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STUDIES ON THE IMMUNOGENIC PROPERTIES OF TISSUE-CULTURE SHEEP POX VIRUS (*)

By

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Sheep pox is one of the serious contagious diseases causing heavy mortality and great economic loss in some parts of the world. This is especially true in most Asian countries where the majority of the farmers and almost all nomads depend on sheep for their livelihood. Their economy suffers greatly from sheep pox which, in some instances, kills 90% of the lambs, reduces production, causes abortion and mastitis and frequently leaves considerable skin defects.

Mass vaccination is the method of choice in checking the spread of the disease. Susceptible animals, fertile hen's eggs and cell cultures are in use as sources of virus for preparation of vaccine.

In vitro cultivation of sheep pox virus (SPV) began with experiments by BRIDE (3), who suspended small pieces of lamb testis in Drew's medium and infected them with SPV.

AGYUN (1) succeeded in cultivating the virus in ovine embryo lung and skin and found that the cultured virus at its 15th passage was safe and immunogenic enough to protect susceptible animals from natural infection or challenge.

BOUE et al. (2) reported the multiplication of SPV in ovine kidney and skin cells.

Great progress has been made in the production of virus using tissue-culture methods since PLOWRIGHT and FERRIS (5) and CILLI and BALDELLI (4) successfully adapted the virus to different cell cultures and studied its characteristics in vitro.

This report deals with the work carried out at this institute on the large-scale production of virus in lamb testis (L. T.) cell cultures and active immunization of sheep using a vaccine prepared from this virus.

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Materials and Methods

Cultures. — Kidneys and testicles were removed aseptically from lambs 1—3 months-old, trypsinized at room temperature for three hours, filtered through two layers of sterile gauze and then washed three times with chilled phosphate-buffered saline at 1,000 rpm.

Packed cells were suspended in Hanks medium containing 0,5% lactalbumin hydrolysate, 10% unheated calf serum, 100,000 I.U. penicillin and 100 mg. streptomycin per litre.

After six days incubation at 37° C. cultures usually have formed monolayers. At this stage, Hanks medium is replaced by VM 3 [Virus Medium 3, SCHWOBEL and SIEDENTOPF (7)], which has the following formula:

NaCl	80.0	gr.
KCl	0.3	gr.
CaCl2, 2 H2O	0.24	gr.
MgCl2, 6 H2O	0.2	gr.
Glucose	1.8	gr.
NaHCO3	2.0	gr.
Lactalbumin hydrolysate (5% Sol.)	100.0	ml.
Phenol red (10% Sol.)	10.0	ml.
Penicillin	100,000	I.U.
Streptomycin	100	mgr.
Twice distilled water	890	ml.

Virus. — The Roumanian strain, received from the Pasteur Institute of Algeria on April 6, 1958, and kept lyophilized after one passage in Iranian fat-tail sheep, was used. This strain is now in its 17th serial passage in LT cells.

Virus Assay. — Culture virus of the 2nd, 4th, 8th, 12th and 15th passages was injected intradermally into susceptible sheep. Inoculated animals showed high fever and specific pox reactions at the site of injection, with generalization in some cases.

The Roumanian tissue-culture virus was titrated simultaneously in cross-bred Merino (highly susceptible) sheep and LT cell cultures at the 4th, 8th and 12th passage levels. For each titration, 4 sheep and 42 culture tubes were inoculated with ten-fold dilutions of the virus (10^{-1}—10^{-7}). The titer of the tissue culture virus of the 8th and 12th passages was 10^{-6}, both in vitro and in vivo, a value higher than the titer of the initial seed virus.

Preparation of Vaccine. — In order to study the immunogenic power of the tissue-culture virus, a batch of aluminium-adsorbed vaccine [RAFYI and MIRCHAMSY (6)], using different amounts of virus, was prepared and tested. Six animals were used per virus dilution. The results of safety and potency tests are shown in the table.

Discussion

Large-scale production of virus is of prime importance in campaigns to control sheep pox.

Inoculation of sheep by the Borrel method is the oldest procedure and is still commonly used to produce the virus. However, the application of this method in infected countries is difficult due to the fact that fully susceptible animals are not readily available, the virus produced is very expensive and there is always a great risk of collecting some other infectious agents together with sheep pox material.

Egg adapted strains of SPV have not been largely used because of the difficulty in adaptation and production and their lower titer when compared with strains grown in animals or tissue culture. Furthermore, laboratory experiments and field investigations on the potency of egg-culture viruses show some contradictory results.

Cultivation of SPV in cell cultures is the most reliable method. It can be used for production as well as research.

From the potency point of view, tissue-culture vaccine has proved quite efficacious in protecting against a challenge dose of virulent virus.

In order to avoid large local reactions and consequent spread of virus, the proportion of tissue-culture virus to aluminium gel should not exceed 1:500.

Summary

A combination of lamb kidney and lamb testis cells was successfully used for the large-scale production of sheep pox virus.

Tissue culture virus from the 8th and 12th passages showed higher titers than the initial seed material.

Vaccine of tissue culture origin established a solid immunity in inoculated animals. From the point of safety, however, the ratio of virus to aluminium gel should not exceed 1:500.

Zusammenfassung

Untersuchungen über die immungenen Eigenschaften von Gewebekultur-Schafpockenvirus

Für die Massenproduktion von Schafpockenvirus wurde mit Erfolg eine Kombination von Lammnieren- und Lammhodenzellen verwendet. Virus der 8. und 12. Passage aus Gewebekultur zeigte höhere Titer als das für die Erstbeimpfung benutzte Material. Vakzine aus Gewebekulturen führte zu einer guten immunität der geimpften Tiere. Aus Sicherheitsgründen sollte jedoch das Verhältnis von Virusmenge zu Aluminiumhydroxyd-Gel 1 : 500 nicht überschreiten.

Résumé

Recherches sur les propriétés immunogènes du virus de la variole ovine obtenu par culture de tissu

Pour la production en grande quantité du virus de la variole ovine, on utilisa avec succès une combinaison de cellules de rein d'agneau et de testicule d'agneau. Le virus des 8e et 12e passages de la culture de tissu présentait un titre plus élevé que le matériel utilisé pour l'ensemencement initial. Des vaccins préparés à partir de la culture de tissu donnèrent une bonne immunité aux animaux vaccinés. Pour des raisons de sécurité il ne faudrait pas dépasser le rapport 1 : 500 entre la quantité de virus et le gel d'hydroxyde d'aluminium.

References

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Safety and potency tests of tissue culture vaccine

Sheep No.	Vaccine : virus / gel ratio	Safety			Potency		
		Dose injected	Thermal reaction	Local reaction	Amount of virulent virus inj.	Thermal reaction	Local reaction
274		0.5 ml. S / C	=		0.5 ml. I / D *	-	-
275		"	= = =	+ + +	"	-	-
276	1 : 100	"	= = =	+ +	"	-	-
277		"	= = =	+ + +	"	-	-
278		"	= =	+	"	-	-
279		"	-	-	"	-	-
280		"	-	-	"		-
281		"	=	+	"	-	-
282	1 : 200	"	-	-	"	-	-
283		"	= = =	+ + +	"	-	
284		"	= = =	+ +	"	-	-
285		"	-	-	"	-	-
286		"	-	-	"	-	-
287		"	= = =	+ + +	"	-	-
288	1 : 250	"	-	-	"	-	-
289		"	=	-	"	-	-
290		"	-	-	"	-	-
291		"	= = =	+ +	"	-	-
292		"	= =	+ +	"	-	-
293		"	= = =	+ + +	"	-	-
294	1 : 300	"	-	-	"	-	-
295		"	-	-	"	-	-
296		"	-	-	"	-	-
297		"	-	-	"	-	-
298		"	-	-	"	-	-
299		"	-	-	"	-	-
300	1 : 500	"	=	+	"	-	-
301		"	-	-	"	-	-
302		"	-	-	"	-	-
303		"	=	-	"	-	-
304		CONTROL			"	= = =	+ + +
305		"			"	= = =	+ + +
306					"	= = =	+ + +
307					"	= = =	+ + +

- : No reaction
= : Body temperature 39.5 - 40 °C.
= = : " " 40 - 41 °C
= = = : " " 41 - 42 °C.

+ : Size of a hazelnut
+ + : " " " walnut
+ + + : " " " tangerine

* : Vaccinated animals challenged with 10,000 R. D. (Reaction Doses) and controls with 100 R. D. of virulent virus