Challenge strains	VACCINE			Unvaccinated controls
	C.c 713	C.c.714	C.c715	
C.c713	5 S.	5 S.	5 S.	2 D.
C.c714	5 S.	5 S.	5 S.	2 D.
C.c715	5 S.	5 S.	5 S.	2 D.

S = Survived D = Died

The haemolytic activity of the isolates were studied using red blood cells of sheep and rabbits. The results of this experiment (Table 2) indicated that the toxins of all strains were more active on red blood cells of sheep than rabbit red blood cells (4).

Table No. 2

HAEMOLYSIS OF SHEEP AND RABBIT RED CELLS BY CLOSTRIDIUM CHAUVOEI TOXIN

Strain	Red cell suspension	Titre of haemolytic activity							
		1/2	1/5	1/10	1/30	1/50	1/100	1/200	1/500
C.c.713	Sheep	+ +	+ +	+ +	+ +	+ +	+ +	+	-
C.c.714	"	+ +	+ +	+ +	+ +	+ +	+ +	+	-
C;c.715	"	+ +	+ +	+ +	+ +	+ +	+ +	+	-
C.c.713	Rabbit	+ +	+ +	+ +	+ +	+	-	-	-
C.c.714	"	+ +	+ +	+ +	+ +	+	-	-	-
C.c.715	"	+ +	+ +	+ +	+ +	+	-	-	-

+ + Complete haemolysis + Partial haemolysis - No haemolysis

Fourteen antibiotics and five sulfonamide disks were selected for testing the sensitivity of isolated strains. The results summarized in Table No. 3. It was found that the isolated strains were more sensitive to Tetracycline, Aureomycin, Terramycin, Ampicillin, Erythromycin, Penicillin, Chloromycetin and Cephalotin. The organisms were found to be resistant or only slightly sensitive to Furacin, Vancomycin, Coly-mycin, Streptomycin, Kanamycin, Neomycin and Sulfonamides.

SUMMARY

A severe outbreak of blackleg occurred in epizootic form, amongst cattle in southern part of Iran. Three strains of Cl. chauvoei were isolated. The study on the morphology, pathogenicity, Immunological and biological properties of the strains confirmed the identification and showed that there was no immunological difference between them. The sensitivity of the isolates to several antibiotics and sulfonamides was also studied.

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Table No. 3

SENSITIVITY TESTS TO ANTIBIOTICS AND SULFONAMIDES

No.of strains tested	Observation Zones	Antibiotics	Sulfonamides
3	sensitive	Tetracycline	---
3	"	Aureomycin	---
3	"	Terramycin	---
3	"	Ampicillin	---
3	"	Erythromycin	---
3	"	Penicillin	---
3	"	Chloromycetin	---
3	slightly sensitive	Furacin	Sulfamerazine
3	" "	Vancomycin	Sulfadiazine
3	" "	Coly-mycin	Sulfadimethoxine
3	" "	---	Triple sulfa
3	" "	---	Sulfathiazole
3	Resistant	Kanamycin	----
3	"	Neomycin	----
3	"	Streptomycin	----