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ISOLATION, TYPING AND RAPID DIAGNOSIS OF PATHOGENIC CLOSTRIDIA FROM INFECTED ANIMALS IN IRAN. (*)

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Summary

Isolation, typing and rapid diagnosis of the pathogenic clostridia from infected animals are described in this paper. About one hundred and four strains of Cl. perfringens type A, twenty two strains of Cl. perfringens type B, eighteen strains of Cl. perfringens type C and seventy seven strains of Cl. perfringens type D were isolated and typed from the intestinal contents of sheep and goats suspected to enterotoxemia. Fifty one strains of Cl. oedematiens were isolated and typed from liver lesions of sheep, cattle and goats suspected to black disease. Thirty strains of Cl. chauvoei and five strains of Cl. septicum were isolated from infected materials suspected to blackleg and malignant oedema in cattle.

The fluorescent labelled antibodies with specific Cl. oedematiens, Cl. chauvoei and Cl. septicum antisera were used for rapid identification of the pathogenic clostridia.

Introduction

The clostridial infections among domestic animals were reported first in 1936 during an outbreak of blackleg of cattle in Iran. The first strain of Cl.chauvoei was isolated from blackleg of cattle in 1938. Further studies proved that the clostridial infections were widespread all over the country, (Kaveh 1957). The first strain of Cl.perfringens type D was isolated from cases of enterotoxemia of lamb and sheep in 1954 (Ardehali, 1969). Later on the infections caused by Cl.perfringens Iranian varient type B was isolated in 1954 from intestinal contents of enterotoxemia of sheep and goats. (Brooks et al 1957). Cl. perfringens type C was isolated in 1971 from cases of necrotic enteritis

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of piglets (Baharsefat et al 1974) and enterotoxemia of sheep 1971 (Ardehali 1982). The first strain of Cl.septicum from cases of gas gangrene (malignant oedema) in cattle was isolated in 1971 (Ardehali et al 1969). Cl.oedematiens type B strain, causative agent of black disease of sheep, was first isolated in 1969 (Ardehali et al 1975). Cl. oedematiens type D was also isolated from cases of liver necrosis in sheep, (Ardehali et al 1978). Cl.botulinum type E has also been isolated from intestinal contents of variety of fishes in Caspian sea (Rouhbakhsh-Khaleghdoust 1975). Cl.botulinum infections have not been encountered among the animals in Iran.

The method of isolation, typing and rapid diagnosis of pathogenic clostridia from carcases and specimens of infected animals is described in this paper.

Materials and Methods

I- Isolation and typing of Cl.perfringens from cases of enterotoxemia of lambs, sheep and goats.

Gram's stained slide was prepared and if organisms resembling Cl.perfringens were observed, the intestinal contents of suspected animals were streaked on the fresh sheep's blood agar. The plates of blood agar were incubated anaerobically in the Gas Pak jar at 37°C for 24 hours. Two colonies resembling Cl.perfringens were picked up and transferred in to the fresh liver broth and incubated for 24 hours. To determine the major lethal toxins, namely alpha, beta and epsilon of each isolate, the culture was transferred in a fresh cooked meat tube and incubated for 5-6 hours. Each culture was centrifuged at 3000 rev/min and the supernatant was used for identification of Cl. perfingens types A, B, C and D by the method of neutralization tests in mice (Table No. 1) described by Sterne and Batty (1975).

Antiserum — The specific Cl.perfringens diagnostic antisera (*) were used for typing of the isolated strains.

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Table 1

Typing of Cl.perfringens: Neutralization tests in mice (Sterne and Batty)

No.		ml of Filtrate	Antisera 0.1ml A α	C β	D ε	ml of Diluent	Types A	B	C	D
1.	Untrypsinized	0.5	–	–	–	0.3	d	d	d	d
2.		0.5	+	–	–	0.2	L	d	d	L*
3.		0.5	+	+	–	0.1	L	L*	L	L*
4.		0.5	+	–	+	0.1	L	d	d	L
5.		0.5	+	+	+	0.0	L	L	L	L
6.	Trypsinized	0.5	–	–	–	0.3	d	d	d	d
7.		0.5	+	–	–	0.2	L	d	L'	d
8.		0.5	+	+	–	0.1	L	d	L	d
9.		0.5	+	–	+	0.1	L	L'	L'	L
10.		0.5	+	+	+	0.0	L	L	L	L

L = lived d = died += addition of 0.1 ml antiserum

* Deaths could occur if epsilon toxin has been activated naturally. This is likely with intestinal contents.

' = Although trypsinization usually destroys beta toxin. A significant amount may remain if the original titre was exceptionally high.

II- Isolation, typing and rapid identification of Cl.oedematiens from cases of black disease of sheep, goat and cattle.

For rapid identification of the organism, smear was taken from necrotic liver lesions and stained by specific Cl.oedematiens labelled antiserum (*) (Batty and Walker 1964). The organisms are stained brightly and clearly distinguishable by fluorescence microscope. For isolation of the extremely fastidious Cl.oedematiens, the causative agent of black disease, the liver lesion was immediately streaked on a fresh solidified horse's blood agar (Moore 1968). The plates were incubated anaerobically for 48 hours at 37°C. The colonies resembled to Cl. oedematiens were picked up and cultured into a fresh liver medium and the organisms are kept as freeze dried ampoules for further study.

Typing of Cl.oedematiens

Each strain was inoculated in a flask of 250 ml of freshly prepared medium containing 3.0 percent proteose peptone, 1.0 percent maltose, 0.5 percent meat particles at pH=8 (Nishida et al 1964). The culture was incubated for 72 hours to obtain maximum yield of the toxin. Each culture was centrifuged at 3000 rev/min and the supernatant was tested for typing of Cl.oedematiens types A, B and D (Table 2) according to the method described by Sterne and Batty (1975).

Table 2

Methods of typing of strains

Identification of Cl.oedematiens types A, B and D by the test of their dermonecrotic action in guinea-pigs and their lecithinase activity

No. of mixture	ml of filtrate	ml of antiserum anti-*Cl.oedematiens*			ml of diluent	Results of lecithinase and skin reactions					
		Type A	Type B	Type D		L	Skin test	L	Skin test	L	Skin test
1.	0.3	0	0	0	0.2	+	+	+	+	+	+
2.	0.3	0.1	0	0	0.1	–	–	+	–	+	–
3.	0.3	0	0.1	0	0.1	+	–	–	–	+	+
4.	0.3	0	0	0.1	0.1	+	+	–	+	–	–
5.	0.3	0	0.1	0.1	0	+	–	–	–	–	–
		Toxin identified				γ	α	β	α	β	β
		Types of *Cl.oedematiens* identified				A		B		D	

L= lecithinase reaction

III- Isolation and rapid identification of Cl.chauvoei

Two smears were taken from the infected muscle and stained by specific Cl.chauvoei and Cl.septicum labelled antisera to differentiate between two organisms, (Batty et all 1963).

The muscle lesion was streaked on the surface of fresh sheep's blood agar. The plates were incubated anaerobically for 48 hours. The resembled colony was confirmed by using Cl.chauvoei and Cl. septicum fluorescent labelled antisera. Three colonies were picked up and inoculated into fresh liver broth and incubated anaerobically for 24-48 hours. The bone marrow was also inoculated into fresh liver broth and incubated anaerobically for 48 hours. Smear was taken and stained by Cl.septicum and Cl.chauvoei specific fluorescent labelled antisera.

The isolated strains of Cl.chauvoei were freeze dried in ampoules for further studies.

IV- Isolation and rapid identification of Cl.septicum

The above mentioned method was also used for identification of Cl.septicum organism.

Results

Cl.perfringens type A

One hundred and four strains of Cl.perfringens type A have been isolated and typed from intestinal contents of sheep, goat, cattle and fish, (Ardehali 1967). They seem to be normal inhabitants of digestive tract of animals (Sterne et al 1975).

Most of the isolated strains were not toxigenic but some produced lethal alpha toxin. The maximum titre of alpha toxin obtained was 20 M.LD.. per ml in mice. The isolated strains were not heat resistant.

Cl.perfringens type B

Twenty two of Cl.perfringens type B strains were isolated from intestinal contents of ten sheep, six lambs, three goats and three kids which died from fatal enteritis and lamb dysentery, (Ardehali et al 1982). All strains of Cl.perfringens type B isolated in Iran produced the major alpha and beta toxins. The strains isolated from adult sheep, goats and kids were found to be different from classical type B strains in their production of kappa and non production of lambda toxin and hyaluronidase, (Brooks et al 1975). Three strains which had been isolated from cases of lamb dysentery were identified in the classical group of type B, (Table 3).

Table 3

Distribution of toxins in Iranian isolates of Cl.perfringens type B

TYPE	DISEASE	Major lethal and non lethal antigens											
		α	β	ε	ι	γ	δ	η	θ	κ	λ	μ	ν
Classical type B	Lamb dysentry	++	++	++	0	++	0	—	++	0	++	++	++
Variant type B	Haemorrhagic enteritis of sheep and goats	++	++	++	0	—	—	—	++	++	0	0	+

++ Proluced by all strains
+ Produced by some strains
0-Not produced by any
— Not tested

Cl.perfringens type C strains

Eighteen strains of Cl.perfringens type C were isolated from intestinal contents of infected animals. Seven strains from fatal entero-

toxemia in young sheep, three strains from enterotoxemia of goats, five from infectious enteritis of piglets and two from fecal materials of sow. All the isolated strains produced major alpha and beta toxins.

Cl.perfringens type D strains

Seventy seven strains of Cl.perfringens type D had been isolated and typed from intestinal contents of lambs sheep, goats and kids suspected to enterotoxemia. The major alpha and epsilon toxins were identified by seru-neutralization tests in mice. Sixty seven strains were isolated from enterotoxemia of lambs and sheep, ten strains from enterotoxemia of kids and goats.

Cl.oedematiens types A, B and D strains

Fifty one strain of Cl.oedematiens were isolated and typed from liver lesions of sheep, cattle and goats suspected to black disease. The fluorescent labelled antibodies with specific Cl.oedematiens antisera were used for identification of Cl.oedematiens from liver lesions of suspected black disease. Among isolated strains, forty three were type B, four type A and three type D, (Ardehali and Darakhshan 1977).

Cl.chauvoei strains

Thirty strains of Cl.chauvoei were isolated from infected muscles and bone marrow from cases of blackleg. The fluorescent labelled antibodies technique has been used for the identification of Cl.chauvoei from pathological materials and cultures. This technique is very valuable for differentiation between Cl.septicum and Cl.chauvoei from the decomposed materials.

Cl.septicum strains

Five strains of Cl.septicum were isolated from cases of gas gangrene of cattle in Iran. The isolated organisms were identified by specific Cl.septicum labelled antiserum.

Discussion

Clostridial infections were diagnosed first in 1936 among the domestic animals in Iran. First stain of Cl.chauvoei was isolated in 1938, later on other pathogenic clostridia were isolated and typed from carcases and pathological specimens. Cl.perfringens type B (classical)

was the causative agent of lamb dysentery and Cl.perfringens Iranian varient type B which caused haemorrhagic enteritis of sheep and goats in Iran. Iranian type B strains produced beta and epsilon toxins which is the characteristic of type B but in minor antigens it produced kappa toxin (collagenase) instead of lambda toxin (proteinase) and also failed to produce hyaluronidase which was produced by all classical type B strains (Brooks et al 1957). Cl.perfringens type C which caused enterotoxemia in young sheep and necrotic enteritis in piglets have not been encountered all over the country. Many strains of Cl. perfringens type D which have been isolated from cases of enterotoxemia of lamb and sheep is very widely spread in sheep husbandry in Iran. Cl.oedematiens type B is the causative agent of very fatal black disease of sheep and goats. Most of the isolated strains were found to be highly toxigenic in mice. Specific Cl.oedematiens labelled antiseum was used for the rapid identification of the organisms in liver lesions and cultures. Two strains of Cl.oedematiens type D have also been occasionally isolated from the liver necrosis of sheep. Blackleg in cattle caused by Cl.chauvoei occurs in most parts of Iran. The fluorescent labelled antibodies was used as a routine for the rapid identification of Cl.chauvoei from infected muscle and bone marrow. This method is also most helpful in differentiation between Cl.chauvoei and Cl.septicum from decomposed specimens. Cl.septicum was isolated from bone marrow of cattle. The organisms caused gas gangrene (malignant oedema) in cattle. Cl.tetani has not been isolated but tetanus occured in wide variety of animals. The other clostridial infections of animals have not been encountered in Iran.

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