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OBSERVATIONS ON THE USE OF LIVE-MODIFIED TISSUE CULTURE VACCINE AGAINST SHEEP POX (*)

by

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INTRODUCTION

Little information is available on the number of outbreaks caused by Sheep Pox, in the past, in Asian countries including Iran.

It could hardly be reporteed from where and during what century the the disease has been introduced in this continent. However, it is generally believed that Sheep Pox has always been enzootic in Asia since the early times and spread to Europe by movement of diseased animals or infected aniimal products.

Presently, according to the Animal Health Year Book published by FAO/ OIE/WHO in 1972 Sheep Pox occurs in the following countries:

Africa	*Asia*	*Europe*	*America*
Algeria	Afghanistan	Greece	Colombia
Chad	China	Turkey	Mexico
Ethiopia	India		
Kenya	Iran		
Lybian Arab	Iraq		
Republic	Israel		
Mali	Jordan		
Morocco	Kuwait		
Nigeria	Lebanon		
Senegal	Nepal		
Sudan	Pakistan		
Tunisia	Saudi Arabia		
U.A.R. (Egypt)	Syrian Arab Republic		

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NUMBER OF FOCUSES AND MORTALITIES 1965-1973

PROVINCE	YEAR																	
	1965		1966		1967		1968		1969		1970		1971		1972		1973	
	Focus	Mortality	Focus	Mortality	Focus	Mortality	Focus	Mortality	Focus	Mortality	Focus	Mortality	Focus	Mortality	Focus	Mortality	Focus	Mortality
Tehran	177	253	138	109	233	129	148	58	86	63	223	135	99	40	84	43	33	27
Guilan	–	–	4	6	4	7	2	72	–	–	1	3	–	–	2	–	2	–
Mazendaran	–	–	1	1	–	–	6	22	–	–	2	13	2	12	2	8	–	–
Azerbaijan (East)	96	141	54	62	17	14	26	14	91	67	159	109	114	93	88	103	30	48
Azerbaijan (West)	285	265	180	103	138	63	20	5	37	4	59	48	28	2	9	2	–	–
Kirmanshah	30	–	47	122	56	96	16	22	10	42	89	123	52	32	64	10	4	2
Khuzistan	34	112	21	20	16	17	7	3	27	21	76	151	161	181	5	3	2	–
Fars	9	2	19	6	25	15	1	2	18	43	121	201	101	147	85	66	25	49
Kirman	32	2	62	30	16	15	11	9	15	14	25	80	39	5	36	22	4	18
Khorassan	128	122	138	165	56	36	68	95	42	30	87	109	27	32	26	8	32	27
Isphahan	18	13	30	20	28	54	10	8	22	7	68	87	50	41	24	46	20	128
Baloochistan	6	–	6	9	4	8	8	11	11	70	7	2	–	–	25	9	19	5
Kurdistan	46	118	35	42	53	49	32	80	11	24	130	248	91	93	26	4	16	25
Lorestan	16	8	42	62	50	53	14	10	–	–	17	46	58	124	137	149	25	16
Semnan	–	–	–	–	5	–	2	–	–	–	14	–	2	–	2	–	1	4
Hamadan	45	16	109	70	112	81	70	66	26	7	50	25	15	12	–	–	6	16
Tcharmohal	–	–	–	–	17	10	5	–	–	–	–	–	–	–	–	–	–	–
Gorgan	31	5	18	3	11	6	13	50	–	–	–	–	4	6	3	–	–	–
Saheli	–	–	–	–	–	–	–	–	–	–	–	–	–	–	68	11	20	13
Booshehr	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Zanjan	–	–	–	–	–	–	–	–	–	–	–	–	–	–	4	5	10	1
Yazd	–	–	–	–	–	–	–	–	–	–	18	19	2	–	16	4	8	8
TOTAL ...	953	1057	904	853	841	653	459	527	396	392	1146	1435	845	820	726	493	257	387

Different kinds of prophylactics have been developed in countries where the disease exists. But the majority of these vaccines have not commonly been used because of poor keeping quality and very weak immunizing power or due to high virulence of virus which has been, in many cases, responsible for severe outbreaks.

In Iran, where the number of ovine population outpasses 40 million heads, Sheep Pox infectin has caused heavy mortality and economic losses in the past. In recent years, the Iran Veterinary Organization has taken energetic steps to control the disease by mass vaccination of sheep, using live-modified tissue culture vaccine. The attached table shows the number of focuses and mortalities reported during the last 9 years.

Since 1930 up to 1965, the following vaccines have been used in Iran:

a) Diluted virulent virus. Lymph harvested from sheep inoculated by Borrel method was diluted in 1/200 in physiological saline.

b) D.C.C. (Combined Sheep Pox and Anthrax vaccine). Mixture of virulent lymph and Anthrax bacilli were distributed in 100 vaccinal doses and lyophilized.

c) Inactivated vaccine. Infected tissues and lymph harvested by Borrel method were ground, inactivated by formaldehyde and adsorbed on aluminium gel.

d) Live tissue vaccine. Ground tissue and lymph adsorbed on aluminium gel.

Due to the difficulties encountered in virus production and unsatisfactory results reported from the field use of these vaccines, extensive studies were conducted at the Razi Institute since 1963 to adapt the virus in a sutable cell culture. First investigations were directed at obtaining a modified strain which could be used for immunization of sheep without any untoward reaction(4).

Large scale production of virus in cell culture is easy and less costly. The titre of virus grown *in vitro* is always monotonous and higher when compared with the virus harvested from animals or egg embryos. Furthermore, tissue culture vaccine is free from contaminants which may cause severe problems in the field.

MATERIALS AND METHODS.

Cells.

Kidneys aseptically removed from healthy lambs are trypsinized at room temperature for 4–5 hours. During the operation, trypsinized cells are pipetted

into sterile erlenmayers containing 10 ml fresh calf serum and kept at 4°C. At the end of the trypsinization period, cell suspension is filtered through 2 layers of gauze and then washed 3 times with chilled PBS at 1000 rpm.

Packed cells are suspended in Hanks' BSS containing 0.5 percent lactalbumine hydrolysate, 10 percent unheated calf serum and conventional amounts of penicillin, streptomycin and mycostatin.

Under sterile operating conditions the cell suspension is distributed in 160 ml amounts into 2 litres Povitsky bottles and transferred to incubator room(1)

After formation of complete monolayers which usually takes 4 to 6 days, Hanks' medium is replaced by VM3 (5) containing seed virus.

Virus.

Strain RM/65 originating from natural outbreak of Sheep Pox has been attenuated by serial passages in primary ovine kidney cells.

Early passages of this virus, when inoculated in susceptible sheep, caused large reaction, fever and sometimes generalized. At the 30th passage level the virus lost its virulence and conferred solid immunity in animals which were inoculated under laboratory conditions (2).

PRODUCTION OF VACCINE.

When the sterility test and titration of culture virus is carried out, the material is distributed in 2 ml amounts in 5 ml vials and lyophilized in Stokes freeze-drying machines at – 40° under 50 micron vacuum within 24 hours.

A single dose of vaccine which contains 10^4 TCID 50 is sufficient to produce a nodule at the site of inoculation and induce solid immunity in vaccinated animals.

Ten random samples of lyophilized vaccine are used to check sterility and titre.

The vaccine keeps its immunizing power for more than one year when stored at 4°C.

SAFETY AND POTENCY TEST.

Each batch of vaccine which consists of 2–3 million doses must be tested for safety and potency.

To carry out safety test, 8 susceptible sheep are inoculated as follows:

2 sheep with 1/10th vaccinal dose

4 sheep with 1 vaccinal dose

2 sheep with 100 vaccinal doses

After 14 days, these animals together with 4 non-inoculated, contact controls were inoculated with virulent virus and kept under close supervision for 3 weeks.

The 8 vaccinated sheep must show neither local reaction nor fever, while large reaction ending in vesicles and scabs should appear in the 4 control animals.

FIELD APPLICATION.

Each vaccine vial contains 200 vaccinal doses. A sterile diluent (100 ml of physiological saline) is supplied with each vaccine vial. Vaccine is delivered in ice boxes (+ 4°C) to the field, reconstituted with diluent previously chilled and 0.5 ml inoculated sub-cutaneously.

142,983,000 doses of freeze-dried tissue culture vaccine have been produced and used with encouraging results in Iran and some other countries (Algeria, Dubai, India, Jordan and Kuwait).

In no case did the vaccine cause generalized symptoms or abortion in highly pregnant animals. Lactating ewes do not suffer a temporary decrease in milk yield and no case of Sheep Pox has been observed in untreated sheep having contact with vaccinated animals.

The immunity estabished by this vaccine lasts for 2 years (3).

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SUMMARY

Preparation of live-modified Sheep Pox tissue culture vaccine and its field application is easy and economical. The antigenicity of vaccine remains intact for more than one year when stored at 4°C.

Immunity established in vaccinated animals is solid and lasts up to 2 years.

Administration of vaccine in highly susceptible or pregnant sheep is harmless. Following vaccination only a small nodule appears at the site of inoculation which will be absorbed within a week or so.

REFERENCES

1. RAMYAR (H.) – *Zbl. Vet. Med. B.,* 1965, **12**, 573.

2. RAMYAR (H.) & HESSAMI (M.) – *Zbl. Vet. Med. B.,* 1967, **14,** 516.

3. RAMYAR (H.) & HESSAMI (M.) – *Zbl. Vet. Med. B.,* 1970, **17,** 869.

4. RAMYAR (H.) – Proc. Symp. Médit. sur les Maladies Infectieuses du Mouton, 12–15 octobre 1970, 1970, 475.

5. SCHWÖBEL (W.) & SIEDENTOPF (V.) – *Zbl. Bakt. I Orig.,* 1961, **181,** 3.