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Studies on the Duration of Immunity conferred by a Live-Modified Sheep Pox Tissue Culture Virus Vaccine (*)

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Sheep pox, an infectious disease of sheep, often becomes epizootic in sheep raising areas in some Asian and African countries. For many years different kinds of vaccines have been used extensively to control the disease (1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24, 26, 27, 29, 31). Some of these vaccines have not proven to be safe and are poorly antigenic. The immunity established by some vaccines has been short. Little precise information was available on the potency of vaccines produced in recent years.

Extensive studies were conducted during the past five years at the Razi Institute to produce a safe and potent sheep pox vaccine by means of virus propagated on monolayers of ovine kidney cells (25) and to develop a reliable method for testing its efficacy. The object of this communication is to report the results of experiments on the immunity of a lyophilized tissue culture vaccine which has been produced and used in Iran since 1966.

Material and Methods

Vaccine: Modified-live sheep pox virus, strain RM/65, was grown in primary sheep kidney cultures, lyophilized and stored at 4° C until used. The dose of reconstituted vaccine was 0.5 ml. containing 100 $TCID_{50}$ viral particles.

Animals: Three hundred Balootchi sheep were selected for this study. They ranged in age from 6 to 9 months. They had no previous vaccination against sheep pox. Clinical signs of sheep pox were not observed.

The sheep were divided into 3 groups as follows:

1. 120 sheep inoculated intradermally (I/D) with one vaccinal dose.
2. 120 sheep inoculated with the same dosage but subcutaneously (S/C).
3. 60 sheep kept as controls without inoculation.

Vaccinated sheep were closely observed until 12 days post-vaccination. Body temperatures were taken twice daily during this period.

Challenge: Twenty sheep (10 vaccinated I/D and 10 S/C) were challenged by the intradermal route at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 24 months after immunization. The vaccinated sheep each received 10,000 R. D. (Reaction dose) of virulent sheep pox virus and 5 controls each received 100 R. D. of the same agent. The challenged sheep were observed for 12 days and thermal and local reactions recorded.

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In addition to the sheep maintained at the Institute, 4 flocks totalling 4,330 sheep in 4 different regions of the country were ear-marked and vaccinated. These flocks were examined at regular intervals for 2 years.

Serology: Serum-neutralization test (SN) — Attempts were made to apply this procedure by using primary ovine kidney cell cultures. The sheep were bled before vaccination and one day prior to challenge. Sera were heated at 56° C for 30 minutes. Two-fold serum dilutions were prepared and mixed with equal amounts of tissue culture material containing 100 $TCID_{50}$ /ml viral particles. The tubes were incubated at room temperature (24° C) for 1 hour and then overnight at 4° C. The following day 2 ml. of each dilution were inoculated into each of 5 primary lamb kidney culture tubes and incubated in a stationary position at 37° C. The inoculated cultures were examined for the presence of CPE for 10 days. The highest serum dilution in which 3 of 5 tubes showed no CPE was considered as the titer.

Complement fixation (CF) test — Both tissue culture and lymph virus were used as antigens. Later in the studies they were concentrated 10 X by centrifugation at 40,000 rpm. for 2 hours in a Spinco ultracentrifuge. Sera were collected from immunized and control sheep and heated prior to the test at 56 or 58° C for ½ hour.

Results and Discussion

Because of the dermotropic nature of sheep pox virus, administration of a live virus vaccine by the intradermal route results in more and larger reactions than if it is given subcutaneously. Consequently, the immunity established will be comparatively greater. However, the difficulty of administering vaccine intradermally in mass vaccination programmes makes its field use practically impossible. Therefore, regardless of the superiority of the first method, sheep should be immunized by subcutaneous inoculations.

No untoward reactions were observed and there was no apparent spread of the virus to contact control animals.

Post-vaccinal reactions were recorded as follows:

Route of inoculation	No reaction %	1 cm reaction %	2 cm reaction %	3 - 4 cm reaction %	Scabs %
Sheep inoculated I / D	23.4	29.14	43.3	4.16	26.66
" " S / C	41.0	32.35	25.0	1.65	3.33

Sheep pox did not appear in the 4 flocks maintained under field conditions. However sheep pox outbreaks occurred in non-vaccinated flocks grazing in the same areas.

The results of the challenges summarized in Table 1 show that sheep vaccinated by either route were 100% protected against the challenge dose of virulent lymph for up to 22 months. Only one sheep of the first group and three of the second showed thermal and local reactions 24 months post-vaccination.

The results of serum-neutralization tests were compared to the simultaneous challenge of sheep pox virus. No correlation was observed between

the two procedures. This raises doubts about the efficacy of the SN test in accurately detecting neutralizing antibodies and evaluating degrees of immunity. In our experiments sera obtained from immunized sheep often failed to show neutralizing effects.

A number of workers have successfully applied the CF test for diagnosis of sheep pox (10, 21, 28 & 30). There are conclusions that this procedure, as in many other viral diseases, can be used for evaluating the degrees of immunity.

These studies failed to find consistent results in more than 750 CF tests of sera from immunized and control sheep and we must conclude that the test cannot replace challenge as a method of evaluating immunity. No differences were observed in viral antigens obtained by tissue culture method and lymph harvested from infected sheep.

Table 1

Results of Vaccination and Challenges

Vaccination				Challenge				
Number of animals	Date of vaccination	Route of vaccination	Post-vaccinal reactions	Number of vaccinated animals	Number of controls	Months after vaccination	Number of controls reacted	Number of vaccinated animals reacted
120	6. 2. 67	I / D	76.6 %	20	5	2	5	Nil
				20	5	4	5	"
				20	5	6	5	"
120	"	S / C	59.0 %	20	5	8	5	"
				20	5	10	5	"
				20	5	12	5	"
60	Controls			20	5	14	5	"
				20	5	16	5	"
				20	5	18	5	"
				20	5	20	5	"
				20	5	22	5	"
				20	5	24	5	4
						Total:	60 / 60	4 / 240

Summary

Three hundred young and healthy sheep were used to study the duration of immunity of a modified live virus sheep pox vaccine.

Experimental sheep vaccinated with the test vaccine resisted a challenge dose of virulent virus for up to 22 months. Some apparent loss of protection occurred after 24 motnhs.

Four large flocks of 4,330 sheep were vaccinated and remained healthy for up to 24 months when pastured in areas where non-vaccinated sheep contracted the disease.

Challenge is the method of choice for evaluating immunity. There was poor correlation between SN and CF tests and immunity produced by the test vaccine.

These studies suggest that the resistance produced by the live modified sheep pox tissue culture vaccine may last beyond the 24 months observation period.

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Zusammenfassung

Untersuchungen über die Dauer der Immunität nach Impfung mit einer Schafpocken-Lebendvaccine aus modifiziertem Kulturvirus

300 junge, gesunde Schafe, die mit einer Lebendvaccine aus modifiziertem Schafpocken-Kulturvirus geimpft worden waren, wurden zur Untersuchung der Immunitätsdauer eingesetzt. Experimentell mit der Versuchsvaccine geimpfte Schafe widerstanden bis zu 22 Monaten einer Testinfektion mit virulentem Schafpockenvirus. Ein merklicher Immunitätsrückgang trat nach 24 Monaten ein.

Vier grössere Herden mit insgesamt 4330 Schafen wurden ebenfalls geimpft. Sie blieben 24 Monate lang gesund, nachdem sie in Gebieten geweidet worden waren, wo nichtgeimpfte Schafe von der Seuche befallen wurden.

Die Testinfektion ist die Methode der Wahl zur Prüfung der Immunität. Es ergaben sich kaum Beziehungen zwischen den Ergebnissen von Serumneutralisation und Komplementbindungsreaktion einerseits und der durch die Lebendvaccine hervorgerufenen Immunität.

Auf Grund der der Untersuchungen kann angenommen werden, dass die durch die Schafpocken-Lebendvaccine aus modifiziertem Kulturvirus bewirkte Immunität noch über die Beobachtungsperiode von 24 Monaten hinaus anhalten dürfte.

Résumé

Recherches sur la durée de l'immunité après vaccination avec un vaccin contre la variole ovine, préparé avec un virus modifié

On emploi 300 moutons jeunes et sains pour vériifer la durée de l'immunité obtenue avec un vaccin vivant préapré avec un virus modifié de la variole ovine. Les moutons vaccinés expérimentalement avec ce vaccin sont protégés pendant 22 mois contre une infection test avec du virus virulent de la variole ovine. On observe un affaiblissement sensible de l'immunité après 24 mois.

On vaccine également quatre troupeaux plus importants, comprenant 4330 moutons. Ces derniers sont restés pendant 24 mois en bonne santé, après avoir été dans des pâturages où des moutons non vaccinés avaient subi l'épizootie.

L'infection test est la méthode de choix pour vérifier l'immunité. On ne trouve pratiquement pas de relations entre les résultats de la neutralisation du sérum et de la réaction de fixation du complément d'une part et d'autre part l'immunité induite par le vaccin vivant.

Sur la base de ces recherches, on peut admettre que la résistance obtenue par le vaccin vivant anti-variole ovine, préparé avec une virus modifié, pourrait persister même au-delà de la période d'observation de 24 mois.

Resumen

Estudios sobre la duración de la inmunidad tras aplicar una vacuna viva contra la viruela ovina elaborada a base de virus cultural modificado

Se emplearon 300 ovejas jóvenes sanas para examinar la duración de inmunidad dispensada por una vacuna viva a base de virus cultural modificado de viruela ovina. Las ovejas protegidas experimentalmente con la vacuna de ensayo resistían hasta 22 meses a la infeccíon de prueba con virus virulento de viruela ovina. La regresión manifiesta de la inmunidad sobrevino al cabo de los 24 meses.

También se vacunaron cuatro rebanos mayores con un total de 4.330 ovejas. Permanecieron sanas durante 24 meses tras haber pastado en zonas, en las que ovejas no vacunadas habían sido atacadas por la epizootía.

La infección de prueba es la técnica idónea para contrastar la inmunidad. Apenas se infirieron relaciones entre los resultados de la seroneutralización y la reacción di fijación del complemento por un lado y la inmunidad conferida por la vacuna viva por otro.

Con arreglo a los estudios realizados, se puedo admitir que la resistencia producida por la vacuna viva antivariólica ovina a base de virus cultural modificado aun podría perdurar más allá del periodo de observación de 24 meses.

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