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PRODUCTION AND STANDARDIZATION OF POLYVALENT *CLOSTRIDIUM PERFRINGENS* VACCINE IN IRAN (*)

M. Ardehali and H. Darakhshan

ABSTRACT

In this presentation an account of the large-scale production and the standardization of polyvalent *Clostridium perfringens* vaccine in Iran is described. Over 20 million doses of this vaccine are produced and utilized in Iran every year. Certain modifications have been made to the culture media used in our laboratory for the production of this vaccine in order to bring down its cost. The prepared vaccine is highly immunogenic as determined by the laboratory examination on the quality of the vaccine according to British Veterinary Codex and the field reports.

RESUME

Il est fait un compte rendu de la production à grande échelle et de la standardisation du vaccin polyvalent contre *Clostridium perfringens* en Iran. Plus de 20 millions de doses de ce vaccin sont produites et utilisées en Iran chaque année. Certaines modifications ont été apportées aux milieux de culture employés, dans notre laboratoire, pour la production de ce vaccin afin d'en diminuer le coût. Le vaccin préparé est hautement immunogène comme l'attestent les examens de laboratoire portant sur la qualité du vaccin conformément au Codex Vétérinaire Britannique ainsi que les rapports concernant son emploi sur le terrain.

(*) Joint OIE – IABS symposium on clostriadial products in Veterinary Medicine, Paris 1975. Develope. biol. standard., Vol. 32. pp. 31–34 (Kargel, Basel 1976).

Clostridial infections are among the most important diseases of cattle and sheep in Iran. Among the various diseases caused by these groups of bacteria, lamb dysentery, struck, pulpy kidney and black disease in sheep and blackleg in cattle are often observed in this country. We have isolated the strains of *Cl. perfringens* types B,C and D, *Cl. oedematiens* types B and D, *Cl. chauvoei* and *Cl. septicum* from the specimens of infected animals at the Razi Institute.

Since 1939 we have been preparing vaccines against clostridial infections. The first clostridial vaccine we prepared was against blackleg of cattle; later, other kinds of clostridial vaccines were introduced. Today, three kinds of vaccines are prepared in the Razi Institute; polyvalent vaccine against lamb dysentery and pulpy kidney diseases, vaccine against black disease and vaccine against blackleg. In 1974, the Razi Institute delivered to the Veterinary Organization of Iran 18 million doses of polyvalent vaccine for lamb dysentery, struck and pulpy kidney, 3 million doses for black disease of sheep and 3 million doses of vaccine for blackleg of cattle.

This presentation is mainly concerned with the preparation and standardization of *Clostridium perfringens* vaccines in the Razi Institute.

PRODUCTION OF *CLOSTRIDIUM PERFRINGENS* VACCINES

Media

Both the following media are used:

1st medium (synthetic)

used for the production of *Cl. perfringens* types B, C and D vaccine

Peptone (Evans or Oxoid)	3 %
NaCl	0.25 %
Na2HP04	1 %
Glucose (for types B and C)	1 %
Dextrin (for type D)	1 %
Trace element solution	0.5 ml%
Vitamin solution	0.2 ml%

Final pH: 7.6

The peptone, sodium chloride and sodium phosphate are dissolved in distilled water and distributed into 20 litres of Pyrex bottles (15 litres/bottle). The bottles are autoclaved at 110°C for 40 min. The solutions of trace elements (FeSO4, 7H20:05 g%; CuSO4, 5H20:0.02 g%; ZnSO4, 7H20:0.02 g%; MnCl2, 4H20: 0.008 g%; MgSO4, 7H20:4 g%), vitamins (biotin 0.05 mg%; thiamine

50 mg%; nicotinic acid 50 mg%; pyridoxine 50 mg%; vitamin B12 0.0025 mg%), glucose and dextrin are prepared separately. They are Seitz-filtered and then added to the sterilized bottles.

2nd medium (Manhouri & Ardehali, 1966)

used for the production of *Cl. perfringens* type D vaccine

Horse plasma residue pancreatic digest	0.3	g	T.N.%
NaCl	0.25	g	%
PO4HNa2, 7H20	0.7	g	%
Dextrin	1.0		%
Trace element solution	0.5	ml	%
Vitamin solution	0.2	ml	%
Final pH 7.8			

Horse plasma residue pancreatic digest, sodium chloride and sodium phosphate are dissolved in distilled water and distributed into 20 litres of Pyrex bottles (15 litres/bottle). The bottles are autoclaevd at 110°C for 40 min. The solutions of trace elements, vitamins and dextrin are prepared separately and then added to sterilizebottles.

Preparation of vaccines

For the production of vaccines in our Institute we use the strains of *Cl. perfringens* type B (C.W.B. 207), *Cl. perfringens* type C (C.W.C. 301) and *Cl. perfringens* type D (C.W.D. 401). A 24–48 hours culture of each type of organism is inoculated in 500 ml flasks containing 350 ml of the medium. After 16 hours of incubation the contents of each flask are then added to each bottle of the medium which has been left in the incubator for a few hours before inoculation. The optimum times required for maximum production of each toxin are for types B and C: 5–7 hours, for type D: 18–20 hours. The pH of the culture of type D is adjusted twice during its incubation period. Incubation samples are taken from each bottle for titration and control of non-specific organisms; contaminated bottles are discarded. A volume of 90 ml of 40% of commercial formaldehyde solution is added to each bottle and the pH is adjusted to 7.0 with 20% of sodium hydroxyde. After 7–14 days when the conversion of toxin to toxoid is completed, a sample of each bottle is taken for potency and safety tests and the bottles are stored at 4–10° C for two months before use.

For production of a polyvalent *Cl. perfringens* vaccine, the three types of the prepared vaccines are mixed with the proportion of one part of types B and C and two parts of type D in a 3 ml dose of vaccine.

QUALITY CONTROL OF VACCINES

The potency and safety of the vaccines are determined according to the British Veterinary Codex (1970) briefly described as follows.

Preparation of beta and epsilon toxins

For the preparation of beta toxin the strain of *Cl. perfringens* type C (C.W.C. 301) and for epsilon toxin the strain of *Cl. perfringens* type D (C.W.D. 401) are grown separately on two litres of the medium consisting of 3% pancreatic digest of casein (N.Z. casein BDH), 0.5% extract of yeast, 0.5% dipotassium phosphate, 0.01% magnesium sulphate and 1% glucose at pH 7.6. After 6 hours incubation each culture is centrifuged and the toxin is separated from the supernatant by ammonium sulphate precipitation. After the addition of 700 g of ammonium sulphate to one litre of the supernatant and mixing well the mixture is kept in a 4° C refrigerator overnight. The following day the precipitated toxin is collected and dried *in vacuo* over phosphorus pentoxide. The dried toxin is powdered in a mortar and washed three times with chloroform. Finally the powdered toxin is stored in a dessicator at room temperature.

Preparation of standard antitoxin

In our laboratory sheep are used for the preparation of standard antitoxin against *Cl. perfringens* types C and D toxins (1). The animals are hyperimmunized according to the method described by Bittner et al. (1966). Sheep developing high titres of antitoxin are selected; the antitoxin present in each serum is purified and concentrated according to Pope (1938). Finally the prepared antitoxins are calibrated against the International Standard Antitoxins: *Clostridium perfringens* type B antitoxin and *Clostridium perfringens* type D antitoxin obtained from the International Laboratory for Biological Standards, Central Veterinary Laboratory, Weybridge.

Potency test

The immunogenic potency of each batch of *Cl. perfringens* types B, C and D combined vaccine is determined according to the British Veterinary Codex (1970). Twelve rabbits are given two injections of the vaccine; fourteen days after the second injection the animals are bled, sera are separated from the collected blood and 1 ml of each serum is taken and pooled. The units of antitoxin present in the pooled serum are estimated by titration in Swiss white mice. Dilutions of beta and epsilon toxins in physiological-saline are prepared from the dried materials. The test dose of toxins is determined by standard antitoxins. To assay the unit of antitoxin in the pooled serum, the test dose toxin is mixed with serial dilutions of serum. The mixtures are kept at room temperature for

30 min and 0.5 ml. of each mixture is injected intravenously into each of 3 mice. The 50% end point is calculated and considered as the unit of antitoxin in pooled rabbit serum.

The results of potency tests routinely obtained from different batches of *Cl. perfringens* vaccines show that the pooled rabbit sera contain an average of 15 International Units of beta antitoxin per ml and 6 International Units of epsilon antitoxin per ml.

The reports obtained from the field indicate that lamb dysentery and pulpy kidney diseases could be effectively controlled by this vaccine. There have been no cases of disease reported from the field after immunization of the sheep by two injections of the vaccine.

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