Arch. Inst. Razi, 1970, 22, 123-128

A new Approach to active Immunization of Sheep by a Combined Sheep Pox and Anthrax Vaccine (*)

By

H. RAMYAR and M. BAHARSEFAT

With one table

Introduction

Effective control of contagious diseases in countries where the number of field veterinarians is limited, good roads and suitable means of transportation are not fully developed, and skilled management of animals is not yet available can be very difficult or even impossible.

To overcome these difficulties and obtain the greatest possible success in the eradication, or at least the control, of outbreaks one should look for the simplest and most economic procedures. Systematic vaccination should be carried out using safe and potent prophylactics with good keeping qualities under unfavourable conditions.

For many years much attention has been given to the campaigning against infectious diseases in animals and great steps forward have been made in the last two decades in the development of effective biologics.

Following the fruitful experiments of G. RAMON on the combination of diphtheria and tetanus toxoids, efforts have been made to mix different antigens with the aim of confering immunity against two or more diseases by a single injection (10, 12, 13 and 14).

CORDIER (2), PLACIDI et al. (11) and DELBE (3) reported the successful immunization of sheep with a combined vaccine against foot-and-mouth disease and sheep pox. DUBOIS (7) demonstrated the possibility of vaccinating sheep by means of combined sheep pox and brucella vaccine. DELPY et al. (4 and 5) developed a combined vaccine which consisted of the Roumanian strain of sheep pox virus and the Pasteur strain of *Bacillus anthracis*. ERTÜRK and BESE (8) reported the safety and potency of a combined anthrax and blackleg vaccine.

Combined vaccines have largely been used in the control of avian diseases such as fowl pox, Newcastle disease and pullorum disease (1, 6 and 9) and this type of vaccine plays a large role in vaccinating dogs against infectious diseases. However, combined vaccines have not yet become of routine use in immunization of farm animals against epizootics except for the use in sheep of combined Clostridial vaccines.

This paper describes the development of a combined sheep pox and anthrax vaccine which induces solid and long lasting immunity against both diseases.

(*) Zbl. Vet. Med., B, **16**, 588—592, 1969.

Materials and Methods

Sheep pox virus: Strain RM/65 which has been attenuated by serial passages in monolayers of ovine kidney cells and used for immunization of Iranian sheep since 1966 (15).

Virus is produced in batches of 10 litres using Povitsky bottles for the cultivation and infection of cells. Lamb kidney cells are grown in Hank's medium with 10% unheated calf serum and the usual range of antibiotics. VM 3 (16) has been used as maintenance medium for infected cells.

Sterility tests, in vivo and in vitro titration, and safety and potency tests of culture virus have been carried out for each batch separately.

Anthrax: Local strain of *Bacillus anthracis* was used, modified in our Institute according to the technique described by STERNE (17), lyophilized and kept in the collection until needed.

Lyophilized non-capsulated bacilli are cultivated in a suitable number of bottles containing peptone broth (seed material). After 24 hours, Roux flasks containing peptone-free agar medium are infected with the liquid seed material and kept at 37° C for 3 days. The infected cultures are then transferred to the laboratory room at about 24° C for 4 more days. At this time the surface of the solid medium is gently washed with 0.85% physiological saline and the suspension is subjected to a sterility test, calculation of spores per ml and safety and potency tests. Four million spores of this strain induce satisfactory immunity when injected into sheep or goats by the intradermal route.

Combined sheep pox and anthrax vaccine

As a result of several experiments, it has been concluded that each vaccinal dose of the combined vaccine should contain 100 TCID/50 of live-modified sheep pox virus and 10,000,000 spores of uncapsulated strain of *Bacillus anthracis.*

Sufficient amounts of virus and bacilli are mechanically mixed and homogenized in a sterile stainless steel container placed in a cold room. The mixture is distributed in 2 ml. amounts in 5 ml. vials which are immediately frozen at —30° C. The frozen material is then transferred to Stokes freeze-dryers (Model 24 P., F. Stokes Corporation, 5500 Tabor Road, Philadelphia 20, Pa.). After lyophilization, which lasts for 32 hours, the vitals are evacuated and stoppered in the same machine.

Two samlpes are selected at random from each batch of vaccine to carry out sterility, safety and potency test.

The sterility test is carried out by inoculation of reconstituted vaccine into aerobic and anaerobic media. Culture tubes are kept at 37° C for 7 days.

For safety and potency tests, 8 susceptible sheep are injected subcutaneously with one dose of the combined vaccine. Fourteen days later, these sheep together with 4 controls were challenged intradermally with virulent sheep pox virus (Roumanian strain). The vaccinated animals show neither thermal nor local reactions while the 4 controls develop high fever and a large local reaction ending with vesicles and scabs at the site of inoculation. After 12 days these sheep are challenged subcutaneously with 1000 MLD of a virulent strain (C2) of *Bacillus anthracis.*

Eight vaccinated animals remained normal, whereas the 4 control sheep died within 4 days of injection (Table 1).

Table 1

Safety and potency tests with combined vaccine

Sheep Nr.	Safety				Fotency							
					Sheep pox				Anthrax			
	Date of Vaccination	Dose Injected	Thermal Reaction	Local Reaction	Date of Challenge	Amount of Virulent Virus Injection(+)	Thermal Reaction	Local Reaction	Date of Challenge	Amount of Virulent Strain Injection(‡)	Thermal Reaction	Remarks
47 - 49	16.7.68	0.5 ml. S/C	-	+ Scabs	30.7.68	0.5 ml I/D	-	-	11.8.68	1 ml S/C	-	Resisted
47 - 50	"	"	-	++ "	"	"	-	-	"	"	-	"
47 - 51	"	"	-	++ "	"	"	-	-	"	"	-	"
47 - 52	"	"	-	++ "	"	"	-	-	"	"	-	"
47 - 53	"	"	-	+ "	"	"	-	-	"	"	-	"
47 - 54	"	"	-	+ "	"	"	-	-	"	"	-	"
47 - 55	"	"	-	+ "	"	"	-	-	"	"	-	"
47 - 56	"	"	-	+ "	"	"	-	-	"	"	-	"
47 - 57	Control				"	"	= = =	+++Scabs	"	"	= =	Died
47 - 58	"				"	"	= = =	+++ "	"	"	= =	"
47 - 59	"				"	"	= = =	++++ "	"	"	= =	"
47 - 60	"				"	"	= = =	++++ "	"	"	= = =	"

- : No reaction
· : Size of a pea
+ : Size of a hazelnut
+ · : Size of a walnut
+ + : Size of a tangerine

= : Body temperature 40—40.5° C
= · : Body temperature 40.6—41° C
= = : Body temperature 41.1—42° C

*) Vaccinated animals were challenged with 10.000 RD (Reaction dose) and controls with 100 RD of virulent sheep pox virus.

**) Vaccinated animals were injected with 1,000 MLD (Minimal lethal dose) and controls with 1 MLD of virulent B. anthracis.

Field Application

Each vaccine vial contains 200 doses of the combined sheep pox and anthrax vaccine. A sterile diluent (100 ml. of 0.1% saponin* solution in distilled water) is supplied with each vaccine vial. Vaccine vials are delivered in ice boxes to the field, reconstituted with chilled diluent immediately before use and 0.5 ml. inoculated into sheep subcutaneously.

Discussion

Sheep pox and anthrax outbreaks are responsible for heavy economic losses to animal industry in Iran. Due to the presence of nomadic tribes, application of practical sanitary measures is not feasible. The only reliable method which would be successful is mass vaccination. Safe and potent combined vaccines would therefore serve as an important tool for checking infections.

An associated sheep pox and anthrax vaccine (Desseché — Charbon-Clavelée) has previously been used in this country but since the sheep pox virus was a virulent strain produced by the Borrel pustule method many instances of generalization and mortality were reported following vaccination. The new combined vaccine consists of attenuated strains of sheep pox virus and *Bacillus anthracis*.

Three million doses of this vaccine have been used in different parts of Iran with satisfactory results. In no case has the vaccine caused generalization in vaccinated animals and no case of sheep pox or anthrax has been observed in control animals in contact with them.

The local reaction to the injection is often a small nodule which is absorbed within a week or so. The vaccine does not cause abortion in pregnant ewes and lactating animals do not show any decrease in milk yield.

As a single injection gives protection against two main diseases of sheep its use is increasingly demanded by field veterinarians and animal owners.

Summary

A combined vaccine against sheep pox and anthrax has been developed and used with good results in Iran in 3 million sheep of varying susceptibility.

Untreated sheep having contact with immunized ones did not show any signs of disease.

The combined vaccine is of potential economic importance since a single injection confers solid immunity against two major diseases of sheep.

Our experiments show that the combined sheep pox and anthrax vaccine confers the same level of immunity as single vaccines.

Acknowledgements

We wish to express our thanks to Dr. M. KAVEH, Director General of the State Razi Serum and Vaccine Institute for his valuable advice and to Drs. AHOURAI and GHAZARIAN for their technical assistance.

(*) Saponin purified MT. E. Merck AG. Darmstadt. Germany.

Zusammenfassung

Ein neuer Versuch zur aktiven Immunisierung von Schafen mit einer kombinierten Schafpocken-Milzbrand-Vakzine

Es wurde eine neue Vakzine gegen Schafpocken und Milzbrand entwickelt, die an 3 Millionen Schafen unterschiedlicher Empfänglichkeit mit gutem Ergebnis in Iran erprobt wurde.

Ungeimpfte Schafe, die in Kontakt mit immunisierten standen, zeigten keinerlei Krankheitserscheinungen.

Die kombinierte Vakzine hat eine grosse wirtschaftliche Bedeutung. Eine einzige Injektion vermittelt eine solide Immunität gegen zwei wichtige Schafseuchen.

Résumé

Un nouvel essai d'immunisation active des moutons à l'aide d'un vaccin combiné contre la variole ovine et le charbon bactéridien

On met au point un nouveau vaccin contre la variole ovine et le charbon bactéridien qui a été testé sur 3 millions de moutons de réceptivité variable et donné de bons résultats.

Les moutons non vaccinés, qui se trouvaient en contact avec les animaux immunisés, ne manifestèrent aucun signe de maladie.

Le vaccin combiné a une grande importance économique. Une seule injection confère une solide immunité contre deux maladies épizootiques sérieuses du mouton.

On peut déduire des résultats de l'expérience, que le vaccin combiné contre la variole ovine et le charbon garantit une immunité identique à celle que l'on obtient à l'aide de vaccinations séparées avec des vaccins monovalents.

Resumen

Un esayo nuevo para la inmunización activa de las ovejas con una vacuna combinada frente a la viruela ovina y el carbunco bacteridiano

Se desarroló una vacuna nueva frente a la viruela ovina y el carbunco bacteridiano, que se probó con éxito satisfactorio en 3 milliones de ovejas de receptividad diversa.

Las ovejas no vacunadas, que se hallaban en contacto con las inmunizadas, no mostraron síntomas nosológicos de ninguna clase.

La vacuna combinada tiene una importancia económica grande. Una sola inyección proporciona una inmunidad sólida frente a dose enfermedades infectocontagiosas importantes para las ovejas.

De los resultados ecperimentales se puede deducir que la vacuna combinada contra la viruela ovina y el carbunco bacteridiano garantiza la misma inmunidad que se logra mediante inoculaciones por separado con vacunas monovalentes.

REFERENCES

1. BEAUDETTE, F. R., 1949: Proc. 53rd Ann. Meeting U. S. Livestock P. 215. • 2 CORDIER, G., 1940: Rec. Med. Vet. **116**, 254. • 3. DELBE, M. M., 1934: Rec. Med. Vet **110**, 280. • 4. DELPY, L. P., et H. MIRCHAMSY, 1947: C. R. Acad. Sci. **225**, 158. • 5. DELPY, L. P., A. RAFYI et H. MIRCHAMSY, 1951: Bull. Acad. Vet. Fr. **24**, 50. • 6. DELPY, L. P., et E. HARS, 1953: Bull. Acad. Vet. Fr. **26**, 175. • 7. DUBOIS, J. M., 1935: Rev. Gen. Med. Vet. **44**, 635. • 8. ERTÜRK, Ö., and M. BESE, 1961: J. Fac. Vet. Med. Univ. Ankara **8**, 188. • 9. LISSOT, G., 1950: Bull. Acad. Vet. Fr. **23**, 327. • 10. MERIEUX, CH., 1949: Rev. Path. Comp. et d'Hyg. **49**, 82. • 11. PLACIDI, L., J. SANTUCCI, J. HAAG et R. SENDRAL, 1958: Bull. Acad. Vet. Fr. **31**, 351. • 12. RAMON, G., et CHR. ZOELLER, 1926: C. R. Soc. Biol. **94**, 106. • 13. RAMON, G., 1936: Rev. Imm. **2**, 415. • 14. RAMON, G., 1949: Rev. Imm. **13**, 41. • 15. RAMYAR, H., and M. HESSAMI, 1967: Zbl. Vet. Med. B. **14**, 516. • 16. SCHWÖBEL, W., und V. SIEDENTOPF, 1961: Zbl. Bakt. I Orig. **181**, 3. • 17. STERNE, M., 1937: Onderstepoort J. Vet. Sci. **8**, 271.