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THE IMMUNE RESPONSE OF HORSES TO TETANUS TOXOID

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ABSTRACT

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An intramuscular injection of 8–16 Lf tetanus toxoid in water-in-oil emulsion protected adult horses against tetanus for at least 128 weeks. A booster dose of 8 Lf toxoid in aqueous solution protected them for a further period of at least 3½ years.

Colostral immunity protected foals for at least 10 weeks. An intramuscular injection of 8 Lf toxoid in water-in-oil emulsion given to foals from immune dams when they were 10–18 weeks old did not elicit any antibody response. They did respond, however, to a booster injection of 8 Lf toxoid in aqueous solution given 12 weeks after the first dose.

New-born foals were shown to be inherently unable to respond to an injection of tetanus toxoid.

Résumé

LA RÉPONSE IMMUNITAIRE DU CHEVAL À LA TOXOÏDE TÉTANIQUE

Une injection intramusculaire de 8 *à* 16 *Lf de toxoïde tétanique en émulsion huileuse a protégé des chevaux adultes contre le tétanos pendant au moins* 128 *semaines. Une dose de rappel de* 8 *Lf de toxoïde en solution aqueuse les a protégés pour une période subséquente d'au moins* 3 *ans et demi.*

L'immunité colostrale a protégé des poulains pour au moins 10 *semaines. Une injection intramusculaire de* 8 *Lf de toxoïde en émulsion huileuse administrée à des poulains nés de mères immunes et âgés de* 10 *à* 18 *semaines n'a provoqué aucune réponse immunitaire. Il y eut cependant une réaction à une dose de rappel de* 8 *Lf de toxoïde en solution aqueuse administrée* 12 *semaines après la première dose.*

On a pu montrer que des poulains nouveau-nés étaient par hérédité incapables de réagir à une injection de toxoïde tétanique.

INTRODUCTION

Although several species of domestic animals are known to suffer from tetanus, one reason for the high incidence in horses is their far greater susceptibility to tetanus toxin than that of other species (Smith, 1975). Common sites of entry of the infection in horses are nail wounds in the foot, the wounds left at castration, and the umbilicus in new-born animals. Cases are also known to occur as a result of infection of rather trivial wounds.

The control of tetanus depends on 2 approaches; firstly, the treatment of affected animals, and secondly, prophylaxis.

The treatment of clinical tetanus is sometimes disappointing, since many affected animals die in spite of modern therapeutic measures, especially after a short incubation period. Even the injection of large doses of antitoxin [from 100 000 to 200 000 International Units (IU)] is not invariably successful, since it will neutralize only circulating toxin, while that attached to the neuromuscular end plates is irreversibly bound and not affected (Jansen, unpublished results).

Although antiseptic surgical procedures and local and systemic treatment of wounds are important in tetanus prevention, one cannot be certain that such treatment will eliminate all spores from the wound and that favourable conditions for their germination and toxin production will not develop later.

The only reliable way of protecting an animal against tetanus is by providing it with an effective circulating antitoxin level. This can be done by injecting a horse with about 1 500 IU of tetanus antitoxin prior to an operation or at the time of wounding. The duration of this type of protection is, however, never more than 3 weeks (Liefman, 1975).

A decided objection to the use of antitoxin is its expense, more so in view of the availability of a cheaper method which gives a more durable protection, namely, active immunization.

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The active immunization of horses against tetanus was introduced by Ramon & Lemetayer as early as 1931. They proved it to be a practical procedure.

Mason & Schaafsma (1962) advanced a convincing plea for the routine active immunization of all horses against tetanus. Subsequently, several research workers published results of their experiments on the protection of horses against tetanus by the injection of tetanus toxoid. Kerry, Thomson, Epps & Foster (1976) used an aluminium phosphate-precipitated toxoid prepared from a fraction of tetanus toxin obtained by precipitation with potassium phosphate. They gave 1 group of horses a primary and secondary stimulus of either 1,0 or 2,0 mℓ separated by an interval of 4–7 weeks, while a second group received a booster stimulus of 1,0 or 2,0 mℓ 8–12 months after the primary injection. All of the 500 horses injected showed a protective titre 2–4 weeks after the last injection and none showed any local or general adverse reaction following immunization.

Scarnell (1974) showed that in horses that had received 2 doses of 2 mℓ of an aluminium hydroxide-adsorbed toxoid at an interval of one month 5 years previously, a dose of 2 mℓ aluminium phosphate-adsorbed tetanus toxoid elicited high concentrations of circulating antitoxin.

Although it is now generally accepted that horses can be protected against tetanus for prolonged periods by active immunization, a programme providing details of the definite amounts of antigen required per injection and the optimum intervals between injections would be useful. The present study aims at providing some contribution towards satisfying this need.

Of particular relevance to this study is the use of adjuvants to prolong the protective effect of vaccination. At this stage every scientist concerned with the production of vaccines would agree with Hilleman (1966) that emulsified water-in-oil adjuvant preparations provide an enhanced immunity of long duration. But the preparation of an ideal stable emulsion is rather exacting. The choice of the grade of paraffin oil and the types and purity of the wetting agents are critical both from the point of view of the antigenic response and of the provocation of local reactions. Furthermore, the ingredients in no small way may add

to the cost of the final vaccine to the consumer. An attempt was therefore made to limit the use of adjuvants as far as possible without forfeiting the aim of prolonged protection against tetanus.

Materials and Methods

In this study 1 Lf toxin or toxoid was taken as the equivalent of one IU of antitoxin as determined by the Ramon flocculation method (Jansen, 1961).

Preparation of the antigen

Clostridium tetani strain 761* was grown in Visking cellophane tubes of 100 mm diameter suspended in medium prepared according to the method employed by the South African Institute for Medical Research (Anon., 1949). This method is based on the technique developed by Sterne & Wentzel (1950) for the production of *Clostridium botulinum* toxin.

Each 10 ℓ production flask contained 6 ℓ of medium consisting of the following:

	g
Tryptone**	300
Peptone**	150
Glucose	120
NaCl	58
$Na_2HPO_4.12H_2O$	7,2
KH_2PO_4	24,5
$MgSO_4.7H_2O$	0,72
Tap water	15ℓ

This medium was made up in bulk by heating the water to 80 °C and then dissolving the NaCl, buffer salts and $MgSO_4$ in it, and subsequently the glucose, peptone and tryptone. The mixture was then boiled for 5 min and the pH adjusted to 7,4 with 1 N NaOH.

Each cellophane bag contained 1 ℓ of 0,4% NaCl solution at the start.

After the flask and its contents had been assembled, it was autoclaved at 115 °C for 60 min, allowed to cool, and then placed overnight in an incubator at 35 °C.

The stock seed culture was stored at −20 °C and activated in Robertson's meat particle broth. Five mℓ of actively growing culture was pipetted into each cellophane bag on the morning after the preparation of the production flasks and the incubation continued at 35 °C for 8 days. Toxin values of about 100 Lf per mℓ were regularly obtained.

Detoxification was carried out by adding 0,4 mℓ of 40% formaldehyde/100 mℓ of the toxic culture fluid at pH 7,0 and continuing the incubation at 35 °C until 1,0 mℓ injected subcutaneously failed to kill a guinea-pig mass-measuring about 400 g. The guinea-pigs were observed for 14 days. Toxoiding was usually complete in 10 days.

Preparation of adjuvant plus toxoid

A toxoid in water-in-oil emulsion was prepared according to the following formula:

	mℓ
Ondina oil***	60
Lubrol Moa****	8
Tween 80****	2
Antigen solution	30

The Ondina oil was mixed with the lipophilic emulsifier, Lubrol Moa, and the aqueous antigen in the appropriate dilution was mixed with the hydrophilic emulsifier, Tween 80. Then the aqueous phase was slowly added to the oil phase with agitation by means of a syringe.

* Kindly supplied by the South African Institute for Medical Research, P.O. Box 1038, Johannesburg 2000
** Bio. Lab., P.O. Box 15849, Lynn East 0039
*** Shell Chemicals, P.O. Box 494, Johannesburg 2000
**** I.C.I., P.O. Box 11270, Johannesburg 2000

Different regimens of vaccination using toxoid plus adjuvant and toxoid in aqueous solution were applied to groups of horses to arrive at the best possible scheme for continuous protection against tetanus. The details of these procedures will become evident from the account of the results achieved.

Experimental animals

The group of brood mares and their foals belonging to the Onderstepoort Veterinary Research Institute was used for immunization studies. They were light horses of no specific breed and kept on open range.

Toxin neutralization tests for determining the antitoxin titres of the sera of immunized horses were done in the conventional way in white mice having an average mass of 25 g (Jansen, 1961). The injection of toxin-antitoxin mixtures was given intravenously and the final results recorded after 3 days. Antitoxin titres were expressed in IU.

Results

The local effect of tetanus toxoid in water-in-oil emulsion

The subcutaneous injection of tetanus toxoid invariably results in an unsightly swelling which does not happen when the injection is given intramuscularly. Since, however, breeders, and especially the owners of thoroughbred horses, are wary of the possibly detrimental effect of any injection on the subsequent performance of their animals, 4 horses destined for slaughter were injected intramuscularly in their gluteal region with 2 mℓ, that is, a double dose of the final vaccine. The injection site was marked and the horses were slaughtered serially at intervals of 4, 8, 16 and 24 weeks. The musculature at the injection sites was carefully dissected in a search of pathological changes, but none were seen.

The response of horses to one injection of tetanus toxoid in water-in-oil emulsion

Two groups of 5 adult horses which had never been injected with tetanus toxoid or antitoxin were selected. They were injected intramuscularly with 1 mℓ of toxoid in water-in-oil emulsion, one group receiving 8 Lf of toxoid and the other 16 Lf. Two weeks later and subsequently at irregular intervals over a period of 128 weeks they were bled and their antitoxin titres determined at each bleeding. The sampling was stopped after 128 weeks when 2 horses of the 16 Lf group had died unavoidably of horsesickness. The results recorded in Table 1 are sufficient to give an impression of the level and duration of the response. The geometric mean values of the titres at each bleeding are given to serve as an indication of the rise and fall of the average value.

From this table it is clear that, when 0,01 IU antitoxin/mℓ of serum is taken as the minimum protective level (Scheibel, 1955), all horses in the 2 experimental groups were protected for at least 128 weeks. Although the premise that 0,01 IU per mℓ serum is sufficient to protect against clinical tetanus is based on experience with humans, there is no evidence to suggest that the situation is different in horses.

Although the response to 8 Lf was consistently lower than to 16 Lf, both reached their peak level between 4–6 weeks after the injection and then slowly reached a lower level which in turn declined, but more slowly.

It is fair to infer that both doses provided the horses with a sound basic immunity in view of the prolonged substantial response.

TABLE 1 Antitoxin titres (IU) of horses injected with tetanus toxoid in water-in-oil emulsion

Horse No.	Lf Toxoid	Weeks after injection										
		2	12	20	32	44	60	76	84	100	116	128
1	8	1,40	0,33	0,10	0,10	0,10	0,10	0,10	0,07	0,07	0,02	0,02
2	,,	0,10	1,00	1,00	1,00	1,00	1,00	1,00	1,00	1,00	0,50	0,50
3	,,	1,40	0,50	0,10	0,10	0,10	0,10	0,10	0,20	0,20	0,10	0,10
4	,,	5,00	1,00	0,40	0,20	0,20	0,20	0,14	0,14	0,14	0,10	0,10
5	,,	5,00	10,00	10,00	3,30	3,30	1,40	1,40	1,00	1,00	1,00	1,00
Geom. mean.....		1,37	1,11	0,53	0,37	0,37	0,31	0,29	0,21	0,21	0,16	0,16
1	16	4,00	5,00	3,30	2,00	1,40	1,00	1,00	0,50	0,50	0,40	0,33
2	,,	1,40	1,00	0,40	0,33	0,33	0,20	0,10	0,20	0,20	0,10	×
3	,,	4,00	3,30	1,40	1,00	1,00	0,20	0,33	0,33	0,33	0,33	0,33
4	,,	1,40	3,30	1,40	1,00	1,00	0,50	0,33	0,20	0,20	0,20	0,20
5	,,	2,00	10,00	10,00	10,00	5,00	2,00	1,40	1,40	1,40	1,00	×
Geom. mean.....		2,29	3,69	1,88	1,46	1,18	0,53	0,43	0,39	0,39	0,31	—

The effect of a booster injection of tetanus toxoid

In view of, firstly, the possible objection to repeated injections of toxoid together with adjuvant and, secondly, the cost and demands of preparing water-in-oil emulsions, the booster effect of an aqueous solution of toxoid was investigated. The 8 remaining horses from the previous experiment were used on the assumption that they had a sound basic immunity. One hundred and twenty-eight weeks after the first injection they received a 1 mℓ intramuscular booster injection of 8 Lf toxoid in aqueous solution. To enable the anamnestic response to be followed closely, the horses were bled at short intervals as indicated in Table 2 and their antibody titres determined.

From these results it is clear that the booster injection elicited an appreciable increase in the antitoxin titre in as short a period as 3 days. The titre rose sharply to a maximum value on about the 10th day after injection and this peak in all animals was many times higher than the highest value attained during the primary response.

The Onderstepoort Veterinary Research Institute required most of the horses for various experimental purposes, but 2 of them were kept under observation for 3½ years and their serum-antitoxin titres again determined. It will be seen from Table 2 that their titres remained as high as 5,00 and 10,00 IU.

The colostral immunity in foals of immune mares

In view of the occurrence of tetanus in very young foals, it seemed necessary to investigate their passive protection by colostral antibodies.

Ten pregnant mares, which had previously been vaccinated against tetanus, were closely observed for foaling. About 24 h after birth, when the foal had had sufficient opportunity of suckling its dam, both the foal and the mare were bled and their serum-antitoxin titres determined. The foals were subsequently bled at the times indicated in Table 3.

Although there was no direct correlation between the level of the titre in the mare and her foal, in general, mares with high titres had foals with correspondingly high titres and the sera of foals from mares with low titres had low values. All foals were protected for 10 weeks, however, when the titres reached the minimal protective level.

The active immunization of passively protected foals

Since under practical conditions the period of passive protection in some foals may be only 10 weeks, it was necessary to investigate the response of young foals to active immunization, while some of the members of the group still had fairly high titres of colostral antibody. To achieve this, all the foals (except one which had died of an intercurrent condition), after being discharged from the previous experiments, were given an intramuscular injection of 8 Lf toxoid in water-in-oil emulsion when they were from 10–18 weeks old. Their antibody titre was determined at the time of injection and subsequently as indicated in Table 4.

From Table 4 it is evident that the foals did not show any response to the injection and their titres continued to decline over the 11-week sampling period.

Twelve weeks after the primary injection this group of foals received an intramuscular injection of 8 Lf toxoid in aqueous solution. The influence of this injection on their antitoxin titres is shown in Table 5.

Table 5 shows that all the foals responded to the booster dose of antigen and developed an antitoxin titre well above the minimum protective level for up to 13 weeks. Some of them were protected for much longer.

The same group of foals received a 2nd booster of 8 Lf toxoid in aqueous solution at the age of 52 weeks and were bled one week afterwards. The antibody titres are given in Table 6.

Table 6 shows that the reaction was much the same as that after the first booster.

The immune response of young foals without colostral immunity

To determine whether the low antibody response to a primary stimulus displayed by the foals in the previous experiments was due to the presence of passively acquired antibodies or an inherent inability, foals from mares that had definitely not had an injection of tetanus antigen and showed no detectable tetanus antitoxin in their blood were used. For practical reasons they were injected at ages varying from 2 days to 8 weeks. The dose was 8 Lf antigen in water-in-oil emulsion given intramuscularly. They were bled at 6, 8 and 10 weeks after the injection and their antibody titres determined. The results are recorded in Table 7.

From Table 7 it is clear that the lack of response to an antigenic stimulus is an inherent characteristic of new-born foals.

TABLE 2 The effect of a booster injection of 8 Lf toxoid in aqueous solution

Horse No.	Titre before booster (IU)	Days after booster											Titre after 3,5 years (IU)
		1	2	3	4	7	9	10	11	14	16	18	
1	0,02	0,02	0,02	1,00	1,40	100,00	100,00	100,00	200,00	100,00	100,00	50,00	
2	0,50	0,50	0,50	1,00	10,00	100,00	100,00	100,00	200,00	100,00	50,00	50,00	5,00
3	0,10	0,10	0,10	0,20	1,00	50,00	333,00	200,00	200,00	100,00	100,00	100,00	
4	0,10	0,10	0,10	0,14	0,40	100,00	100,00	100,00	100,00	100,00	100,00	100,00	
5	1,00	1,00	1,00	1,00	2,00	200,00	333,00	200,00	200,00	100,00	100,00	100,00	
6	0,33	0,33	0,33	0,33	1,00	1 000,00	1 000,00	1 000,00	1 000,00	333,00	333,00	333,00	
7	0,33	0,33	0,33	0,50	1,40	100,00	100,00	100,00	100,00	33,00	33,00	33,00	10,00
8	0,20	0,20	0,20	0,14	1,00	500,00	500,00	333,00	333,00	200,00	200,00	200,00	
Geom. mean	0,20	0,20	0,20	0,42	1,41	163,10	220,30	184,40	225,60	110,40	101,20	93,01	

TABLE 3 The passive immunity of foals born from immune dams

Mare No.	Titre (IU)	Foal titre							
		1 day	1 wk	2 wks	3 wks	4 wks	6 wks	8 wks	10 wks
1	0,10	0,10	0,10	0,10	0,05	0,04	0,03	0,02	0,014
2	40,00	50,00	50,00	50,00	50,00	50,00	40,00	40,00	20,00
3	3,30	20,00	14,00	10,00	10,00	5,00	3,30	3,30	0,40
4	10,00	10,00	10,00	5,00	3,30	2,00	1,40	1,00	0,40
5	2,00	3,30	3,30	2,00	2,00	1,00	1,00	0,33	0,14
6	4,00	20,00	20,00	14,00	10,00	5,00	3,30	1,40	0,50
7	10,00	10,00	10,00	10,00	5,00	3,30	1,00	1,00	0,33
8	10,00	1,40	2,00	1,40	1,40	1,40	0,50	0,10	0,01
9	3,30	10,00	10,00	10,00	5,00	3,30	2,00	1,00	0,50
10	1,00	1,00	0,50	0,33	0,14	0,10	0,10	0,10	0,014

TABLE 4 The response of passively immune foals to an injection of 8 Lf toxoid in water-in-oil emulsion given when 8–10 weeks old

Foal No.	Titre before injection (IU)	Weeks after injection						
		1	2	3	4	5	8	11
1	0,004	0,003	0,003	0,001	0,001	0,001	0,10	0,20
2	10,00	10,00	10,00	5,00	5,00	4,00	3,30	2,00
3	0,14	0,14	0,14	0,10	0,10	0,02	0,01	0,001
4	0,20	0,14	0,20	0,14	0,10	0,01	0,01	0,003
5	0,01	0,01	0,002	0,002	0,002	0,002	0,05	0,10
6	0,50	0,50	1,00	1,00	0,33	0,10	0,01	0,05
7	0,33	0,33	0,10	0,10	0,10	0,10	0,10	0,10
8	0,01	0,004	0,003	0,002	0,001	0,001	0,001	<0,001
9	0,50	0,50	0,10	0,33	0,33	0,33	0,04	0,04

TABLE 5 The effect of a booster injection of aqueous antigen 12 weeks after the primary injection in foals born from immune mares

Foal No.	Titre before booster (IU)	Weeks after booster								
		1	2	3	4	8	13	21	26	36
1	0,20	2,00	4,00	5,00	4,00	1,40	0,33	0,05	0,03	0,033
2	2,00	2,00	1,40	1,00	1,00	0,50	0,14	0,05	0,01	0,003
3	0,001	5,00	3,30	3,30	2,00	1,00	0,14	0,04	0,01	0,01
4	0,004	1,00	0,50	0,50	1,00	0,05	0,03	0,005	0,001	0,001
5	0,10	20,00	14,00	14,00	5,00	3,30	1,00	0,05	0,025	0,033
6	0,05	14,00	10,00	5,00	3,30	2,00	0,33	0,33	0,03	0,033
7	0,10	20,00	33,00	14,00	10,00	4,00	1,40	0,10	0,10	0,10
8	<0,001	3,30	2,00	0,33	0,20	0,10	0,004	0,004	0,002	0,002
9	0,04	5,00	10,00	5,00	3,30	1,40	0,20	0,05	0,014	0,01
Geom. mean		4,97	4,12	3,71	2,12	0,797	0,167	0,038	0,013	0,011

TABLE 6 The effect of a second booster in foals

Foal No.	Titre one week after booster (IU)
1	4,0
2	1,40
3	10,00
4	2,00
5	4,00
6	14,00
7	20,00
8	2,00
9	10,00
Geom. mean	5,14

TABLE 7 The response of foals without a passive immunity to an injection of 8 Lf tetanus toxoid in water-in-oil emulsion

Foal No.	Age	Titre after weeks (IU)		
		6	8	10
1	8 wks....	<0,0001	<0,0001	<0,0001
2	8 „	„	„	„
3	6 „	„	„	„
4	5 „	„	„	„
5	4 „	„	„	„
6	2 days.....	„	„	„

Discussion

Vaccinating children with diphtheria and tetanus toxoid and pertussis vaccine at 2, 4, 6 and 18 months of age has become a generally accepted practice and establishes a sound basic immunity against tetanus. Also, immunizing an adult without childhood immunization by giving 3 diphtheria and tetanus toxoid injections at one-month intervals and a booster injection one year after the 3rd injection (Rothstern & Baker, 1978) is sound practice. According to Peebles, Levine, Eldred & Edsall (1969), an adequately immunized person does not need another booster for at least 10 years. A booster will produce protective antibody titres in 24 h or less in a person previously adequately immunized (Brown, 1968). This rapid response precludes the necessity for hyperimmune serum therapy with its well-known attendant disadvantages, except under special circumstances such as serious, dirty wounding of the head and face.

Although in horses there is no danger associated with the use of homologous antitoxin, there seems to be no reason why the same scheme as currently used for the prevention and treatment of tetanus in humans should not be applied *mutatis mutandis* to horses. Tetanus toxoid is easier and cheaper to prepare than antitoxin. The effect of an injection of toxoid lasts much longer than one of antitoxin and it can furthermore act as a booster stimulus to an existing basic immunity.

The above contention is supported by the results obtained in this study. The results recorded in Table 1 show that adult horses can be protected for at least 128 weeks by a single injection of 8 Lf tetanus toxoid in a water-in-oil emulsion. This immunity can be boosted effectively by an injection of toxoid in aqueous solution, thus avoiding such objections as could exist against the repeated use of water-in-oil emulsions. It has been proved that the anamnestic response is already evident on the 3rd day after the booster injection. Since the incubation period of tetanus in horses is usually long, and may be as long as 7–15 days (Henning, 1949), a booster injection in an animal with a basic immunity at the time of wounding would be a most valuable adjunct to the treatment of tetanus. Furthermore, according to Table 2, the antitoxin titres of horses after a single booster dose are so far above the protective level for 3,5 years that during this phase there is no reason to consider giving a booster dose, not even after wounding. The duration of immunity after this period still has to be worked out either during the course of clinical practice or through a purposeful experiment.

From Table 3 it can be seen that colostral immunity can play an important part in protecting young foals against tetanus for at least 10 weeks. Where the titres of foals from mares with identical titres vary, an explanation could be that all foals do not suckle their dams equally soon after birth and that the lower titres result where the first sucking has been delayed.

From the results recorded in Tables 4 and 7 it can be inferred that the inability of young foals to respond to a primary stimulus is not accounted for by the presence of colostral antibodies, but rather by a state of immaturity of the immune mechanism of the body. The immune mechanism does, however, become primed by this first injection of toxoid plus adjuvant, as is shown by the results in Table 5. These latter results must be regarded as being evidence of an anamnestic response, since, by comparison with Table 4, the geometric mean value of the titres showed a decided increase. Table 7 confirms this notion in that it shows that foals 8 weeks old show no response to a primary injection of toxoid plus adjuvant.

The logical inference from these results is that foals should be given their primary injection at the age of about 10 weeks so that they can be given a booster injection soon after the titres of a number of them have declined to below the protective level. This should protect them for at least a further 13 weeks. But from Table 6 it is clear that even a 2nd booster, when the foals are 1 year old, does not elicit a response as high as the one produced by the 1st booster in adult horses. The stage at which the transition from an immature to a mature immune status takes place shoud be determined more precisely.

Although it is not yet possible to state the minimum number of booster injections and their spacing required to keep a group of foals continuously protected, the results of this study clearly show that a foal shows an anamnestic response to a booster injection when it has had an injection of toxoid in adjuvant during early life. This information could be useful in treating fresh wounds.

In general, the results achieved show that by means of a primary stimulus of 8 Lf toxoid in water-in-oil emulsion and of appropriately spaced booster injections, tetanus in horses can be effectively and cheaply prevented.

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