

SOME OBSERVATIONS ON CLOSTRIDIUM PERFRINGENS STRAINS ISOLATED IN IRAN (*)

by

M. ARDEHALI

Since diseases caused by anaerobic organisms were recognized in sheep and goats in Iran in 1938 (RAFYI and ARDEHALI [1961]) more than 110 toxigenic strains of *Cl. perfringens* (*welchii*) have been isolated at the Razi Institute.

These strains have been classified as type A, classical type B, Iranian type B, and type D. No strain of *Cl. perfringens* type C has so far been isolated and there is no report or evidence on the existence of disease related to type C in this country.

This paper presents the summary of some preliminary studies on the types of *Cl. perfringens* isolated in Iran, for reference in future studies in this field.

MATERIALS AND METHODS

Source of materials :

Materials from suspected cases of anaerobic diseases were received from different parts of Iran by the Veterinary Department. The materials consisted mostly of intestinal contents to which chloroform had been added at the rate of 0.5 percent.

Antiserum :

The specific Burroughs Wellcome Diagnostic and monospecific antisera were used in this investigation.

Isolation of the organisms :

The specimens received were seeded on the surface of blood agar and also in chopped meat broth containing 1 percent glucose. The seeded blood agar was incubated anaerobically for 18 to 20 hours at 37° C in a Brewer anaerobic jar

(*) Presented to the Second World Symposium on Diseases Caused by Anaerobes, Paris, 5-7 July 1967.

Reprinted from Bull. Off. int. Epiz., 1967, 67 (9-10), 1207-1214.

(BREWER, HEER and MCLAUGHLIN [1955]). The infected chopped meat broth was subcultured on blood plates which were treated as above. The isolated colonies which showed the characteristic properties of the organisms were subcultured to produce pure cultures. The isolated strains were eventually tested for their biochemical properties in peptone water sugar, serum and azocoll agar plates in the usual manner (SMITH [1954]). The strains were kept as freeze dried ampoules at 4° C.

Examination for major lethal toxin of strains.

To determine the major lethal toxin, namely α , β , ϵ , and δ of each isolate, the strain was grown in Robertson's meat broth. Then the Seitz filtrates from six hours culture was used for identification of type of *Cl. perfringens* using neutralization tests in guineapigs and mice.

A.— NEUTRALIZATION TEST IN THE SKIN OF GUINEAPIG.

Bacteria free filtrates of the examined strains were used for the necrotizing skin test into the skin of depilated albino guineapigs (OAKLEY and WARRACK [1953]). Each filtrate was divided into two parts. The first part was activated by 0.4 per cent trypsin powder (Difco) for 60 minutes at 37° C and the other part was used unactivated.

The following mixtures were made up and allowed to stand for 30 minutes at room temperature before injection.

Scheme

I

- (1) 0.5 ml filtrate --- 0.3 ml broth.
- (2) 0.5 ml filtrate — 0.2 ml broth — 0.1 ml *Cl. perfringens* type A antiserum.
- (3) 0.5 ml filtrate — 0.1 ml broth — 0.1 ml *Cl. perfringens* type A antiserum — 0.1 ml *Cl. perfringens* type C antiserum.
- (4) 0.5 ml filtrate — 0.1 ml *Cl. perfringens* type A antiserum — 0.1 ml *Cl. perfringens* type C antiserum — 0.1 ml *Cl. perfringens* type D antiserum.

II

- (1) 0.5 ml activated filtrate — 0.3 ml broth.
- (2) 0.5 ml activated filtrate — 0.2 ml broth — 0.1 ml *Cl. perfringens* type A antiserum.
- (3) 0.5 ml activated filtrate — 0.1 ml broth — 0.1 ml *Cl. perfringens* type A antiserum — 0.1 ml *Cl. perfringens* type D antiserum.
- (4) 0.5 ml activated filtrate — 0.1 ml *Cl. perfringens* type A antiserum — 0.1 ml *Cl. perfringens* type C antiserum — 0.1 ml *Cl. perfringens* type D antiserum.

All tests were performed in four injections per side of depilated albino guineapigs in volumes of 0.2 ml. Necrotic reactions were read after 24 and 48 hours. Yellowish necrotic lesions, purplish necrotic lesions and whitish necrotic lesions with small patches purple from haemorrhages were attributed to α , β and ϵ toxin, respectively.

B. — SERUM NEUTRALIZATION TESTS IN MICE.

Similar mixtures as above were prepared for intravenous injection into groups of two mice in volumes of 0.3 ml. The results were read after 48 hours.

All results in both tests were recorded and interpreted as follows (see Table I).

Tests for minor lethal and non-lethal antigens.

Each strain was grown in medium described by BROOKS *et al.* (1957) and tested for production of minor antigens as below:

1. Lecitho-vitellin tests for detection of α toxin as described by MAC-FARLANE, OAKLEY and ANDERSON, 1941.
2. Haemolytic tests using sheep red blood cells for α , θ and δ antigens (OAKLEY and WARRACK [1953]).
3. Collagen paper and azocoll tests for λ and λ toxins as described by OAKLEY, WARRACK and WARREN, 1948.
4. ACRA tests using horse synovial fluid as substrate for μ toxin and for deoxyribonuclease tests, sodium deoxyribonucleate used as substrate (OAKLEY and WARRACK [1951]).

TABLE I

FILTRATE			TRYPSINISED filtrate			ANTIGEN present	TYPE
Diluent	Anti- α	Anti- α and β	Diluent	Anti- α	Anti- α and ϵ		
+	—	—	+	—	—	α	A
+	+	—	+	+	—	β and ϵ	B
+	+	—	+	—	—	β	C
—	—	—	+	+	—	ϵ	D

TABLE II

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 dysentery	++	++	++	0	++	0	--	++	0	++	++	++
Variant Type B	Haemorrhagic enteritis of goats and sheep	++	++	++	0	—	—	—	++	++	0	0	+

++ Produced by all strains;
0 not produced by any;

+ produced by some strains;
— not tested.

RESULTS

Types of "Cl. perfringens" isolated in Iran.

Of the one hundred and ten specimens examined, four types of *Cl. perfringens* were classified as follows:

Cl. perfringens type A, 94 strains.

Cl. perfringens type B, Iranian variety 8 strains.

Cl. perfringens type B, classical type, 2 strains.

Cl. perfringens type D, 6 strains.

Type A strains. From 110 specimens examined, 104 isolates were identified as type A. These were isolated from intestinal contents of fish, cattle and sheep. They seem to be normal inhabitants of the digestive tract. They were not heat resistant.

Type B strains. From 10 specimens classified as *Cl. perfringens* type B, one was isolated from the intestine of a six week-old kid, three from the intestines of goats, four from the intestines of adult sheep, and two from the intestines of young lambs.

These isolates could be typed into two groups according to their minor antigens :

1. *Classical type B.* The major lethal and minor lethal and non-lethal antigens produced by classical type B are described by OAKLEY and WARRACK (1953).

Two isolates were identified in this group. These have recently been isolated from cases of lamb dysentery in Iran (RAFYI and ARDAHALI [1963]).

2. *Iranian type B strains.* Isolates belonging to this group produce major lethal toxin similar to those of classical type B, but in production of minor antigens they fail to produce kappa and hyaluronidase toxins (BROOKS and ENTESSAR [1957]). Eight isolates were found to belong to this type.

Type D strains. *Cl. perfringens*, type D, was isolated from the intestines of six young lambs and adult sheep. None of the *Cl. perfringens* type D showed any difference from the classical type D whose properties have been described by OAKLEY and WARRACK (1951).

* * *

SUMMARY

Types A, B and D of *Cl. perfringens* have been isolated from 110 specimens received from different parts of Iran.

Classical type B strains have recently been isolated from cases of lamb dysentery. The isolates of *Cl. perfringens* type B which differ from the classical type B in production of some minor toxins have been found in cases of haemorrhagic enteritis of goats and adult sheep but not in young lambs in Iran. Type A and D strains found in Iran show no difference from the classical types A and D.

* * *

RESUME

Les types A, B et D de *Cl. perfringens* ont été isolés de 110 prélèvements provenant de différentes régions d'Iran.

Des souches classiques du type B ont été récemment isolées dans des cas de dysenterie des agneaux. Des souches de type B, différent du type B classique par la production de quelques toxines mineures, ont été trouvées en Iran dans les cas d'entérite hémorragique de la chèvre et de la brebis adultes, mais pas chez de jeunes agneaux. Les souches de types A et D trouvées en Iran ne montrent pas de différence avec les types A et D classiques.

* * *

RESUMEN

Se aislaron los tipos, A, B y D de *Cl. perfringens* de 110 tomas procedentes de distintas regiones de Irán.

Recientemente se han aislado cepas clásicas del tipo B, en casos de disenteria de los corderos. En Irán, se han encontrado cepas de tipo B, diferente del tipo B clásico por la producción de algunas toxinas menores, en casos de enteritis hemorrágica de la cabra y de la oveja adulta, pero no en corderos jóvenes. Las cepas de tipos A y D encontradas en Irán no muestran diferencia con los tipos A y D clásicos.

* * *

ACKNOWLEDGMENTS

The writer is greatly indebted to Dr. KAVEH, Director of Razi Institute, for permission to publish this paper, to Dr. G. H. WARRACK for supplying antisera and to Mr. DURAN for his technical assistance.

* * *

REFERENCES

- BREWER (J.H.) HEER (A.A.) & MCLAUGHLIN (C.B.)— *Appl. Microbiol.*, 1955, **3**, 136.
BROOKS (M.E.) & ENTESSAR (F.)— *Arch. Inst. Hessek*, 1957, **11**, 20.
MACFARLANE (R.G.), OAKLEY (C.L.) & ANDERSON (C.G.)— *J. Path. Bact.*, 1941, **52**, 99.

- OAKLEY (C.L.) & WARRACK (G.H.)—*J. Path. Bact.*, 1951, **63**, 45.
OAKLEY (C.L.) & WARRACK (G.H.)—*J. Hyg. Camb.*, 1953, **51**, 102.
OAKLEY (C.L.) WARRACK (G.H.) & WARREN (M.E.)—*J. Path. Bact.*, 1948,
60, 495.
RAFYI (A.) & ARDAHALI (M.)—*Bull. Off. int. Epiz.*, 1961, **55**, 999,
RAFYI (A.) & ARDAHALI (M.)—*Bull. Off. int. Epiz.*, 1963, **59**, 1283,
SMITH (L. DS). — Introduction to the Pathogenic Anaerobes, 1955, *Chicago*,
University of Chicago Press.