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The effect of the route of administration on the immunity produced by the T_1 strain of *Mycoplasma mycoides* sub-species *mycoides*

by R. S. WINDSOR (*) and W. N. MASIGA

RÉSUMÉ

Influence de la voie d'administration sur l'immunité produite par la souche T_1 de *Mycoplasma mycoides* subsp. *mycoides*

Partout en Afrique, on se sert encore communément de l'injection dans le toupillon de la queue pour l'administration du vaccin T_1 en bouillon contre la péripneumonie. Les auteurs décrivent les motifs historiques de l'emploi de cette voie ; y a-t-il une justification scientifique à la poursuite de cette habitude ?

On a vacciné les bovins à leur extrémité caudale, en sous-cutanée en arrière de l'épaule, et par voie intra-dermique à l'épaule en usant toutefois du même lot de vaccin T_1 en bouillon.

Quinze mois plus tard, on a déterminé le niveau d'immunité de ce bétail par une épreuve infectante par contagion directe. Les 24 animaux des groupes vaccinés au bout de la queue et par la voie sous-cutanée étaient, tous, entièrement réfractaires à l'infection d'épreuve. Trois des 12 animaux vaccinés par la voie intra-dermique ont développé des lésions de la péripneumonie au cours de cet essai. On essaie d'expliquer ces résultats.

L'efficacité de la voie sous-cutanée est démontrée ; dès à présent, on devrait pouvoir se servir de cette méthode, plus convenable, plus rapide et plus propre, pour la vaccination contre la péripneumonie. Il est conseillé cependant, d'en faire l'essai en Afrique orientale, pour établir la sûreté de la voie sous-cutanée avant de la faire appliquer à grande échelle.

WILLEMS (16) had shown that the inoculation of virulent *Mycoplasma mycoides* subspecies *mycoides (M. mycoides)* under the skin of the thorax could result in the death of the animal. Rider HAGGARD (7) in his novel « King Solomon's Mines » gives the earliest description of field vaccination against contagious bovine pleuropneumonia (CBPP) :

« As for the lung sick which is a dreadful form of pneumonia, very prevalent in this country, they had all been inoculated against it. This is done by cutting a slit in the tail of an ox, and binding in a piece of the diseased lung of an animal which had died of the sickness. The result is that the ox sickens, takes the disease in a mild form, which causes its tail to drop off... It seems cruel to rob an animal of his tail especially in a country where there are so many flies, but it is better to sacrifice the tail and keep the ox than to lose both tail and ox. »

Today the tail-tip route is still widely used throughout English-speaking African countries. There are several disadvantages to this method. It is difficult to inject 0.5 ml into the tip of the tail : often the pressure required to empty the syringe results in the needles being forced off the nozzle. The tail is often dirty, or soiled

(*) East African Veterinary Research Organisation, Muguga, P. O. Kabete, Kenya. On secondment from the Ministry of Agriculture, Fisheries & Food, U. K. under United Kingdom Overseas Development Administration Research Scheme N° R 2396.

Present address : Veterinary Investigation Centre, Madingley Road, Cambridge.

with faeces, with the concurrent risk of the needle introducing anaerobic bacteria with the vaccine. The more difficult a job is to do the less well it will be done. It is much simpler and personally safer to give a sub-cutaneous injection behind the shoulder than to inject into the tail. It was therefore decided to investigate the possibility of using other routes of vaccination against CBPP.

Before carrying out large scale safety trials in the field it was decided to study the immunity produced by vaccination via routes other than the tail-tip. The sub-cutaneous route was selected because it is the simplest method of vaccination for large scale campaigns. The intra-dermal route was chosen because it was thought that when animals were vaccinated in the tail some proportion of the dose must be given into the skin. It was further thought that a depot of organisms might be required for slow release over a period of time.

METHODS

Animals

Forty-eight European, or high grade cattle were obtained from areas of Kenya free from CBPP.

Vaccination

The animals were arranged in groups of 4 according to weight, breed and sex. Following a method of random selection one member of each group was vaccinated in the tail-tip with 0.5 ml of T_1 Broth Vaccine (2) (Batch 365 containing 3.2×10^9 colony forming units (CFU) per ml) ; one received 0.5 ml of the same batch of vaccine by the sub-cutaneous route behind the shoulder ; the third received 0.2 ml of the same batch into the skin of the shoulder while the fourth was left as an unvaccinated control. These 4 groups of 12 animals were grazed together and bled before vaccination. After vaccination they were bled each week until the sera when examined by the complement-fixation (CF) test showed no antibodies to *M. mycoides*. Thereafter they were bled once a month until the « in contact » challenge trial commenced, when weekly bleedings were reintroduced.

It was not possible to obtain 48 animals of the same age and the age range varying from one year to well over three years, was much greater than in previous trials carried out in this laboratory. A further 12 control animals were introduced into the experiment during this period between vaccination and challenge. Two control animals died from anaplasmosis during this time, and a further one died just after the challenge commenced.

Complement fixation tests

The sera were examined by the CF test of CAMPBELL and TURNER (3), using antigen prepared from the T_1 strain of *M. mycoides*.

Challenge

The immunity was challenged after 15 months by mixing the vaccinated and control animals with others artificially infected by the endobronchial route (1) using the Gladysdale strain of *M. mycoides*. There were 24 artificially infected animals at the start of the trial and a further 10 animals were added during its course to replace those intubated animals that died. The challenge was carried out in the building described by GILBERT and WINDSOR (6) ; however the doors of the two rooms were left open the whole time and the animals were allowed to move freely between the 2. Fourteen days after the animals had been artificially infected, the control and vaccinated animals were brought into the building.

Unless they died, vaccinated and control animals were killed 6 weeks after the development of a CF titre, so that the scoring system of HUDSON and TURNER (8) could be applied. *Post mortem* and cultural examinations were carried out as described by GILBERT, DAVIES, READ and TURNER (5). The experiment was terminated once it appeared that no further serological reactions were developing.

RESULTS

Vaccination

Tail-tip route

Two weeks after vaccination 10 of the animals had CF titres in the range 2/10 to 3/40 but after 4 weeks all were negative to the CF test. Thereafter animals D579 and D627 gave

intermittent positive reactions to the test until 10 weeks after vaccination. From then on all remained negative until they were challenged. Animals D253 and D607 never produced CF antibody following vaccination.

Intra-dermal route

Two weeks after vaccination 9 of the animals had CF titres in the range 2/10 to 2/160. By 4 weeks all the sera were negative to the test. Animals D193, D461 and D576 gave intermittent positive reactions until 11 weeks after vaccination. Thereafter all remained negative until they were challenged. Animals D402, D572 and D580 did not produce CF antibody following vaccination.

Sub-cutaneous route

Nine of the animals had CF titres in the range 2/10 to 4/2560 by one week after vaccination. By the second week 10 animals had antibody (highest titre 2/160) and at 4 weeks all were negative. Animal D878 gave a fleeting positive reaction at week 9 and D577 was also positive at week 11. Thereafter the animals all remained negative until they were challenged. Animals D600 and D754 did not produce CF antibody following vaccination.

At no time before challenge did any of the control animals show a positive CF titre.

Challenge

The results of exposure to challenge of the 21 control and 36 vaccinated animals are summarised in tables I, II, III, IV.

Control Animals

Nineteen developed CF antibodies in their sera, 6 at 5 weeks, 8 at 6 weeks, 2 at 7 weeks and 3 at 8 weeks after the start of the challenge. Two animals never developed CF antibody in their sera and when examined *post mortem* lesions of CBPP were not seen. *M. mycoides* and its antigens were not demonstrated in the tissues of these 2 animals.

Three control animals died, and 3 showed overt clinical signs of CBPP. These 6 animals all had large lesions in the lungs from which *M. mycoides* was isolated. Thirteen animals with CF antibody in their sera showed no overt signs of disease but on *post mortem* examination 10 had unequivocal lesions of CBPP in the lungs, from which *M. mycoides* was isolated. The remaining 3 animals which produced CF antibody did not have typical lesions of CBPP in the lungs. *M. mycoides* and its antigens were not demonstrated in these 3 animals.

Tail-tip route

Four animals developed CF antibodies after challenge. D253 had CF antibody in its sera 4 weeks after the trial commenced but it was negative when slaughtered 6 weeks later. The CF reaction of D250 was transient but those of D327 (commenced 13 weeks after start of challenge) and D627 (6 weeks after start of challenge) persisted until the animals were slaughtered. No animal had lesions of CBPP and *M. mycoides* was not isolated, neither were its antigens demonstrated in the tissues of any animal in this group.

Intra-dermal route

Six animals developed CF antibody and in all cases it persisted until the animal was slaughtered. The first appearance of such antibodies was between 5 and 10 weeks after the challenge commenced. Three of these 6 animals had typical, if small, sequestra, from which *M. mycoides* was isolated. Two further animals (one which developed CF antibody and one which did not) had areas of fibrosis in the lungs from which *M. mycoides* was not isolated. Antigens were only detected in animals from which *M. mycoides* was isolated. Five animals did not respond to the challenge in any way.

Sub-cutaneous route

Three animals developed CF antibodies between 5 and 10 weeks after the start of the challenge. One animal had intermittent titres, one had a transient titre and in one the CF titre persisted for the entire 6 weeks. This last had an extensive area of fibrosis in its lung. No other animal in this group had lesions in its lungs and from none was *M. mycoides* isolated. The antigens of *M. mycoides* were not demonstrated in any animal in this group.

Statistical Analysis

Analysis of the challenge results was carried out and the animals were divided into two groups for each treatment, those having any signs of disease (score > 0) and those with none (score $= 0$). Chi square tests show

Table 1-In-contact challenge fifteen months after vaccination.

Experimental animals	Maximum C.F. titres	Clinical ≠	Post-mortem	*M. mycoides* isolated	H & T score ≠	
					Pathology	Total
Control animals						
C 309	3/1280	-	Sequestrum 1 cm in diameter	+	2	7
C 746	-	-	No lesions	-	0	0
C 748	2/40	-	Consolidation 2 cm containing pus. Not typical CBPP	-	1	3
D 73	1/1280	Temp. 3-7 days	Massive subacute lesion Lobes Left Lung	+	6	16
D 213	2/640	Died	3 large sequestra	+	6	18
D 314	1/640	-	4 sequestra - maximum size 5 cm	+	4	11
D 398	3/2560	Died	Massive acute lesion	+	6	18
D 422	3/2560	-	Sequestrum 6 cm in diameter	+	4	11
D 483	2/640	-	No lesions	-	0	3
D 573	4/2560	Temp. > 7 days	2 massive sequestra in both lungs	+	6	17
D 689	1/2560	-	5 sequestra, largest 6 cm in diameter	+	4	11
D 732	3/2560	-	Sequestrum 10 cm x 8 cm in intermediate lobe	+	4	11
D 792	-	-	No lesions	-	0	0
D 867	2/1280	Temp. > 7 days	Sequestrum - whole cardiac lobe right lung	+	6	17
D 968	2/2560	Died	Massive acute lesion	+	6	18
E 45	2/40	-	Area of consolidation 1 cm in diameter	-	1	3
E 429	2/640	-	2 sequestra 1 x 5 mm and 1 x 2 cm in diameter	+ (lesions only)	2	7
E 488	3/2560	-	Sequestrum 5 cm in diameter	+	2	7
E 491	2/640	-	Sequestrum 2 cm in diameter	+	2	7
E 495	1/640	-	Sequestrum 4 cm in diameter	+	2	7
E 501	2/2560	-	2 sequestra : 2 cm and 5 cm in diameter	+	4	11
			Total		66	203
			Mean		3.14	9.67

≠ Temp. = temperature of 103°F or more ; ≠ H & T score : Hudson & Turner Score (Hudson, J.R. & Turner, A.W. (1963). *Aust. vet. J.* 39. 373)

very highly significant (P = 0.001) differences between the control and vaccinated groups. When the treatments were compared the differences were not significant. However, when a t-test was used to compare the different treatment groups, the differences between the intra-dermal group and the other two groups showed a difference that almost reached the 5 p. 100 level of significance.

DISCUSSION

There was a marked difference between the groups in response to vaccination although all the animals received vaccine from the same bottle. A similar number of animals in each group produced CF antibody following vaccination but the group vaccinated by the subcutaneous route, in the main, developed CF anti-

TABLE II

Experimental animals	Maximum C.F. titres	Clinical ≠	Post-mortem	*M. mycoides* isolated	H & T score ≠	
					Pathology	Total
Animals vaccinated in Tail-tip						
D 250	2/20	-	No lesions	-	0	1
D 253	2/20	-	No lesions	-	0	1
D 270	-	-	No lesions	-	0	0
D 326	-	-	No lesions	-	0	0
D 327	1/160	-	No lesions	-	0	3
D 579	-	-	No lesions	-	0	0
D 599	-	-	No lesions	-	0	0
D 605	-	-	No lesions	-	0	0
D 607	-	-	No lesions	-	0	0
D 627	2/2560	-	Area fibrosis 2 cm in diameter	-	1	4
D 648	-	-	No lesions	-	0	0
D 662	-	-	No lesions	-	0	0
			Total		1	9
			Mean		0.083	0.75

TABLE III

Experimental animals	Maximum C.F. titres	Clinical ≠	Post-mortem	*M. mycoides* isolated	H & T score ≠	
					Pathology	Total
Animals vaccinated by sub-cutaneous Route						
D 321	-	-	No lesions	-	0	0
D 438	-	-	No lesions	-	0	0
D 497	-	-	No lesions	-	0	0
D 577	3/20	-	No lesions	-	0	2
D 596	-	-	No lesions	-	0	0
D 600	2/2560	-	No lesions	-	0	3
D 682	2/160		Fibrosis 10 x 4 cm	-	2	5
D 701	-	-	No lesions	-	0	0
D 754	-	-	No lesions	-	0	0
D 807	-	-	No lesions	-	0	0
D 878	-	-	No lesions	-	0	0
D 976	-	-	No lesions	-	0	0
			Total		2	10
			Mean		0.17	0.83

body a week before the other two groups. In 4 of the 10 animals in this group antibody was demonstrated on three successive occasions. In the other two groups no animal had demonstrable antibody for more than 2 consecutive weeks. The tail-tip and intra-dermal groups both showed a similar response to vaccination and they developed very much lower titres than the animals in the group vaccinated sub-cutaneously. A possible explanation is that when the vaccine is given by the sub-cutaneous route it is taken up by the reticulo-endothelial system much more quickly and in greater quantity than when given by the other two routes.

TABLE IV

Experimental animals	Maximum C.F. titres	Clinical ƒ	Post-mortem	*M. mycoides* isolated	H & T score ≠	
					Pathology	Total
Animals vaccinated by intra-dermal route						
C 723	1/160	-	No lesions	-	0	3
D 193	-	-	No lesions	-	0	0
D 242	2/640	-	No lesions	-	0	3
D 248	-	-	No lesions	-	0	0
D 303	-	-	Fibrosis 1 cm in diameter	-	1	1
D 333	-	-	No lesions	-	0	0
D 400	2/320	-	Sequestrum 3 cm in diameter	+	2	7
D 402	-	-	No lesions	-	0	0
D 461	-	-	No lesions	-	0	0
D 572	2/2560	-	Fibrosis 4 cm in diameter	-	1	4
D 576	2/320	Temp. 3-7 days	Sequestrum 1.5 cm in diameter	+	2	8
D 580	2/640	Temp. 3-7 days	Sequestrum 5 mm in diameter	+	2	8
			Total		8	34
			Mean		0.67	2.83

WINDSOR, MASIGA and READ (18) stipulated that a mean pathology « score » of less than 2.5 in the control animals indicated that the challenge had been inadequate. In the present study, the control animals had a mean pathology score of 3.14 indicating the vaccinated cattle had received an adequate challenge. The statistical analyses showed that all vaccine regimes had resulted in a significant reduction in disease. However, there was a suggestion, not confirmed by the statistical evidence that the intra-dermal route of vaccination was not as good as the other two. No unequivocal lesions of CBPP were seen in the animals vaccinated by the tail-tip or subcutaneous routes, whereas in those vaccinated by the intra-dermal route, 3 of 12 animals had typical, if small, CBPP sequestra. These findings suggest that immunity in the group vaccinated by the intra-dermal route was on the wane. Windsor, *et al* (18) reported the results of an « in-contact » trial carried out 2 years after primary vaccination, when immunity was on the wane and 5 of 16 vaccinated animals had small sequestra in the lungs.

It is possible that if the trial had been carried out 12 rather than 15 months after vaccination all treatment groups would have shown a similar lack of response to the challenge. An intra-dermal vaccination group was included in the trial because it was thought that a slow release of organisms might be essential for the development of a satisfactory resistance to the disease. These results have completely disproved that theory. It should be remembered that the intra-dermal group received a smaller vaccinating dose than did the other two groups, 6.4×10^8 as opposed to 1.6×10^9 CFU. However, GILBERT and WINDSOR (6) as a result of their vaccine dosage trial recommended a minimum vaccinating dose of 10^7 CFU and in that trial challenge was carried out 6 months after vaccinating whereas in the present study the animals were not challenged for 15 months. With a greater period of time, after challenge, it is possible that the results might have been different. This poses the question, does vaccination produce a true immunity ? The animals had no demonstrable circulating antibody in their blood at the time the trial commenced. Yet, all groups showed a significant resistance to challenge when compared to the control group. WINDSOR, MASIGA and BOARER (17) showed that animals vaccinated with the T_1

broth vaccine did not give a positive response to the comparative intra-dermal allergic test and ROBERTS and WINDSOR (14) used a whole battery of tests in an attempt to distinguish between vaccinated and control animals, without success. This suggests classical concepts of immunity do not apply to CBPP. It would seem that neither humoral nor cellular immune mechanisms play any part in resistance to the disease. Perhaps there is a premune status in which the presence of viable mycoplasms is required to prevent the establishment of disease. However, MASIGA and WINDSOR (10) were able to protect two animals for 8 and 9 months by a single intravenous injection of 1.5 and 2.0 litres of serum from animals recovered from CBPP but which did not contain antibodies demonstrable by the CF test. MASIGA, ROBERTS, KAKOMA and RURANGIRWA (9) repeated this work with a larger number of cattle and showed a similar protection for 4 months. The means whereby such protection is effected is still in doubt. The work of MASIGA and WINDSOR (10) shows that the protection can persist for considerable periods of time. These findings do not rule out the possibility that a premune status exists. Possibly viable organisms in the body produce a chemical that circulates in the blood and prevents other mycoplasms from establishing themselves : such a compound could be present in the serum of recovered animals but not be measurable by immunological tests. If such is the case then the length of time that an animal remains resistant may bear a simple numerical relationship to the number of organisms in the vaccinating dose. This would explain why the intra-dermal group showed a waning resistance whereas the other two groups were both solidly resistant. Killed vaccines have been shown to offer no protection (15) which is further evidence for this theory. A vaccinating dose of 1.6×10^9 CFU produced solid immunity for 15 months whereas with a dose of 1.8×10^9 CFU immunity was waning 2 years after vaccination (18). More work into the mechanisms of resistance to CBPP is required.

In many of the French-speaking countries it was the practice to vaccinate cattle in the muzzle. PROVOST, VILLEMOT and QUEVAL (13) describe muzzle vaccination with the T3 strain of *M. mycoides,* they stated that if the vaccine is injected into the correct site, it is perfectly safe. However, if the vaccine is administered incorrectly it can result in a very severe reaction, and today this route is only used in the face of an outbreak when a rapid production of immunity is required (12). ORUE and MEMERY (11) stated that in taurins, the vaccine reactions were « often fatal, always serious and in consequence unacceptable ». They recommended intra-dermal vaccination into the distal portion of the external surface of the ear. Apart from being subject to some of the drawbacks of tail-tip vaccination the intra-dermal route of vaccination has now been shown to be markedly less efficient than either the tail-tip or sub-cutaneous routes.

Since there was no significant difference between the immunity produced by the tail-tip and sub-cutaneous routes when the animals were challenged 15 months after primary vaccination, it is reasonable to suggest that this latter route of administration could be used in the field. GILBERT *et al.* (5) recommend that CBPP vaccination should be carried out on an annual basis and WINDSOR *et al.* (18) concurred with this view and furthermore considered that less frequent vaccination might lead to the production of « carrier » animals. Since resistance to CBPP in animals vaccinated by the sub-cutaneous route was total 15 months after vaccination it is reasonable to assume that it will be no less at 12 months. This 15 month interval between vaccination and challenge was chosen specifically since it was longer than the recommended interval between vaccinations.

The results of this trial are in agreement with those of DOUTRE and CHAMBRON (4) who showed that 14 months after sub-cutaneous vaccination with the T1 strain of *M. mycoides,* 9 out of 10 of the animals were resistant to challenge. The present study has demonstrated the efficacy of the sub-cutaneous route but more knowledge of the safety of this method in the field in East Africa is required. The T1 vaccine has been known to produce reactions following tail-tip vaccination, including the loss of the tail. It would therefore be prudent to introduce sub-cutaneous vaccination with caution, particularly in areas where reactions have been known to occur. Providing this precaution is taken it should now be possible to use the more convenient, quicker and cleaner sub-cutaneous route for vaccination against CBPP.

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SUMMARY

The effect of the route of administration on the immunity produced by the T_1 strain of *Mycoplasma mycoides* sub-species *Mycoides*

The tail-tip route of administration of the T_1 broth vaccine against contagious bovine pleuropneumonia (CBPP) is still widely used throughout Africa. The historical reasons for this route of administration are described as is an experiment designed to determine whether there is any scientific necessity for the continued use of this route.

Cattle were vaccinated in the tail-tip, under the skin and into the skin of the shoulder, using the same batch of the T_1 broth vaccine. Fifteen months later their immune status was determined by an « in-contact » challenge trial. The 24 animals in the groups vaccinated in the tail-tip and by the sub-cutaneous route were all completely refractory to the challenge. Three of the 12 animals vaccinated by the intra-dermal route developed lesions of CBPP during the challenge. An attempt is made to explain these results.

The efficacy of the sub-cutaneous route of administration has been demonstrated and it should now be possible to use this more convenient, quicker and cleaner route for vaccination against CBPP. A word of caution is inserted ; a suggestion is made that a field trial to determine the safety of the sub-cutaneous route be carried out before its widespread introduction into the field.

RESUMEN

Influencia de la via de administración sobre la inmunidad producida por la cepa T_1 de *Mycoplasma mycoides* subsp. *mycoides*

En todas partes de Africa, comunmente se sigue utilizando la inyección en la borla de la cola para la administración de la vacuna T_1 en caldo contra la perineumonia.

Los autores describen las causas históricas del empleo de dicha via ; ¿ Justificase de modo científico el seguimiento de este uso ?

Se vacunaron bovinos en su extremidad caudal, atrás de la espalda por via subcutánea, en la espalda por via intradermica, al utilizar vacuna T_1 en caldo del mismo lote.

Quince meses más tarde, se determinó el nivel de inmunidad de estos bovinos mediante una prueba infectante por contagión directa. Los 24 animales de los grupos vacunados en el cabo de la cola y por via subcutanea resistieron a la infección de prueba. 3 de los 12 animales vacunados por via intradérmica mostraron lesiones de perineumonia durante este ensayo. Se intenta explicar dichos resultados.

La eficacia de la via subcutánea está demostrada, desde ahora seria posible utilizar dicho método, más apropiada, más rapida y más limpia, para la vacunación contra la perineumonia. Sin embargo, se aconseja ensayarlo en Africa oriental, para asegurarse la eficacia de la via subcutánea antes de aplicarlo en gran escala.

BIBLIOGRAPHIE

1. BROWN (R. D.). Endobronchial inoculation of cattle with various strains of *Mycoplasma mycoides* and the effects of stress. *Res. vet. Sci.*, 1964, **5** : 393-404.
2. BROWN (R. D.), GOURLAY (R. N.), MACLEOD (A. K.). The production of T_1 broth culture contagious bovine pleuropneumonia vaccine. *Bull. epizoot. Dis. Afr.*, 1965, **13** : 149-155.
3. CAMPBELL (A. D.), TURNER (A. W.). Studies on contagious pleuropneumonia of cattle. IV. An improved complement fixation test. *Aust. vet. J.*, 1953, **29** : 154-163.
4. DOUTRE (M. P.), CHAMBRON (J.). Valeur de l'immunité conférée par un vaccin antipéripneumonique lyophilisé préparé à l'aide de la souche T_1. *Rev. Elev. Méd. vét. Pays trop.*, 1970, **23** (2) : 163-179.
5. GILBERT (F. R.), DAVIES (G.), READ (W. C. S.), TURNER (G. R. J.). The efficacy of T_1 strain broth vaccine against contagious bovine pleuropneumonia : in-contact trials carried out six and twelve months after primary vaccination. *Vet. Rec.*, 1970, **86** : 29-33.
6. GILBERT (F. R.), WINDSOR (R. S.). The immu-

nizing dose of T_1 strain *Mycoplasma mycoides* against contagious bovine pleuropneumonia. *Trop. anim Hlth. Prod.*, 1971, **3** (2) : 71-76.
7. HAGGARD (R. H.). « King Solomon's Mines » 1885 p. 49, Collins, 1959.
8. HUDSON (J. R.), TURNER (A. W.). Contagious bovine pleuropneumonia : a comparison of the efficacy of two types of vaccine. *Aust. vet. J.*, 1963, **39** : 373-385.
9. MASIGA (W. N.), ROBERTS (D. H.), KAKOMA (I.), RURANGIRWA (F. R.). Passive immunity to contagious bovine pleuropneumonia. *Res. vet. Sci.*, 1975, **19** (3) : 330-332.
10. MASIGA (W. N.), WINDSOR (R. S.). Immunity to contagious bovine pleuropneumonia. *Vet. Rec.*, 1975, **97** (18) : 350-351.
11. ORUE (J.), MEMERY (G.). Note sur la vaccination intradermique contre la péripneumonie contagieuse bovine. *Bull. Acad. vét. Fr.*, 1960, **33** : 411-418.
12. PROVOST (A.). Prophylaxie et vaccination dans la péripneumonie bovine. Evolution des techniques et applications pratiques actuelles. *Rev. Elev. Méd. vét. Pays trop.*, 1974, **27** (2) : 145-161.
13. PROVOST (A.), VILLEMOT (J. M.), QUEVAL (R.). Recherches immunologiques sur la péripneumonie. VII. Immunisation au moyen d'une souche avianisée de *Mycoplasma mycoides* var. *mycoides* inoculée par la voie du mufle. *Rev. Elev. Méd. vét. Pays trop.*, 1959, **12** (4) : 381-404.
14. ROBERTS (D. H.), WINDSOR (R. S.). Attempts to differentiate *Mycoplasma mycoides* var. *mycoides* immune cattle from susceptible cattle. *Res. vet. Sci.*, 1974, **17** (3) : 403-405.
15. SHIFRINE (M.), BEECH (J.). Preliminary studies on living culture and inactivated vaccines against contagious bovine pleuropneumonia. *Bull. epizoot. Dis. Afr.*, 1968, **16** : 47-52.
16. WILLEMS (L.). Mémoires sur la pleuropneumonie enzootique du gros bétail. *Recl. Méd. vét.* Ecole Alfort, 1852, **28** : 401.
17. WINDSOR (R. S.), MASIGA (W. N.), BOARER (C. D. H.). A single comparative intradermal test for the diagnosis of contagious bovine pleuropneumonia. *Res. vet. Sci.*, 1974, **17** (1) : 5-23.
18. WINDSOR (R. S.), MASIGA (W. N.), READ (W. C. S.). The efficacy of T_1 strain broth vaccine against contagious bovine pleuropneumonia : incontact-trials carried out two years after primary vaccination. *Vet. Rec.*, 1972, **90** (1) : 2-5.