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VIRAL HAEMORRAGIC DISEASE (VHD) IN RABBITS : PROTECTION CONFERRED BY INTRADERMAL VACCINATION WITH DERCUNICAL® IN COMPARISON WITH S.C. OR I.M. VACCINATION WITH ARVILAP®, LEPORIPHYL® AND HEBOVAC-88-T®

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Introduction

The viral hemorragic disease (VHD) is a highly lethal disease which is caused by a small calicilike virus (Ohlinger et al., 1990). VHD causes 80 to 100 % of mortality in rabbits of 10 weeks and older. The disease was first reported in 1984 from China (Liu et al., 1984), then spread to Italy in 1986 (Cancelotti et al., 1988) and subsequently all over Europe. The disease has also been reported from Egypt, Korea, Libanon, Mexico and Tunesia (Morisse et al., 1991). Chinese authors established excellent protection against the disease after vaccination of 12-week-old rabbits with inactivated tissue vaccines (Liu et al., 1984, Gu et al., 1986). In recent years various commercial vaccines became available in Europe (Argüello et al., 1989, Pagès Manté, 1989, Mocsari, 1990). The virus is being inactivated by various methods and adjuvants are incorporated (table 1). Most vaccines are administered by the subcutaneous (s.c.) or intramuscular (i.m.) route, which implicates a lot of handicraft and frequent change of needles. Recently Mérieux developed a new inactivated vaccine, Dercunical®, for intradermal (i.d.) application using a special needle-less device (Sani-jet M10) allowing simultaneous injection at 3 different sites. The sani-jet avoids accidental rabbit-to-rabbit transmission of intercurrent diseases during vaccination.The purpose of this study is to compare the efficacy of intradermal vaccination with other routes of vaccination at weaning or at 12 weeks of age.

Materials and methods

Animals and husbandry. A total of 414 conventional New Zealand white rabbits were purchased from the Rijksstation voor Kleinveeteelt, Merelbeke (RVK). During 3 vaccination trials 168 four-week-old and 168 twelve-to-fourtheen-week-old rabbits were used. Another 24 twelve-week-old animals served as contact-controls and 3 groups of 4-, 8- and 12-week-old animals were used to test the influence of maternal antibodies on vaccination. During vaccination trial 1 and during the contact-trial rabbits issued from non-VHD-vaccinated does were used. During trials 2 and 3 rabbits originated from does vaccinated with Arvilap *2 months before*. The rabbits used during the maternal antibody test were issued from does vaccinated *6 months before*. All rabbits were housed individually in heat-sterilized, wire-floored metal cages at 22 °C ambient temperature, were fed standard rabbit pellets and received water *ad libitum*. The pellets contained 16.7 % of crude protein, 15.6 % of crude fiber and 2350 kcal of apparently digestible energy. Before starting the trials, the rabbit houses were thoroughly cleaned, disinfected with formol and subsequently fumigated with NH_3. All rabbits were treated against pasteurellosis with 40 mg/kg of terramycine (Pfizer) i.m. the day of arrival and against coccidiosis on days 0, 1, 7, 8, 14 and 15 after arrival with 1 ml of toltrazuril (Baycox, Bayer) per liter of drinking water.

Vaccines. Four different commercial vaccines (table 1) were tested : Arvilap (lot 89/21, Laboratorios Ovejero, León, Spain), Leporiphyl (lot K-030888, Phylaxia, Budapest, Hungary), Hebovac-88-T (State Veterinary Services, Sofia, Bulgaria, lot 449/11.1990) and Dercunical (lot 1DNL 5A01, Mérieux, Lyon, France). All vaccines showed an HA-titer of 512.

Table 1. Commercial vaccines

Vaccine	Manufacturer	Country	Inactivation	Adjuvant
Arvilap*	Ovejero	Spain	ß-propiolactone	Al(OH)3
Cunipravac	Hipra	Spain	formol	Mineral oil
Cylap	Sobrino	Spain	ß-propiolactone	Mineral oil
Dercunical*	Mérieux	France	formol	Al(OH)3
Hebovac-88-T*	Veterinärwesen	Bulgary	68 °C, 90 min.	Saponine
Leporiphyl*	Phylaxia	Hungary	formol	Al(OH)3

* Vaccines tested by the National Institute of Veterinary Research, Belgium

Mode of administration. Dercunical was injected i.d. with a needle-less device (Sani-jet M10, Sanitec, Les Herbiers, France) with an injection head (type nr 373) showing 3 injection points, ejecting each 0.033 ml of vaccine simultaneously. About 50 % of the ejected volume reaches the dermis. The vaccine was applicated i.d. to the inner-side of each ear. This means that vaccination with twice a volume of 0.1 ml resulted in a real vaccination dose of 0.1 ml. Other vaccines were administered s.c. or i.m. : rabbits were vaccinated s.c. between both shoulderblades or i.m. in the left thigh muscles.

Challenge. Two strains were used : most rabbits vaccinated with Arvilap or Leporiphyl (table 2) were challenged with the Hungarian Mohava-strain isolated in 1988. During further challenge experiments the Belgian 90/97/1-strain isolated during a field outbreak in 1990 was used (Peeters et al., 1990). Animals were challenged by the intra-peritoneal or subcutaneous route (table 2) with 1,000 LD_{50}.

Haemagglutination (HA) test. Human type O red blood cells (RBCs) were washed 3 times in PBS containing 0.5 % (w/v) of bovine albumine (fraction V) and suspended at 0,75 % (v/v) in PBS. Two-fold dilutions of clarified supernatant of a 10 % (w/v) homogenate of liver in PBS were incubated with an equal volume of washed RBCs in microtitre plates at ambient temperature. After an incubation of one hour, agglutination at titres greater than 10×2^4 was considered positive. Specificity of end point dilutions between 10×2^2 and 10×2^6 was tested by inhibition of the haemagglutination with hyperimmune anti-VHD rabbit sera.

Haemagglutination-inhibition (HI) test. Clarified supernatant of a 10 % (w/v) homogenate of an infected liver in PBS was used as antigen. Sera were inactivated for 30 minutes at 56 °C. The inactivated serum (0.1 ml) was mixed with 0.4 ml of PBS and 0.5 ml of 25 % kaolin in PBS (w/v) and allowed to stand for 20 minutes and then centrifuged. The kaolin-adsorbed serum was then treated with one drop of 50 % packed human type O RBCs. After a further 20 minutes, the serum was clarified by centrifugation and used in the HI-test. Two-fold dilutions of serum-samples (original dilution 1:10) in PBS were incubated in microtitre plates. An equal volume of antigen (8 HA units/0.025 ml) was added and incubated at ambient temperature for 30 minutes. Human O RBCs, prepared as described above, were added to each well and allowed to settle for one hour. The titre was calculated as the reciprocal of the highest dilution of serum that produces inhibition of haemagglutination. All reactions were done at ambient temperature.

Table 2. Experimental design

VACCINATION					CHALLENGE		
Exp.	Age (weeks)	Vaccine	Route	ml	Age (weeks)	Route	Strain
1	4	Arvilap Leporiphyl	s.c. s.c.	0.5 1	11	i.p.	Mohava
	4 + 14	Arvilap Leporiphyl	s.c. i.m.	1 1	34	i.p.	Mohava
	14	Arvilap Leporiphyl	s.c. i.m.	1 1	16, 22 30 44, 66	i.p. s.c. s.c.	Mohava Mohava 90/97/1
2	4	Hebovac	s.c.	0.5	5, 12	s.c.	90/97/1
	4 + 12	Hebovac	s.c.	1	38	s.c.	90/97/1
	12	Hebovac	s.c.	1	13, 24, 38	s.c.	90/97/1
3	4	Dercunical	i.d.	0.2	8, 12	s.c.	90/97/1
	4 + 12	Dercunical	i.d.	0.2	38	s.c.	90/97/1
	12	Dercunical	i.d.	0.2	13, 24, 38	s.c.	90/97/1

Vaccination trials. The rabbits were separated into 56 homogeneous groups of 6 animals with equal mean weight. They were tested as set out in table 2. Possible adverse vaccination reactions were monitored during 4 weeks post-vaccination (p.v.). Body temperature was evaluated 48 and 72 hours p.v. at 9 a.m. Blood samples were taken at regular intervals for evaluation of HI-titers after vaccination and for rapid screening of possible infections of control rabbits by wild virus. Rabbits were challenged with wild VHD-virus at various times after vaccination (table 2). Therefore animals were housed in isolation rooms in individual cages inhibiting contact between animals. Each challenge experiment consisted of 6 infected vaccinated, 6 infected unvaccinated controls (IUC) and 6 uninfected unvaccinated controls (UUC). Body temperature was monitored daily from 24 to 72 hours post-infection (p.i.). Blood samples were tested by HI at challenge and 14 days later. All dead animals were necropsied and tested by HA. Surviving animals were sacrificed 14 days p.i., necropsied and tested by HA. Liver was subjected to histopathological examination.

Contact infection. Arvilap and Leporiphyl vaccinated animals which were challenged at the age of 44 weeks received an 11-week-old rabbit as cage-mate 7 and 14 days p.i. respectively. The purpose was to test if vaccinated animals become healthy carriers p.i. The contact-controls were observed during 3 weeks and then subjected to the same examinations as the vaccinated and challenged cage-mates.

Maternal immunity. A total of 54 rabbits were separated into 3 equal groups of 4-, 8- and 12-week-old animals. They were weaned from does which had been vaccinated 6 months before. Within each age-group, 6 rabbits were vaccinated s.c. with 1 ml of Arvilap, 6 with 1 ml of Hebovac, while 6 rabbits served as unvaccinated control-group. Animals were challenged with the 90/97/1-strain 8 weeks p.v. and tested as described above. In addition feed intake and weight gain was evaluated.

Results

Observations after vaccination

None of the vaccinated rabbits showed a rise of body temperature p.v. with any vaccine. Local post-vaccination reactions were not noticed either. Only in Dercunical vaccinated rabbits a small nodule of 2-4 mm appeared at the inoculation site which disappeared within 7-14 days p.v. Similar observations were made after revaccination of the rabbits at the age of 12-14 weeks. No difference of feed-intake with unvaccinated rabbit was noticed during the whole observation period. Vaccination was followed by a quick rise of serum HI-titers. Titers rose to higher levels in rabbits vaccinated at 12 weeks than in rabbits vaccinated at 4 weeks. They also remained detectable for a longer time : 10-30 weeks against 4-8 weeks respectively. In non vaccinated rabbits no HI-titers were observed at any time during the 6-12 months of observation.

Table 3. Experiment 1 : clinical signs and HA-values after challenge infection of rabbits vaccinated with Arvilap or Leporiphyl

Parameter	Uninfected controls	Infected controls	Leporiphyl	Arvilap
7 weeks after vaccination at 4 w				
Mortality by VHD	0/6	.5/6	.1/6	0/6
Mean rectal °T 3 d p.i.	39.1 ± 0.2	40.7 ± 0.6	39.3 ± 0.8	39.1 ± 0.2
N with rectal °T ≥ 40 °C	0/6	.5/6	.2/6	.1/6
log2 HA-titre liver (dil. 1:10)	-	11 - 21	13	-
22 weeks after vaccination at 4/14 w				
Mortality by VHD	0/6	.5/5	.1/5	0/6
Mean rectal °T 3 d p.i.	39.5 ± 0.2	40.8 ± 0.8	39.8 ± 0.3	39.6 ± 0.3
N with rectal °T ≥ 40 °C	0/6	.3/5	.1/5	.1/6
log2 HA-titre liver (dil. 1:10)	-	15 - 18	15	-
2-52 weeks after vaccination at 14 w				
Mortality by VHD	0/27	16/28	.2/27	0/28
Mean rectal °T 3 d p.i.	39.5 ± 0.2	40.2 ± 0.6	39.6 ± 0.6	39.4 ± 0.3
N with rectal °T ≥ 40 °C	0/27	13/28	.5/27	.2/28
log2 HA-titre liver (dil. 1:10)	-	13 - 23	11 - 12	-

Experimental infection of non vaccinated rabbits

Experimental infection of non vaccinated rabbits did not kill 5 to 9-week-old rabbits, although some animals showed a rise of body temperature and reduction of feed intake (table 4). Nevertheless the infection was associated with a strong rise of serum HI-antibodies, indicating viral replication. In rabbits older than 11 weeks 82 % (74/90) died within 3-7 days after the experimental infection (tables 3, 4 and 5). There was no difference of mortality between i.p. and s.c. challenged animals. Within 48-72 hours p.i. body temperature increased to 40.0-41.7 °C. Most animals died after a short episode of increased respiration and/or nervous symptoms or showed no clinical signs at all. Necropsy revealed marked hypertrophy of thymus and kidneys with petechiae, hæmorrhagic pneumo-tracheitis as well as hypertrophy and degeneration of the liver. Most liver extracts showed high HA-titers varying between 8 and 24. Surviving animals showed high serum HI-titres p.i.

Table 4. Clinical signs and HA-values after challenge infection of rabbits vaccinated with Dercunical (i.d.) or Hebovac (s.c.) at 4 weeks of age

Parameter	Experiment 2			Experiment 3		
	Uninfected controls	Infected controls	Hebovac	Uninfected controls	Infected controls	Dercunical
*1 or 4 weeks after vaccination**						
Mortality by VHD	0/4	0/6	0/6	0/6	0/6	0/6
Mean rectal °T 2 d p.i.	39.7 ± 0.1	39.7 ± 0.2	39.6 ± 0.1	39.6 ± 0.2	39.4 ± 0.1	39.4 ± 0.2
N with rectal °T ≥ 40 °C	0/4	.1/6	0/6	0/6	0/6	0/6
log2 HA-titers liver (dil. 1:10)	-	0	0	-	0	0
8 weeks after vaccination						
Mortality by VHD	0/5	.5/5	.2/6	0/6	.6/6	0/6
Mean rectal °T 2 d p.i.	39.5 ± 0.1	41.0 ± 0.1	40.1 ± 0.7	39.5 ± 0.1	40.8 ± 0.1	39.7 ± 0.3
N with rectal °T ≥ 40 °C	0/5	.3/5	.4/6	0/6	.3/3	.1/6
log2 HA-titers liver (dil. 1:10)	-	8 - 24	0 - 24	-	3 - 22	0
26 w after revaccination at 12 w						
Mortality by VHD	0/6	.5/5	0/6			
Mean rectal °T 2 d p.i.	39.3 ± 0.1	40.4 ± 0.9	39.6 ± 0.1			
N with rectal °T ≥ 40 °C	0/6	.3/5	0/6			
log2 HA-titers liver (dil. 1:10)	-	14 - 17	0			

* Hebovac : challenge 1 week p.v., Dercunical : challenge 4 weeks p.v.

Table 5. Clinical signs and HA-values after challenge infection of rabbits vaccinated with Dercunical (i.d.) or Hebovac (s.c.) at 12 weeks of age

Parameter	Experiment 2			Experiment 3		
	Uninfected controls	Infected controls	Hebovac	Uninfected controls	Infected controls	Dercunical
1 week after vaccination						
Mortality by VHD	0/5	.6/6	0/6	0/6	.4/6	0/6
Mean rectal °T 2 d p.i.	39.5 ± 0.2	40.6 ± 0.3	39.5 ± 0.1	39.5 ± 0.1	40.7 ± 0.9	39.5 ± 0.2
N with rectal °T ≥ 40 °C	0/5	.6/6	0/6	0/6	.5/6	0/6
log2 HA-titers liver (dil. 1:10)	-	2 - 18	0	-	12 - 14	0
12 weeks after vaccination						
Mortality by VHD	0/6	.4/5	0/6	0/6	.6/6	0/6
Mean rectal °T 2 d p.i.	39.9 ± 0.1	40.3 ± 1.1	39.4 ± 0.2	39.4 ± 0.1	40.1 ± 0.9	39.4 ± 0.2
N with rectal °T ≥ 40 °C	.1/6	.2/6	0/6	.0/6	.2/6	0/6
log2 HA-titers liver (dil. 1:10)	-	18 - > 24	0	-	12 - 21	0
26 weeks after vaccination						
Mortality by VHD	0/6	.6/6	0/6	0/6	.6/6	0/6
Mean rectal °T 2 d p.i.	39.5 ± 0.1	40.0 ± 0.7	39.5 ± 0.1	39.6 ± 0.1	40.2 ± 0.8	39.5 ± 0.2
N with rectal °T ≥ 40 °C	0/6	.4/6	0/6	0/6	.3/6	0/6
log2 HA-titers liver (dil. 1:10)	-	5 - 20	0	-	10 - 21	0

Protection of rabbits vaccinated at 4 weeks of age

Rabbits issued from non-vaccinated does. All Arvilap-vaccinated rabbits survived the challenge infection 7 weeks later. In the Leporiphyl-vaccinated group one rabbit out of six died with typical lesions and clinical signs of VHD described above (table 3). A few animals showed rise of body temperature. Animals revaccinated at the age of 14 weeks showed good protection against challenge 20 weeks later : 6/6 Arvilap-vaccinated rabbits and 5/6 Leporiphyl-vaccinated rabbits survived the challenge infection. All animals showed clear-cut seroconversion 2 weeks p.i.

Rabbits issued from vaccinated does. All Dercunical-vaccinated rabbits survived the challenge infections 4 and 8 weeks p.v. (table 4). None of the animals showed rise of body temperature 4 weeks p.v. and only one out of six 8 weeks p.v. Feed intake was not influenced. At necropsy no lesions nor HA-titres were noticed either. All Hebovac-vaccinated animals survived the challenge infection one week p.v., but 2 out of 6 animals died with typical lesions and signs of VHD when challenged 7 weeks p.v. Four out of 6 animals also showed significant rise of body temperature, indicating that protection after vaccination with Hebovac at 4 weeks was only partial. Revaccination at 12 weeks with both Dercunical and Hebovac was followed by full protection against VHD, as established by challenge 26 weeks later.

Table 6. Influence of age on mean HI-titres (log_2) after vaccination of rabbits issued from vaccinated does

Age at Vaccination	4 weeks after vaccination*			7 weeks after vaccination*		
	Unvaccinated controls	Arvilap	Hebovac	Unvaccinated controls	Arvilap	Hebovac
4 weeks	0	1.8 ± 1.1a	0a	0	1.0 ± 1.4a	0a
8 weeks	0	6.2 ± 2.4a	1.0 ± 1.7b	0	4.3 ± 1.0a	0b
12 weeks	0	7.2 ± 1.8a	3.5 ± 1.2b	0	4.0 ± 1.3a	3.2 ± 0.4a

* Means with different superscript are significantly different ($p < 0.05$)

Table 7. Animals issued from vaccinated does showing weight loss and reduced feed intake after vaccination and subsequent challenge with VHD

Age at Vaccination	Weight loss 7 days p.i.*			Reduced feed intake 4-9 days p.i.*		
	Infected controls	Arvilap	Hebovac	Infected controls	Arvilap	Hebovac
4 weeks	6/6 dead	.1/5	.1/5	6/6 dead	.1/5	.3/5
8 weeks	6/6 dead	.0/6	.2/6	6/6 dead	.0/6	.2/6
12 weeks	6/6 dead	.0/6	.2/6	6/6 dead	.0/6	.2/6
Total	18/18 dead	.1/17a	.5/17b	18/18 dead	.1/17a	.7/17b

* Means with different superscript are significantly different ($p < 0.05$)

Protection of rabbits vaccinated at 12-14 weeks of age

Animals vaccinated at 12 to 14 weeks showed good protection : between 2 to 52 weeks p.v. 28/28 Arvilap-vaccinated rabbits and 25/27 Leporiphyl-vaccinated rabbits survived the experimental infection (table 3). A total of 5 out of 27 Leporiphyl-vaccinated rabbits showed a rise of body

temperature against 2 out of 28 Arvilap-vaccinated rabbits. All animals vaccinated with Dercunical or Hebovac survived the infection from the first week p.v. till at least 6 months later (table 5). Challenge infection was not associated with a rise of body temperature, nor with a negative influence on feed intake either. At necropsy no lesions were noticed and HA of liver extracts remained negative. All animals showed a clear-cut seroconversion p.i.

Contact trial

None of the unvaccinated rabbits acquired VHD-infection from the infected-vaccinated cage-mates during the 3 weeks of observation. They showed no fever nor seroconversion. At necropsy no lesions were noticed and HA remained negative. Also histopathology of liver, thymus, lungs or kidney did not show any pathological change.

Influence of maternal immunity

None of the 4-, 8- or 12-week-old rabbits issued from vaccinated does (6 months before birth) showed a rise of body temperature p.v. Most rabbits showed seroconversion, although a significant influence of age and vaccine became evident : Hebovac induced a significant lower HI-response than did Arvilap. Antibody response was stronger as rabbits were vaccinated at later age (table 6). Although all non vaccinated rabbits died of VHD after challenge, all vaccinated rabbits resisted challenge. None of them showed hyperthermia. Yet, challenge caused significant weight loss and reduced feed intake in 5 and 7 out of 17 Hebovac vaccinated rabbits respectively (table 7). Arvilap vaccinated rabbits remained almost unaffected.

Table 8. Protection against challenge after vaccination : total results

Vaccine	Administration	Vaccination at 4 weeks		Vaccination at 12-14 weeks	
		% survival	% fever	% survival	% fever
Dercunical	i.d.	100	17	100	0
Arvilap	s.c.	100	8	100	6
Hebovac	s.c.	83	33	100	0
Leporiphyl	i.m.	83	33	93	19

Discussion

VHD causes high economic losses in rabbits older than 10 weeks. Our experiments confirm that infection of 4 and 8-week-old rabbits is not followed by mortality, while an 82 % mortality was established in older rabbits. To avoid losses by VHD at the end of the fattening period, rabbits should be vaccinated at weaning age. Among the vaccines tested, only Arvilap and Dercunical protected the rabbits completely against mortality during the whole fattening period. Nevertheless a few animals showed rise of body temperature when challenged at 12 weeks (table 8). Moreover, rabbits vaccinated at 4 weeks showed lower HI-titres than rabbits vaccinated at 12 weeks (table 6) and HI-titres remained detectable for a shorter time. This indicates that rabbits vaccinated at 4 weeks, which are kept for reproduction purposes need a booster vaccination at 12 to 15 weeks of age. Our data confirm the findings of Argüello et al. (1989) that this booster vaccination does not increase the risk of anaphylactic shock, although we are dealing here with a tissue vaccine.

The results indicate that residual maternal immunity may interfere with vaccination at 4 weeks. Indeed, when rabbits issued from does vaccinated **two** months before kindling were vaccinated

with Hebovac at 4 weeks and subsequently challenged 8 weeks later (exp. 2), two out of six rabbits died from the challenge infection and 4 rabbits showed increased body temperature. On the other hand, when the rabbits were issued from does vaccinated **six** months before kindling no mortality nor rise of body temperature was established after challenge. Yet, vaccination with Dercunical in rabbits issued from does vaccinated two months before kindling was not followed by mortality after challenge. This may be related with a lower antigenic stimulation by Hebovac as suggested by the data of table 6.

In 12-week-old rabbits, Arvilap, Dercunical and Hebovac conferred full protection against VHD as early as 7 days after vaccination and for at least 6 months. Only 2 out of 27 Leporiphyl-vaccinated rabbits died from the infection. Six months of protection is sufficient to cover the economic life of most does in commercial rabbitries. The results with Arvilap indicate that protection may even last 12 months. Yet, rupture of immunity was reported in infected environment in does vaccinated 8 months before with Arvilap (Rosell et al., 1990). So revaccination every 6 to 8 months seems advisable.

Maternal immunity did not interfere with the protection obtained after vaccination at 12 weeks. As non vaccinated control animals housed in the same environment as the vaccinated rabbits remained fully sensitive to the virus and did not show seroconversion, we can also exclude possible interference of intercurrent infections with wild virus which could boost immunity after vaccination. So it is clear that protection in vaccinated rabbits was related with vaccination only. It also shows that vaccinated animals do not eliminate live virus after vaccination, which proves full inactivation of the vaccines used. Moreover, the data from contact-animals support the hypothesis that vaccinated rabbits do not become healthy carriers after infection. This probably explains why rapid vaccination after the first clinical signs of VHD can stop the spread of the disease within a rabbitry (Rosell et al., 1990).

The recently developed Dercunical vaccine for intradermal application represents a significant improvement of vaccination against VHD in commercial rabbitries. Table 8 clearly indicates that the vaccine induces full protection in 4- and 12-week-old rabbits. Moreover, as outbreaks of VHD in commercial rabbit units require urgent intervention, there always exists a danger of rabbit-to-rabbit transmission of the virus. This can be overcome by using intra-dermal vaccination with a needle-less device. Such technique does not only allow quick vaccination, but also avoids transmission of other intercurrent diseases as myxomatosis, colibacillosis, pateurellosis and staphylococcosis which frequently occur in commercial rabbitries.

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Summary

Four commercial inactivated viral hæmorrhagic disease (VHD) vaccines have been tested : Arvilap (Ovejero, León, Spain), Dercunical (Mérieux, Lyon, France), Hebovac-88-T (Veterinärwesen, Sofia, Bulgaria) and Leporiphyl (Phylaxia, Budapest, Hungary). Rabbits were vaccinated at the age of 4 and/or 12-14 weeks. Arvilap and Hebovac were injected subcutaneously, Leporiphyl intramuscularly, whereas Dercunical was administered intradermally with a needle-less device which saves labour and avoids rabbit-to-rabbit transmission of intercurrent diseases. None of the vaccinated rabbits showed rise of body temperature after vaccination (p.v.). Revaccination 8-10 weeks later did not increase the risk of anaphylactic shock. Post-vaccination HI-titres rose to higher levels in rabbits vaccinated at 12-14 weeks than at 4 weeks and remained detectable for a longer time. Challenge infection of rabbits vaccinated at 4 weeks with Arvilap and Dercunical resulted in complete protection 8 weeks p.v. Hebovac and Leporiphyl showed only 83 % of protection and 33 % of animals developed fever. Maternal immunity influenced protection after vaccination at weaning distinctly, which makes revaccination at 12-14 weeks of age necessary. Revaccination with Arvilap, Dercunical and Hebovac was followed by complete protection. Primovaccination with the latter vaccines at 12-14 weeks also induced complete protection against challenge as early as 7 days p.v. Protection lasted at least 6 months p.v. Leporiphyl vaccinated animals showed 93 % of survival. In non vaccinated rabbits 82 % of the animals died as a result of the experimental infection. No virus excretion has been established in vaccinated animals one week after challenge with wild VHD.