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Bovine Pasteurellosis in Iran

Methods of Vaccination

by

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Haemorrhagic Septicaemia occurs in Iran in areas with special climatic and soil conditions. It shows a preference for humid and marshy zones; particularly in the North, along the coast of the Caspian Sea, in the north-west, near to the Lake of Rezaie and also in the south-west towards the basin of the river Karoun; on the other hand it has never been recorded on the plateau where the soil is usually very dry.

In the infected area the disease has an enzoo-epizootic nature and has been responsible for thousands of bovine deaths. Since 1935 a profound study of the condition has been made by the research workers at the Razi Institute and several strains of Pasteurella have been isolated by the usual procedures. In 1938, Delpy and Rastegar (1 and 2) and later Delpy and Mirchamsy (3), after having tried the types of vaccine prepared in all countries of the world, adopted a vaccine prepared with very virulent strains of cattle and buffalo origin, lysed and treated with saponin in the manner later described by these authors. The immunising value of this vaccine was demonstrated and proved very satisfactory.

Vaccinations were practised methodically in the infected regions with a success that increased day by day; it has also been found that in addition to its rapid action in the lysis of the organism, saponin is also a

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first-class adjuvant in retarding the absorption of the vaccine, thereby provoking a solid and durable immunity.

The number of doses used since 1938 increases yearly so that in 1958 and 1959 we have issued respectively 566,000 and 600.000 doses of vaccine against haemorrhagic septicaemia; as a result the disease was controlled effectively and only rare cases are now seen in the infected area. One may add that no case of the disease has ever been seen in an animal vaccinated methodically.

Characteristics and type of the strains of Pasteurella from Iran

Several strains of Pasteurella multocida have been isolated from cattle, buffaloes, pigs and fowls. These strains have been typed using the technics of Roberts (1947) (4) and Carter (1959) (5). Strains of bovine, bubaline and porcine origin have been found to be of type I of Roberts or B. Carter. Strains isolated from cattle, pigs and fowls in dry regions where the disease is virtually non-existant have been of type II or III or unidentifiable. The biochemical characters have confirmed the biological results. Table I summarises the type of the strains isolated in Iran.

TABLE I

Host	Region where strain isolated	Number of strains examined	Type determination				
			I	II	III	IV	unclassifide
Cattle	damp soil where HR is enzootic	8	8	—	—	—	—
Cattle	dry area	2	—	—	2	—	—
Buffaloes	damp soil	3	3	—	—	—	—
Pigs	damp soil	4	4	—	—	—	—
Pigs	dry area	1	—	—	—	—	1
Birds	dry area	2	—	1	—	—	1
	Total	20	15	1	2	—	2

Technique and the method of preparation of the Haemorrhagic Septicaemia Vaccine used in Iran

In order to have a vaccine of good antigenicity and high efficacity the following points must be kept in mind:-

1.) Utilise very virulent strains of Roberts' group I of bovine or buffaloe origin. They should be capsulated in phase F. They are maintained in the heart-blood of calves collected after death from an experimental infection. They may be kept lyophilised but it is necessary to make passages in calves before use to prepare vaccine.

2) Convert the suspension of **Pasteurella** into vaccine in such a way that the capsular antigen remains intact. To this end we have found that saponin alone or associated with merthiolate has a very suitable bactericidal effect. In this way certain antigenic factors in the **Pasteurella,** which play an important role in immunization, are not damaged. The saponin also serves as an adjuvant.

3) The number of bacteria in a dose of vaccine is very important and should be at least 5 X 10^9 organisms or 2.0 mg dry weight of organisms.

Culture and Preparation of Vaccine

The organisms are Cultured on nutrient agar for 24-36 hours at a temperature of 35. The growth is harvested by washing off with distilled water containing 1:10,000 merthiolate and diluted to give a concentration of 2,500 millions of organisms per ml.

The suspension so prepared is sterile up to staying for 48 hours at room temperature. To it is then added a concentrated solution of saponin so that the final concentration of the latter is 2:1,000. The saponin used is the English preparation of B.D.H. It is advisable to find the most suitable concentration experimentally for each lot of this product.

The vaccine so prepared is ready for testing on experimental animals. Before inoculating cattle it is necessary to control the sterility by culture and injection to rabbits. Occasionally the vaccine is sterile on culture but still is found to contain live organisms in inoculation to rabbits. Once sterility is confirmed, we inject increasing doses of vaccine to 12 calves (table II). For the test we use two-year old calves weighing 100-120 Kg. The dose used at the Razi Institute is 2 cc for cattle and buffaloes which corresponds to 5X109 organisms transformed into vaccine antigen or 2.0 mg dry **Pasteurella.** Animals vaccinated with this dose are challenged 14 days later with 100 m. l.d. ✡

★ m.l.d. corresponds to 1 cc of dilution of a 1: 8,000 6-hour broth culture of **P. multocida**; this corresponds to 100,000-120,000 organisms of Pasteurella for a calf of 90-100 kg.

Test of the Vaccine

Each batch of vaccine should be titrated and tested from the point of view of safety and potency Table III illrstrates the type of protocol of tests as followed by the Razi Institue.

Table II

No. of calves	Safety				Potency	
	Material	dose	Local reaction	Date	Material	Result
1	Vaccine batch 37	0,5cc	oedema 6x8 cm	after two weeks	25 M.L.D.	Resisted
2	»	0,5cc	6x6 cm	»	25 M.L.D.	»
3	»	1 cc	8x9 cm	»	50 M.L.D.	»
4	»	1 cc	9x9 cm	»	50 M.L.D.	»
5	»	2 cc	9x18 cm	»	100 M.L.D.	»
6	»	2 cc	6x10 em	»	100 M.L.D.	»
7	»	5 cc	7x16 cm	»	100 M.L.D.	»
8	»	5 cc	7x19 cm	»	100 M.L.D.	»
9	»	10 cc	10x20 cm	»	200 M.L.D.	»
10	»	10 cc	10x30 cm	»	200 M.L.D.	»
11	control	—	—	»	2 M.L.D.	dide from Pasteur-cllosis
12	control	—	—	»	1 M.L.D.	»

Duration of Immunity conferred by the saponized Vaccine

As we have mentioned at the beginning of this report, the immunity conferred by the saponized vaccine is in practice about a year and during the 20 years that we have used it, we have received no complaints of failure to protect. Animals are usually vaccinated once a year in the areas where haemorrahagic septicaemia is known to occur. In order to test the duration of immunity experimentally, we vaccinated 12 calves of the same age and retained them under observation for a year. These animals were divided into four groups one of which was tested after the elapse of each 3 months. At the same time a control animal that had not been vaccinated was tested. Table III gives the results obtained.

Table III

No. of calves	Vaccination				Immunity Test	
	Material	Dose	Local reaction	Duration	Material	Result
1	saponised vaccine	2cc	present	3 months	100 M.L.D.	resisted
2	"	2cc	"	"	"	"
3	"	2cc	"	"	"	"
4	control	–	–	"	2 M.L.D.	died
	vaccine	2cc	"	6 months	100 M.L.D.	resisted
6	"	2cc	"	"	"	"
7	"	2cc	"	"	"	"
8	control	–	–	"	2 M.L.D.	died
9	saponised	2cc	"	9 months	100 M.L.D.	resisted
1	vaccine					
10	"		"	"	"	"
11	"	2cc	"	"	"	died
12	control	–	–	"	2 M.L.D.	died
13	saponised	2cc	"	12 months	100 M.L.D.	resisted
	vaccine					
14	"	2cc	"	"	"	
15	"		"	"	"	died
16	control	–	–	"	2 M.L.D.	died

★ Local reaction : localoedema of 6 - 8 cm. diameter.

Thus as the above table demonstrates all the vaccinated animals resisted challenge after 3 and 6 months; but at 9 months and one year, there was one death in each group of 3 animals. In spite of the apparent fall in immunity starting from the sixth month, 70% of the animals still resisted a fairly severe test dose at the twelfth month.

Comparative Study of the Immunizing Power of some Vaccines

Quite recently our Institute has undertaken a comparative study of the immunizing power of certain vaccines. We took 38 cattle and divided them into 4 groups of nine head, two being retained as controls. Each

group received a vaccine prepared in such a way that the only difference between the vaccines was in the adjuvant. The results will be seen in Table IV.

Table IV

Type of vaccine	No. of animals inoculated	Dose	Interval between vaccination and challenge	Challenge dose	Result	p.c. protected
Merthiolate saponine vaccine	3	1 cc	24 days	100 M.L.D.	3/3	100p.c.
,,	3	,,	,, ,,	150 M.L.D.	3/3	100p.c.
,,	3	,,	,, ,,	200 M.L.D.	3/3	100p.c.
Formolised saponine vaccine	3	,,	,, ,,	100 M.L.D.	3/3	100p.c.
,,	3	,,	,, ,,	150 M.L.D.	3/3	100p.c.
,,	3	,,	,, ,,	200 M.L.D.	3/3	100p.c.
Alum treated formol vaccine	3	,,	,, ,,	100 M.L.D.	3/3	100p.c.
,,	3	,,	,, ,,	150 M.L.D.	3/3	100p.c.
,,	3	2 cc	,, ,,	200 M.L.D.	0/3	0p.c.
Oil adjuvant formol vaccine	3	,,	,, ,,	100 M.L.D.	3/3	100p.c.
,,	3	,,	,, ,,	150 M.L.D.	3/3	100p.c.
,,	3	,,	,, ,,	200 M.L.D.	0/3	0p.c.
,,	3	,,	60 days	200 M.L.D.	3/3	100p.c.
Control	2	—	—	1 M.L.D.	0/2	0p.c.

M.L.D.: 1cc 10^4 dilution, 6 hour broth culture
Numerator: number of survivors
Denominator: number of animals in group.

The difference in volume of the doses corresponds to the type of adjuvant used. The number of organisms per dose was identical for all the types of vaccine. Each animal received $5X10^9$ **Pasteurella** incorporated with the different adjuvants.

It follows from this experiment that the immunity provoked by saponised vaccines whether merthiolated or formolised was more solid after 24 days than that following vaccination with vaccine to which alum had been added or oil-adjuvant vaccine. The immunity established by the oil- adjuvant vaccine was quite strong after 60 days. The vaccinated animals have resisted towards a challenge of 200 M.L.D.

Bibliography

1) L.P. Delpy and R. Rastegar - Bull. Ac. Vet., 1938, 9, 256
2) L.P. Delpy and R. Rastegar Rev. Imm., 1938, 4, 322
3) L.P. Delpy and H. Mirchamsy C.R. Ac. Sci., 1947 225, 158
4) R.S. Roberts - J. Comp. Path., 1947, 57, 261
5) G.R. Carter - Canada J. Mic., 1856, 2, 483
6) R.V.S. Bain - OIE, 1954, 42, 256
7) S. Vancheswara Lyer and D.V. Ranga Roa - Ind. Vet. J., 1959, 36, 415