The protective properties of vaccination against equine herpesvirus 1-induced viremia, abortion and nervous system disorders

Het beschermend effect van vaccinatie tegen equiene herpesvirus 1-geïnduceerde viremie, abortus en zenuwstoornissen

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ABSTRACT

Equine herpesvirus (EHV) 1 is an important pathogen of horses. Upon infection, the virus replicates in the upper respiratory tract. Then it spreads to internal organs via a cell-associated viremia. Local replication in internal organs may result in abortion and nervous system disorders. The currently designed vaccines are not able to induce significant protection against EHV1-induced viremia. However, several vaccines are able to induce a significant level of protection against either abortion or nervous system disorders. Which immune responses correlate with this protection is so far unknown. Besides vaccination, management will remain a crucial factor in the prevention of EHV1-induced clinical signs. Management measures include the vaccination of all horses on the premises to reduce infection pressure; the separate housing of young horses, adult horses and pregnant mares; strict hygiene measures and strict control of contact with horses from outside the premises. If, despite vaccination and thorough management, an outbreak of EHV1-induced disease occurs, treatment with antiviral agents may be useful.

SAMENVATTING

Equiene herpesvirus (EHV) 1 is een belangrijk pathogeen bij paarden. Na infectie vermeerdert het virus eerst in het ademhalingsstelsel. Vervolgens verspreidt EHV1 via een celgeassocieerde viremie naar inwendige organen. Daar kan een lokale virusvermeerdering resulteren in abortus of zenuwstoornissen. De huidige beschikbare vaccins kunnen het optreden van viremie na een infectie met wild-type virus niet voorkomen. Sommige vaccins kunnen wel een bescherming induceren tegen het optreden van abortus of zenuwstoornissen. Welke immunologische respons aan de basis ligt van deze bescherming is nog niet gekend. Naast de vaccinatie is ook het management zeer belangrijk bij de preventie van EHV1-geïnduceerde symptomen. De managementmaatregelen omvatten het vaccineren van alle paarden op het bedrijf om de infectiedruk te verlagen, de gescheiden huisvesting van jonge paarden, volwassen paarden en drachtige merries, een strikte hygiëne en het toezicht op het contact met paarden van buiten het bedrijf. Indien zich, ondanks vaccinatie en uitgebreide managementmaatregelen, toch een uitbraak van EHV1-geïnduceerde symptomen voordoet, dan kan eventueel een behandeling met antivirale geneesmiddelen overwogen worden.

INTRODUCTION

Equine herpesvirus 1 (EHV1), a member of the *Alphaherpesvirinae*, is an important pathogen of horses. Infection with EHV1 occurs via contact with infectious secretions or via inhalation of infectious aerosols. The primary site of replication is located in the epithelia of the upper respiratory tract (Kydd *et al.*, 1994a). Subsequently, the virus invades through the epithelial barrier into deeper tissues of the respiratory tract (Patel *et al.*, 1982; Kydd *et al.*, 1994a and b). Primary replication is accompanied by mild, transient respiratory disease, which is self-limiting within 9 days following infection (Gibson *et al.*, 1992).

Four to 6 days after respiratory infection, EHV1-infected mononuclear cells enter the blood, resulting in a cell-associated viremia. Viremia generally lasts until 9 to 14 days post-infection (Thein and Brown, 1988; Gibson *et al.*, 1992), although McCartan *et al.* (1995) reported viremia lasting for 27 days. Carried by infected mononuclear cells, EHV1 spreads throughout the body (Allen and Bryans, 1986).

When EHV1-infected mononuclear cells reach the pregnant uterus, this may result in abortion, which typically occurs in the last trimester of pregnancy. A key factor in the pathogenesis of EHV1-induced abortion is the infection of endothelial cells in the endometrium by transfer

of EHV1 from infected mononuclear cells. The infection of the endothelial cells results in vascular damage and subsequent dissemination of the virus into the fetus (Jackson *et al.*, 1977; Edington *et al.*, 1991; Smith *et al.*, 1992, 1993; Smith and Borchers, 2001).When a fetus becomes infected late in gestation, a living foal may be delivered. However, such foals are usually weak and depressed, and they die within 24 hours postpartum. Some foals appear normal at birth, but develop severe respiratory distress within 18 to 24 hours and succumb within 3 days.

When EHV1-infected mononuclear cells reach the nervous system, neurological disorders may be induced. Edington *et al.* (1986) provided clear evidence that EHV1 can infect endothelial cells of the nervous system and that this forms the initial step in the induction of vascular lesions. The vascular lesions result in secondary hypoxic degeneration of adjacent neural tissue and, subsequently, in severe nervous system disorders such as ataxia, paralysis, bladder paralysis and urine incontinence, as well as cranial nerve deficits and/or signs of cerebral involvement.

In order to protect horses against EHV1 infection, they can be vaccinated. The purpose of vaccination is twofold. First, vaccination is meant to minimize virus replication in the respiratory tract upon subsequent exposure to virulent EHV1. In this manner, nasal shedding of virus will be reduced, as will the occurrence of respiratory disorders. Second, vaccination should prevent the occurrence of abortion and/or nervous system disorders either by preventing viremia or by preventing transmission and/or subsequent replication of EHV1 in internal organs. The first purpose of vaccination seems to be fulfilled by the available vaccines, as demonstrated by reduced nasal virus titers and the reduced severity of respiratory disease upon challenge infection of vaccinated horses (Burrows *et al.*, 1984, Heldens *et al.*, 2001, Goodman *et al.*, 2006). However, the efficacy of the current commercial and experimental vaccines to protect horses against EHV1-induced viremia and subsequent abortion or neurological disorders is highly variable and none of them can guarantee 100% protection.

The purpose of this article is to review the studies that address the potentials and limitations of the current commercial and experimental vaccines in the prevention of viremia, abortion and nervous system disorders in horses. Beside these studies, many other studies have described new approaches for vaccination against EHV1-induced disease. They include studies on inactivated vaccines comprising one or more major viral glycoproteins such as gB (Kukreja *et al.*, 1998), gC (Tewari *et al.*, 1995) or gD (Weerasinghe *et al.*, 2006), on deletion mutants lacking genes encoding for one or more major viral glycoproteins such as gB or gM (Neubauer *et al.*, 1997), gC (Osterrieder, 1999), gD (Csellner *et al.*, 2000), gE or gI (Tsujimura *et al.*, 2006), and on DNA vaccines based on the coding region for gD (Ruitenberg *et al.*, 1999) or gp2 (Learmonth *et al.*., 2003). However, none of the latter studies described the protective properties of the vaccines against EHV1-induced disease in horses. Therefore, they remained outside the scope of this article.

POTENTIALS AND LIMITATIONS OF VACCINES TO PREVENT VIREMIA

If a vaccine were developed that could limit or block the transport of virus to internal organs via the cell-associated viremia, then the occurrence of abortion and nervous system disorders could be reduced. Many studies have addressed the potential of vaccines to protect horses against cell-associated viremia. These include studies on commercially available and experimentally designed vaccines. An overview of the protective efficacy of these two types of vaccines against viremia is presented in Tables 1 and 2, respectively.

Commercial vaccines

The first study addressing the effect of an inactivated vaccine on EHV1-induced viremia was performed by Burrows *et al.* in 1984. They examined the effectiveness of Pneumabort-K , a whole virus inactivated and oil-adjuvanted vaccine containing EHV1. The incidence of EHV1-induced viremia was similar for vaccinated mares and non-vaccinated control mares (Table 1), as was the duration of viremia (8 to 14 days for vaccinates and controls). Similar results were obtained for yearlings and two-year-old ponies vaccinated with Pneumabort-K . Viremia was apparent in all animals upon challenge and lasted for 4 to 10 days (Burrows *et al.*, 1984). Bürki *et al.* (1990) were also unable to demonstrate a significant reduction in the number of viremic animals upon vaccination with Pneumabort-K (Table 1).

In 2001, Heldens *et al.* performed a vaccination/challenge study with Duvaxyn EHV$_{1,4}$, a whole virus inactivated and carbomer-adjuvanted vaccine containing EHV1 and EHV4. Pregnant mares that had previously been in contact with EHV were vaccinated three times and challenged 4 weeks later. A non-vaccinated group was included as a control. All mares, irrespective of their vaccination status, became viremic upon challenge. Moreover, the duration of viremia was hardly affected by vaccination. Additionally, Heldens *et al.* (2001) examined the occurrence of viremia in vaccinated and challenged foals that had not been previously in contact with EHV. Only 30% of vaccinated foals became viremic upon challenge, compared to 80% of control foals. This significant effect of Duvaxyn EHV$_{1,4}$ on the prevention of viremia in a group of EHV-naive foals seems somewhat surprising. Indeed, the same vaccine was unable to prevent viremia in a group of mares that all had been in contact with EHV earlier in life. The virulence of the challenge virus may possibly have affected the outcome of the challenge infection. The Ab4 strain used to challenge the mares is known to be highly virulent (Crowhurst *et al.*, 1981; Mumford *et al.*, 1994). The 121412 strain used to challenge the foals is likely to be of much lower virulence, since even in the non-vaccinated group not all foals became viremic upon challenge (Heldens *et al.*, 2001).

Very recently, the inactivated vaccine Flu-Vac Innovator 6 has been tested for its efficacy to prevent viremia (Goodman *et al.*, 2006), but also this vaccine was unable

Table 1. Effect of commercially available vaccines on EHV1-induced viremia, abortion and nervous system disorders in horses.

Vaccine	Type	Study group	Challenge post-vaccination	no. of viremic horses	Effect on duration of viremia	Effect on amount of viremia	Effect on no. of abortions	no. of horses with NSD	Source
Duvaxyn EHV1,4	inact.	pregnant mares	4 w	V: 5/5 — 100 % C: 4/4 — 100 %	no effect	Nd	V: 1/5 — 20 % C: 4/4 — 100 %	Nobs	Heldens et al. (2001)
		weaned foals	2 w	V: 3/10 — 30 % C: 4/5 — 80 %	reduced	Nd	-	Nobs	Heldens et al. (2001)
Pneumabort K	inact.	pregnant mares	1-2 m	V: 15/17 — 88 % C: 7/9 — 78 %	no effect	no effect	V: 7/17 — 41 % C: 3/9 — 33 %	Nobs	Burrows et al. (1984)
		1 to 2-year-old	1-2 m	V: 18/18 — 100 % C:11/11 — 100 %	no effect	no effect	-	Nobs	Burrows et al. (1984)
		pregnant mares	3-15 w	V: 6/6 — 100 % C: Nd	Nd	Nd	V: 3/6 — 50 % C: Nd	Nobs	Bürki et al. (1990)
		1 to 4-year-old	3 w	V: 2/3 — 67 % C: 2/2 — 100 %	no effect	Nd	-	Nobs	Bürki et al. (1990)
Flu-Vac Innovator 6	inact	3 tot10-year-old	2 m	V: 5/5 — 100 % C: 5/5 — 100 %	no effect	Nd	-	V: 3/5 — 60 % C: 3/5 — 60 %	Goodman et al. (2006)
Prevaccinol	MLV	pregnant mares	3-15 w	V: 3/4 — 75 % C: Nd	Nd	Nd	V: 2/4 — (50 %) C: Nd	Nobs	Bürki et al. (1990)
		1 to 20-year-old	3-16 w	V: 5/5 — 100 % C: 2/2 — 100 %	no effect	Nd	-	Nobs	Bürki et al. (1990)
Rhinomune	MLV	3 to 10-year-old	2 m	V: 5/5 — 100 % C: 5/5 — 100 %	no effect	Nd	-	V: 0/5 — 0 % C: 3/5 — 60 %	Goodman et al. (2006)

NSD: nervous system disorders; inact.: inactivated vaccine; MLV: modified-live vaccine; w: weeks; m: months; V: vaccinated horses; C: non-vaccinated control horses; Nd: not determined; Nobs: none observed.

to reduce either the number of viremic horses or the amount or duration of viremia upon challenge (Table 1).

Besides inactivated vaccines, live-attenuated vaccines are used for the prevention of EHV1-induced disease. Prevaccinol is a commercial live-attenuated Rac-H virus vaccine developed by attenuating the RacL11 strain of EHV1 via serial passages on heterologous cells (Woyciechowska, 1960; Woyciechowska *et al.*, 1980). Bürki *et al.* (1990) demonstrated viremia in the vast majority of vaccinated horses upon subsequent challenge infection (Table 1). However, in 4 out of the 8 viremic animals, infected mononuclear cells were only detected in the blood on one occasion (at 4 days post-challenge). Since the study lacked a control group of non-vaccinated horses, it could not be concluded whether this was an effect of vaccination. For the live-attenuated vaccine Rhinomune, no significant effect was observed between vaccinates and controls, either on the mean number of viremic animals or on the mean amount and duration of viremia upon subsequent challenge infection (Goodman *et al.*, 2006).

Experimental vaccines

Many researchers have designed and tested vaccines that have not (yet) been commercialized for horses. These include inactivated vaccines based on EHV1-specific glycoproteins, live-attenuated vaccines based on EHV1 mutants and DNA vaccines.

Matsumura *et al.* (1996) examined a vaccine based on the live-attenuated KyA strain obtained by serial passages on heterologous cells (Randall and Lawson, 1962; Perdue *et al.*, 1974). All horses included in the vaccination/challenge study developed viremia upon challenge, irrespective of their vaccination status. However, the maximum duration of viremia was reduced in the vaccinated horses (3 to 6 days) when compared to the control horses (16 to 19 days).

The live-attenuated C147 strain was obtained by growing a German abortion isolate of EHV1 in the presence of 5-bromo-2-deoxy uridine. By subsequent cloning, clone 147 was obtained, which was found to be restricted for growth at temperatures above normal body temperature (Patel *et al.*, 2003a). The results of the vaccination/challenge study were very promising. A single intranasal vaccination was able to induce complete protection against viremia in one- and two-year-old horses upon challenge at 6 weeks post-immunization (Patel *et al.*, 2003b). Also at later time points after vaccination, significant protection against viremia was observed (Table 2). When adult mares were challenged at 4 or 5-6 months post immunization, 50% and 60% of the mares, respectively, did not develop detectable viremia (Patel *et al.*, 2003a). Despite this significant protective effect, practical use of the vaccine has been hindered. This is most likely due to the fact that the vaccine itself can induce viremia in 30-70% of horses (Patel *et al.* 2003 a and b), thereby enabling the mutant virus to reach the internal organs and, in the worst case, to cause abortion.

Slater *et al.* (1993) and Tewari *et al.* (1993) constructed a mutant that specifically lacked the thymidine kinase. As for the C147 mutant, immunisation with the TK⁻ mutant also exerted an effect on viremia upon challenge. The mutant could not prevent viremia, but it was able to induce a 10-fold reduction in the number of infected mononuclear cells in the blood ($1/10^6$) when compared to non-immunized controls ($1/10^5$) (Slater *et al.*, 1993). A live-attenuated vaccine based on a gE⁻/gI⁻ mutant was unable to reduce either the number of viremic animals or the duration of viremia (Matsumura *et al.*, 1998). The extensiveness of viremia was not addressed in this study.

Besides vaccines based on mutants lacking expression of one or more major proteins, several vaccines have been designed instead to express only one or more major proteins, but to lack expression of all other proteins. Cook and colleagues (1990) designed such an inactivated vaccine containing all the major EHV1 glycoproteins presented by immune stimulating complexes or ISCOMs. Hannant *et al.* (1993) demonstrated that all foals vaccinated with the ISCOM vaccine developed viremia upon challenge infection, but that the duration and amount of the viremia were significantly reduced compared to non-vaccinated foals.

Most other studies are less extensive and, unfortunately, only address the number of viremic animals among vaccinates and control, but lack data on amount and duration of viremia. Nevertheless, it is striking that none of these vaccines are able to significantly affect the number of horses with viremia (Table 2).

Another attractive approach to immunizing with one or more EHV1 proteins is via DNA vaccination. This induces both potent CTL and antibody responses, and the safety concerns are minimal (Hassett and Whitton, 1996). However, Minke *et al.* (2006) demonstrated that DNA vaccination with a DNA vaccine containing plasmids for gB, gC and gD did not have a positive impact on the prevention of viremia (Table 2). A particle-mediated DNA vaccination based either on gB/gC/gD or on IE/UL5 was described by Soboll *et al.* (2006). Vaccination and subsequent challenge of 1-year-old ponies resulted in viremia in 4 out of 5 animals for both vaccines. Surprisingly, challenge infection of non-vaccinated control animals resulted in viremia in only 1 out of 5 animals, strongly suggesting that vaccination did not have any impact on the occurrence of viremia.

From the above-mentioned results, we can conclude that the currently available vaccines have only limited or no potential to protect horses against viremia upon subsequent challenge infection. However, several studies report a reduction in the number of horses with abortion or nervous system disorders after vaccination, as discussed below.

POTENTIALS AND LIMITATIONS OF VACCINES TO PREVENT ABORTION

Studies that address the protective potential of vaccines against abortion are summarized in Tables 1 and 2.

Table 2. Effect of experimental vaccines on EHV1-induced viremia, abortion and nervous system disorders in horses.

Vaccine	Type	Study group	Challenge post-vaccination	no. of viremic horses	Effect on				Source
					duration of viremia	amount of viremia	no. of abortions	no. of horses with NSD	
gD/gB (Baculovirus)	inact.	mares	2 m	V: 8/10 C: 4/4 (80 % 100 %)	no effect	Nd	-	Nobs	Foote et al. (2006)
		foals (2 m)	1 m	V: 10/10 C: 3/4 (100 % 75 %)	no effect	Nd	-	Nobs	Foote et al. (2006)
gB/gC/gD (Canarypox-virus)	inact	1 to 2-year-old	2-3 w	V: 15/15 C: 5/5 (100 % 100 %)	Nd	Nd	-	Nobs	Minke et al. (2006)
IE (Vacciniavirus)	inact.	1-year-old	3-9 w	V: 3/4 C: 1/1 (75 % 100 %)	Nd	Nd	-	Nobs	Paillot et al. (2006)
ISCOM	inact.	ponies	3 w	V: 9/9 C: 6/6 (100 % 100 %)	reduced	V<C (x 20)	-	Nobs	Hannant et al. (1993)
TS mutant C 147	MLV	pregnant mares	4-6 m	V: 5/11 C: 5/5 (40-50 % 100 %)	no effect	Nd	V: 2/11 C: 5/5 (18 % 100 %)	Nobs	Patel et al. (2003a)
		1 to 2-year-old	6 w	V: 0/8 C: 8/8 (0 % 100 %)	Nd	Nd	-	Nobs	Patel et al. (2003b)
KyA stam	MLV	weaned foals	4 w	V: 4/4 C: 2/2 (100 % 100 %)	reduced	Nd	-	Nobs	Matsumura et al. (1996)
TK⁻ mutant	MLV	SPF foals (3-4 m)	3.5 m	V: 2/2 C: 2/2 (100 % 100 %)	Nd	V<C (x10)	-	Nobs	Tewari et al. (1993) Slater et al. (1993)
gE⁻/gI⁻ mutant	MLV	SPF foals 3 m		V: 3/3 C: 3/3 (100 % 100 %)	no effect	Nd	-	Nobs	Matsumura et al. (1998)
gB/gC/gD	DNA	1 to 2-year old	2-3 w	V: 19/20 C: 5/5 (95 % 100 %)	Nd	Nd	-	Nobs	Minke et al. (2006)
gB/gC/gD	DNA	1 year old	1 m	V: 4/5 C: 1/5 (80 %) (20 %)	Nd	Nd	-	Nobs	Soboll et al. (2006)
IE/UL5	DNA	1 year old	1 m	V: 4/5 C: 1/5 (80 %) (20 %)	Nd	Nd	-	Nobs	Soboll et al. (2006)

NSD: nervous system disorders; Inact.: inactivated vaccine; MLV: modified-live vaccine; V: vaccinated horses; C: non-vaccinated control horses; Nd: not determined; Nobs: none observed; g: glycoprotein; ISCOM: immune stimulating complexes; TS: temperature sensitive; TK: thymidine kinase; IE: immediate early; UL: unique long; SPF: specific pathogen free.

Commercial vaccines

Heldens *et al.* (2001) examined the protective effect of Duvaxyn EHV$_{1,4}$ against EHV1-induced abortion. As described above, pregnant mares were vaccinated three times and challenged 4 weeks later. It was found that the incidence of EHV1-induced abortion was clearly reduced in the vaccinated (1/5 aborted) compared to the non-vaccinated mares (4/4 aborted). This is an interesting finding, knowing that all mares, irrespective of their vaccination status, did become viremic upon challenge. It could suggest that vaccination with Duvaxyn EHV$_{1,4}$ reduces the number of infected mononuclear cells per mare and, subsequently, reduces the chance of interaction of these infected mononuclear cells with endothelial cells of the pregnant uterus. Alternatively, the vaccine may induce certain protective responses that aid in limiting the transmission of EHV1 from the carrier leukocyte to endothelial cells of the uterus or that aid in limiting the extensiveness of lesions in the endothelium of the pregnant uterus.

In contrast to Duvaxyn EHV$_{1,4}$, the inactivated Pneumabort-K vaccine was unable to induce protection against abortion in a vaccination/challenge study (Burrows *et al.*, 1984) (Table 1). Abortion was also evident in 50% of Pneumabort-K-vaccinated mares in the vaccination/challenge experiment performed by Bürki *et al.* (1990). However, the latter researchers did not include a non-vaccinated control group and, therefore, it is not possible to judge the effect of the vaccine based on their results. Despite the negative findings in the experimental vaccination/challenge studies, Bryans and Allen (1982) demonstrated a positive effect of vaccination in the field. They found that, upon introduction of vaccination with Pneumabort-K in Kentucky, the incidence of EHV1-induced abortion declined from 6.8/1000 in 1977 to 1.8/1000 in 1980.

Also for Prevaccinol there are some contradictory findings concerning its protective effect against EHV1-induced abortion. Von Benten and Petzoldt (1977) performed a six-year field survey in German thoroughbreds and reported that EHV1 abortions occurred as frequently in vaccinated as in non-vaccinated mares. On the other hand, introduction of the vaccine on 6 farms in Poland resulted in a slight, but significant decrease in the number of fetal and neonatal foal losses from 11.8% to 8.9% (Frymus *et al.*, 1986). In a field trial performed over a period of 6 years by Becker (1988), no cases of virus abortion were detected when mares were vaccinated according to the manufacturer's instructions. Bürki *et al.* (1990) carried out an experimental vaccination/challenge study with Prevaccinol. Two out of 4 vaccinated pregnant mares aborted upon challenge. It could not be concluded whether vaccination had reduced the abortion rate, since the study lacked a control group of non-vaccinated pregnant mares.

Experimental vaccines

Among the many studies on the protective effect of experimental vaccines, only one addresses the protective potential against abortion (Patel *et al.*, 2003a). Pregnant mares received a single intranasal vaccination with the live-attenuated C147 mutant. Following this vaccination protocol, only 17% and 20% of mares aborted upon subsequent challenge at 4 and 5-6 months, respectively. In the control group, in contrast, all mares aborted. Despite these promising results, practical use of the vaccine has been hindered, most likely due to safety reasons, as discussed above.

POTENTIALS AND LIMITATIONS OF VACCINES TO PREVENT NERVOUS SYSTEM DISORDERS

Up till now, only one study has addressed the protective potential of vaccines against nervous system disorders (Goodman *et al.*, 2006). The main reason for this limited number of studies is that there are still very few good experimental models to induce nervous system disorders in horses. Goodman *et al.* (2006) immunized horses with the inactivated vaccine Flu-vac Innovator 6 or the modified-live vaccine Rhinomune. Non-vaccinated horses were included as a control. Upon challenge, nervous system disorders were observed in the control horses (3/5) and in the horses vaccinated with the inactivated vaccine (3/5). Among the horses vaccinated with the modified-live vaccine, none developed nervous system disorders. Again, this is an interesting finding, since all horses, irrespective of their vaccination status, became viremic upon challenge. Moreover, the amount and duration of viremia were similar (Goodman *et al.*, 2006). As mentioned above, this may indicate that the modified-live vaccine induced a certain protective response that limited the transmission of EHV1 from the carrier leukocyte to endothelial cells of the nervous system or that limited the extensiveness of lesions in the endothelium of the nervous system. The unraveling of the underlying protective immune mechanism(s) that occur(s) at the level of the endothelium may be an important step in future vaccine development.

CONCLUDING REMARKS

In reviewing the various vaccination/challenge studies, it has become clear that the currently designed vaccines are not able to induce significant protection against EHV1-induced viremia. However, several vaccines are able to induce significant protection against either abortion or nervous system disorders. Which immune responses correlate with this protection is so far unknown. They may include responses that limit the transmission of EHV1 from the carrier leukocyte to endothelial cells of the target organs. Responses that limit the extensiveness of lesions in the endothelium of the target organ and, consequently, limit the devastating effects of thrombosis, vasculitis and anoxemia may also be involved. The unraveling of the underlying protective immune mechanism(s) that occur(s) at the level of the endothelium may be an important step in future vaccine development.

EHV researchers may also find helpful clues by looking more closely into the immune responses raised at the

level of the respiratory tract upon natural infection with EHV1. In any case, these responses were found to be fully protective against viral replication in the respiratory tract for up to 2 months (Gibson *et al.*, 1992; Slater *et al.*, 1993; Tewari *et al.*, 1993), and fully protective against viremia for up to 6 months post-infection (Edington *et al.*, 1990; van der Meulen, unpublished results). If viremia is prevented, then subsequent spread to the pregnant uterus and the nervous system are prevented as well. Once the mechanisms of protection have been unraveled, a vaccine may be designed that induces similar responses. Keeping the protective potentials of the C147 mutant in mind (Patel *et al.*, 2003a), such vaccine is likely to be a locally administered, live-attenuated vaccine.

Besides vaccination, management will remain a crucial factor in the prevention of EHV1-induced clinical signs. Vaccination according to the manufacturer's instructions should be performed for all horses on the premises so that infection pressure on the premises is reduced. Sound management also includes the separate housing of young horses, adult horses and pregnant mares, strict hygiene measures, and strict control of contact with horses from outside the premise. The combination of accurate vaccination with sound management proved effective in significantly reducing the chance of EHV1-induced abortion in Kentucky, USA (Timoney, personal communication). If, despite vaccination and thorough management, an outbreak of EHV1-induced disease nevertheless occurs, recent studies have demonstrated the usefulness of antiviral therapy (Wilkins *et al.*, 2005; Bentz *et al.*, 2006; Garré *et al.*, 2006, 2007).

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Uit het verleden

Nr 31

MINISTERIE VAN BINNENLANDSCHE ZAKEN

17 SEPTEMBER 1935. — Ministerieel besluit.
Vleeschuitvoer.

De Minister van Binnenlandsche Zaken,

Gelet op het bij koninklijk besluit van 24 Augustus 1935 gewijzigd koninklijk besluit avn 23 Maart 1901 houdende algemeen reglement op de vleeschkeuring;

Herzien het ministerieel besluit van 31 Maart 1901 betreffende de vleeschkeuring;

Herzien het ministerieel besluit van 22 April 1914 betreffende de erkende slachthuizen,

Besluit :

Artikel één. — Het slachten en het keuren van dieren waarvan het vleesch, het vet of de afval onder Rijkswaarborg voor den uitvoer bestemd is, mag slechts in de speciaal door den Minister erkende slachthuizen geschieden. Deze erkenning kan steeds ingetrokken worden.

Art. 2. — Erkenning van slachthuizen geschiedt onder de volgende voorwaarden :

1" Er moet regelmatige vergunning verleend zijn krachtens de wetgeving op de gevaarlijke, ongezonde of hinderlijke inrichtingen;

2° Er mag slechts uitsluitend voor den uitvoer geslacht worden, behoudens de uitzonderingen welke de Minister toestaat;

3" De ruimte moet er zoo groot zijn dat het slachten en het bereiden van het in de erkenningsaanvraag opgegeven aantal dieren kunnen geschieden;

4° Het bedrijf moet omvatten :

a) twee stallen, waarvan één om verdachte dieren ter waarneming te stallen;

b) een slacht- en eventueel een waschlokaal;

c) een hanglokaal;

d) een lokaal voor het vleesch dat voor den uitvoer werd geweigerd of ter waarneming wordt gehouden;

e) een lokaal voor het afgekeurd vleesch;

f) een werkplaats ter bewerking van darmen en andere producten van dierlijken aard;

g) een kleedkamer voor de werklieden;

h) een bureel voor de keurders;