«Programmi vaccinali per la MEV»

ISTITUTO ZOOPROFILATTICO SPERIMENTALE
DELLA LOMBARDIA E DELL'EMILIA ROMAGNA
"BRUNO UBERTINI"
ENTE SANITARIO DI DIRITTO PUBBLICO

LA NOSTRA ESPERIENZA, LA VOSTRA **SICUREZZA**.

Nuovi vaccini biotecnologici basati sull'utilizzo di VLPs espresse in baculovirus

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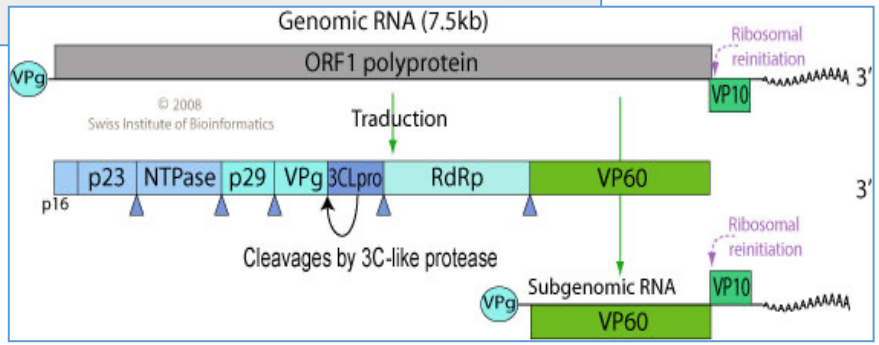
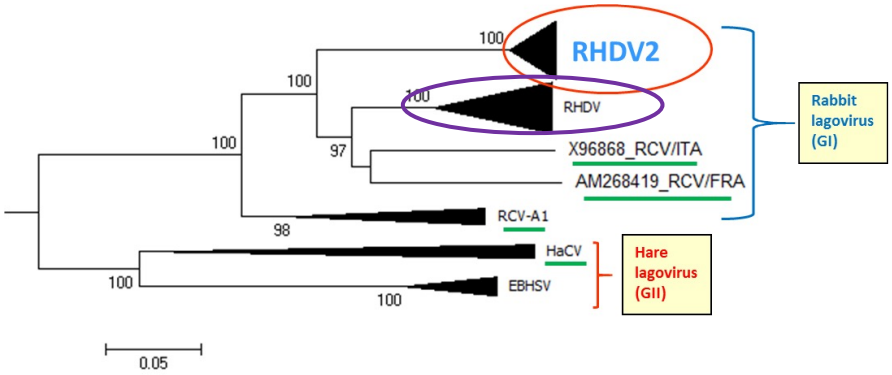
18/03/2022

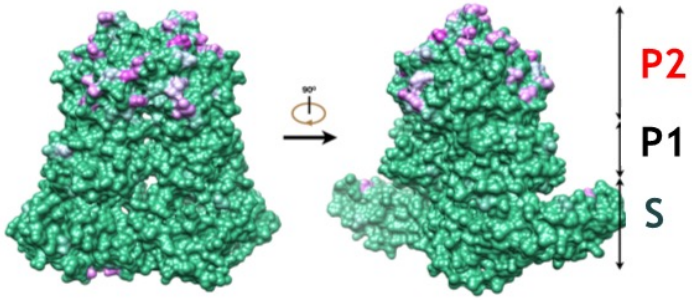
Rabbit Haemorrhagic Disease Virus

GENOME: SS-RNA, positive sense





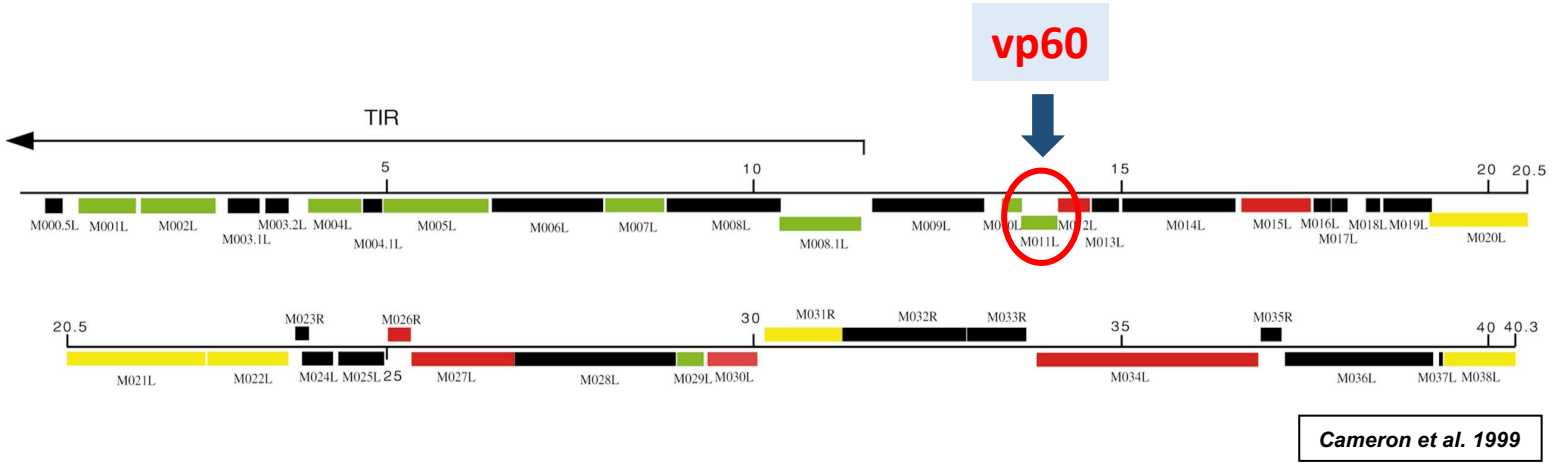
Elevata variabilità genetica e antigenica



Capside: 90 dimeri di VP60

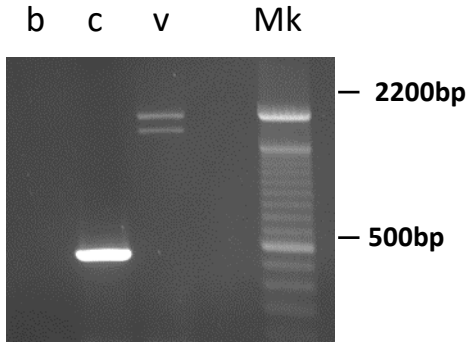
Vaccini biotecnologici per la MEV

- Vaccino vivo “biotecnologico” (Nobivac Plus), costituito da virus della Myxomatosi attenuato in laboratorio che esprime la VP60 sia di **RHDV** (strain 009) che di **RHDV2** (strain MK1899, 2012). Il gene vp60 è stato clonato nel gene M011L (anti-apoptotico) del Myxoma virus (genoma 160kb).



Cameron et al. 1999

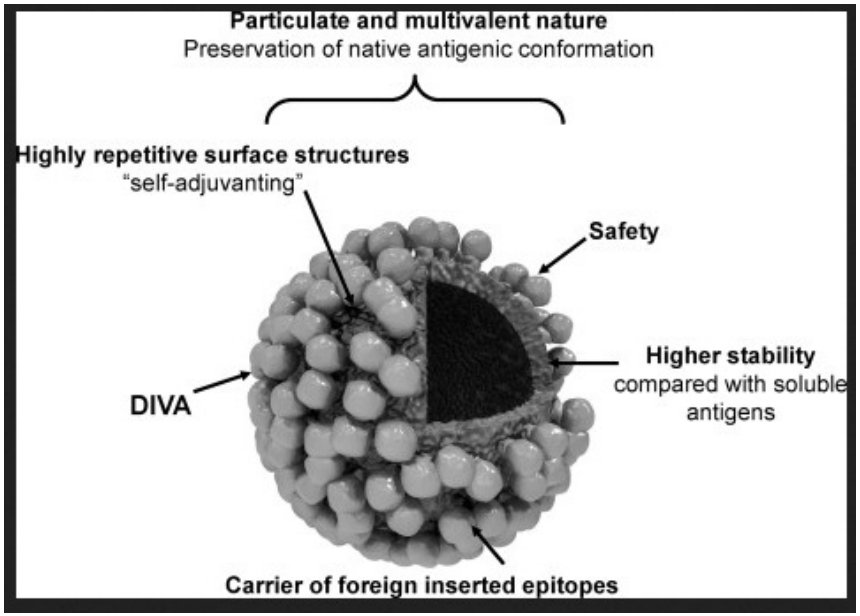
RT-PCR diagnostica



Virus-Like Particles (VLPs)

«VLPs are a form of subunit vaccine consisting of self-assembling shells derived from virus capsid proteins» *(Donaldson et al.; 2015)*

- Geometria ben definita e con strutture di superficie ripetute e ordinate
- Conservazione della conformazione antigenica nativa
- Sicuri, in quanto sono molecole assolutamente non infettive e non replicanti (nessun genoma)
- Maggiore stabilità in condizioni ambientali estreme rispetto agli antigeni solubili
- Possono essere utilizzati come vettori per la presentazione di antigeni diversi



E. Crisci 2012

Vantaggi e limiti dei differenti sistemi di espressione

Table 9.2 VLP expression systems

	Advantages	Limitations
Bacteria (e.g. *Escherichia coli*)	• Rapid cell growth	• No post-translational modification
	• Highest yield	• Limited applications for mammalian VLPs
	• Low production cost	• May form inclusion bodies
	• Scalable	• Requires removal of endotoxins
Yeast (e.g. *Saccharomyces cerevisiae*) 	• Rapid cell growth	• Limited post-translational modification
	• High yield	• May form inclusion bodies
	• Low production cost	
	• Scalable	
	• Already has some regulatory approval	
Insect cells/Baculovirus (e.g. *Spodoptera frugiperda*) 	• Average cell growth	• Requires removal of baculovirus proteins
	• High yield	• May form inclusion bodies
	• Scalable	
	• Complex post-translational modification	
	• Formation of multi-protein VLP	
Plant cells (e.g. *Nicotiana* sp.) 	• Rapid production	• Limited post-translational modification
	• Low production cost	• Relatively new system
	• Scalable	
Mammalian cells (e.g. Chinese hamster ovary cells) 	• Scalable	• Slow growth
	• Complex post-translational modification	• Low yield
	• Formation of multi-protein	• Demanding culture conditions
	• VLP	• High production cost
		• Potential infectious contamination
Cell free 	• Almost exclusive production of target protein	• Very high production cost
	• Limited cellular contaminants	• Limited scalability
	• Enables production of VLPs containing non-natural amino acids or toxic protein intermediates	• Relatively new system, not well characterised

Adapted from Rebeaud and Bachmann (2012)

Cellule procariote

Cellule eucariote

Vantaggi vaccini a VLPs

VLP vaccines

Non-infectious

Safe for immune-compromised individuals

Fast manufacturing process

More stable than other subunit vaccines

No allergens

High yield

No risk of mutation due to lack of genetic material

V S

Conventional vaccines

Reversion to virulent form

Toxicity

Lengthy formulation time

Stability issues

Risk of allergic response

Low yield

Mutation risks

Espressione eterologa di VLPs di RHDV

- *Escherichia coli* (Boga et al.; 1994)
- *Saccharomyces cerevisiae* (Boga et al.; 1997)
- Piante (Castanon et al.; 1999)
- Cellule di mammifero utilizzando poxvirus ricombinanti (Bertagnoli et al.; 1996)
- Cellule di insetti infettate mediante il sistema baculovirus (Perez-Filgueira et al.; 2007)

Le VLPs inducono un' elevata risposta immunitaria sia di tipo umorale che cellulare

RHDV VLPs

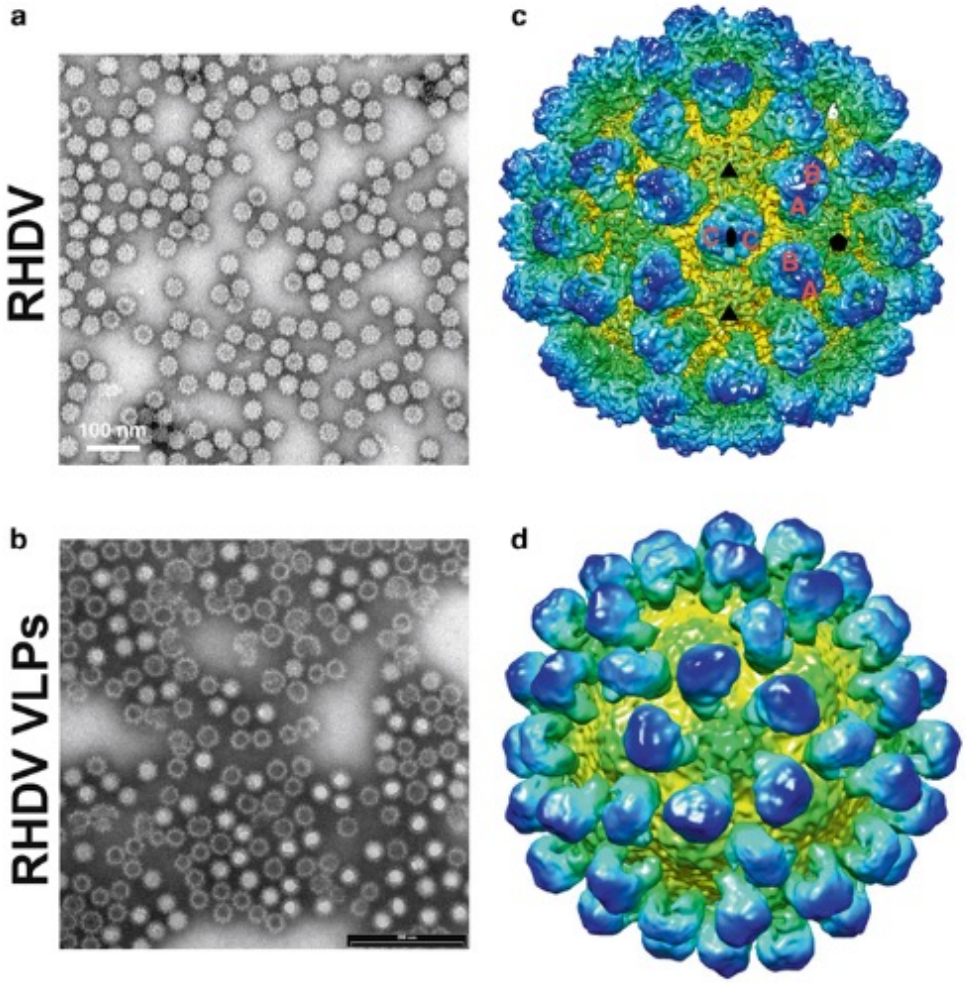
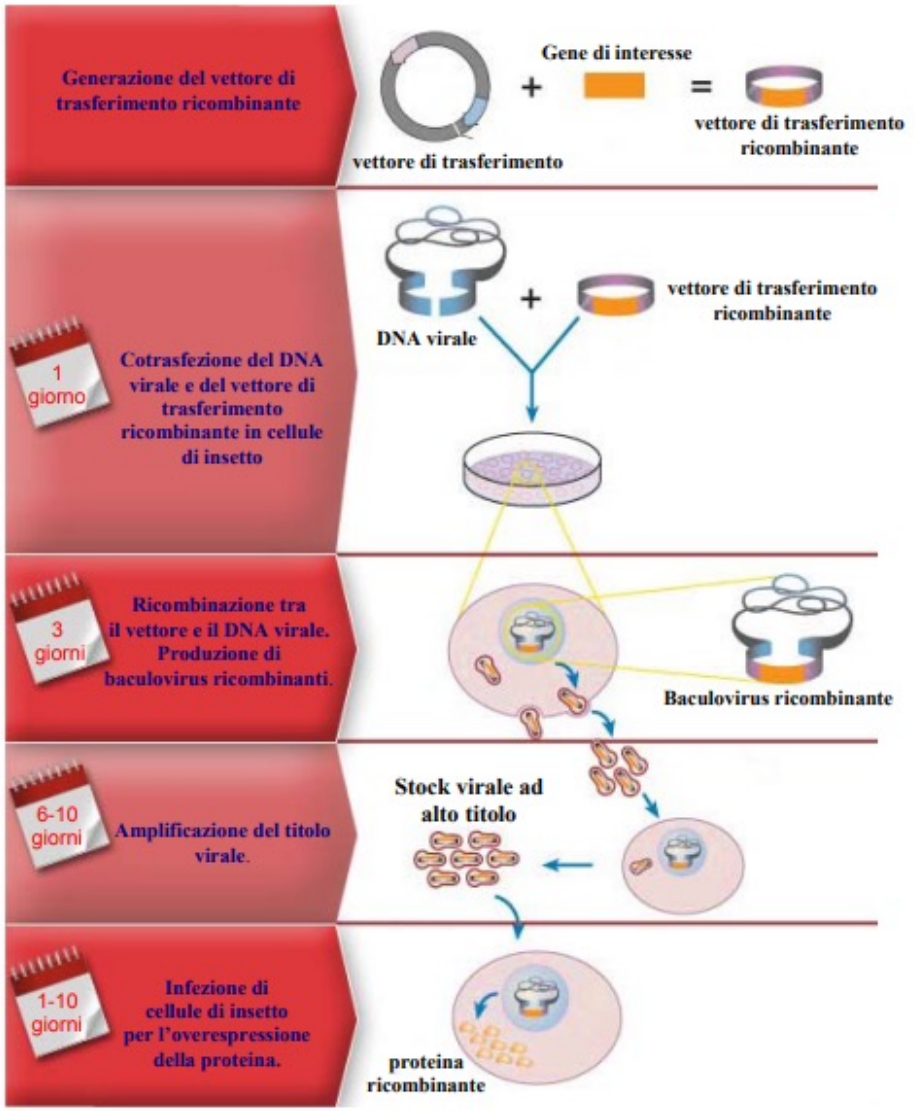


Fig. 9.2 Comparison of RHDV and RHDV VLP structure. RHDV VLPs expressed in insect cells visibly share structural characteristics with the native virus as viewed by transmission electron microscopy (**a**, **b**) and 3D modelling from cryo-electron microscopy and crystallography (**c**, **d**). (**a**, **c**) Adapted from Wang et al. (2013). (**d**) Generously supplied by Thomas J. Smith (Katpally et al. 2010)

Espressione di una proteina ricombinante mediante il sistema baculovirus in cellule di insetto



vaccines

MDPI

Article

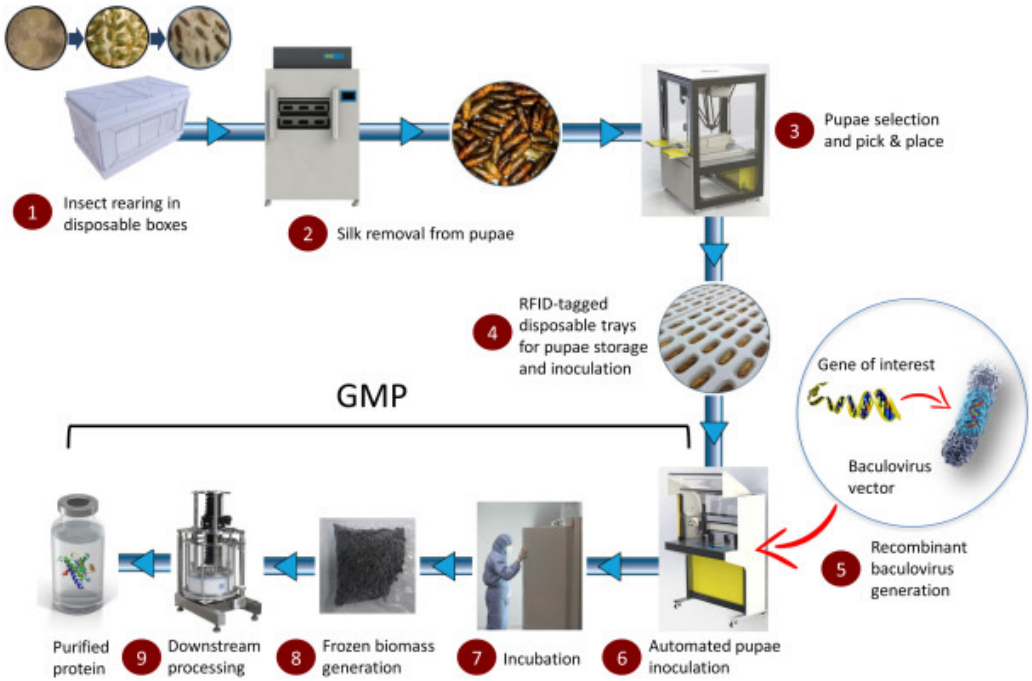
Chimeric VLPs Bearing VP60 from Two Serotypes of *Rabbit Haemorrhagic Disease Virus* Are Protective against Both Viruses

Kevin P. Dalton [1], Carmen Alvarado [2], Edel Reytor [2], Maria del Carmen Nuñez [2], Ana Podadera [1], Diego Martínez-Alonso [2], Jose Manuel Martin Alonso [1], Ines Nicieza [1], Silvia Gómez-Sebastián [2], Romy M. Dalton [2], Francisco Parra [1] and José M. Escribano [2,*]

Settembre 2021

"Al fine di ridurre i costi associati alla produzione, la nostra strategia è stata quella di generare VLPs chimeriche contenenti le proteine VP60 dei due sierotipi ottenuti dalla co-infezione delle pupae di insetto con due baculovirus ricombinanti che esprimono una delle due proteine del capsid (RHDV and RHDV2)"

Construction of recombinant Baculoviruses and expression in Trichoplusia ni Pupae



1) RHDV GI.1 AST89
2) RHDV2 GI.2 Nav10/11

Fig. 1. Schematic CrisBio-based recombinant subunit vaccine production process described in 9 steps, from insect rearing and pupae production to recombinant protein extraction and purification. **Step 1:** Chrysalises production in disposable insect rearing boxes. **Step 2:** elimination of silk from chrysalises. **Step 3** and **4:** Pick & place of pupae into RFID-labeled plastic trays. **Step 5:** recombinant baculovirus generation. **Step 6**: automated pupae inoculation with the recombinant baculovirus. **Step 7:** Infected pupae incubation. **Step 8:** infected pupae harvesting and freezing. **Step 9:** recombinant protein purification.

Escribano et al.; 2020

Expression of VP60 Proteins and VLP Conformation

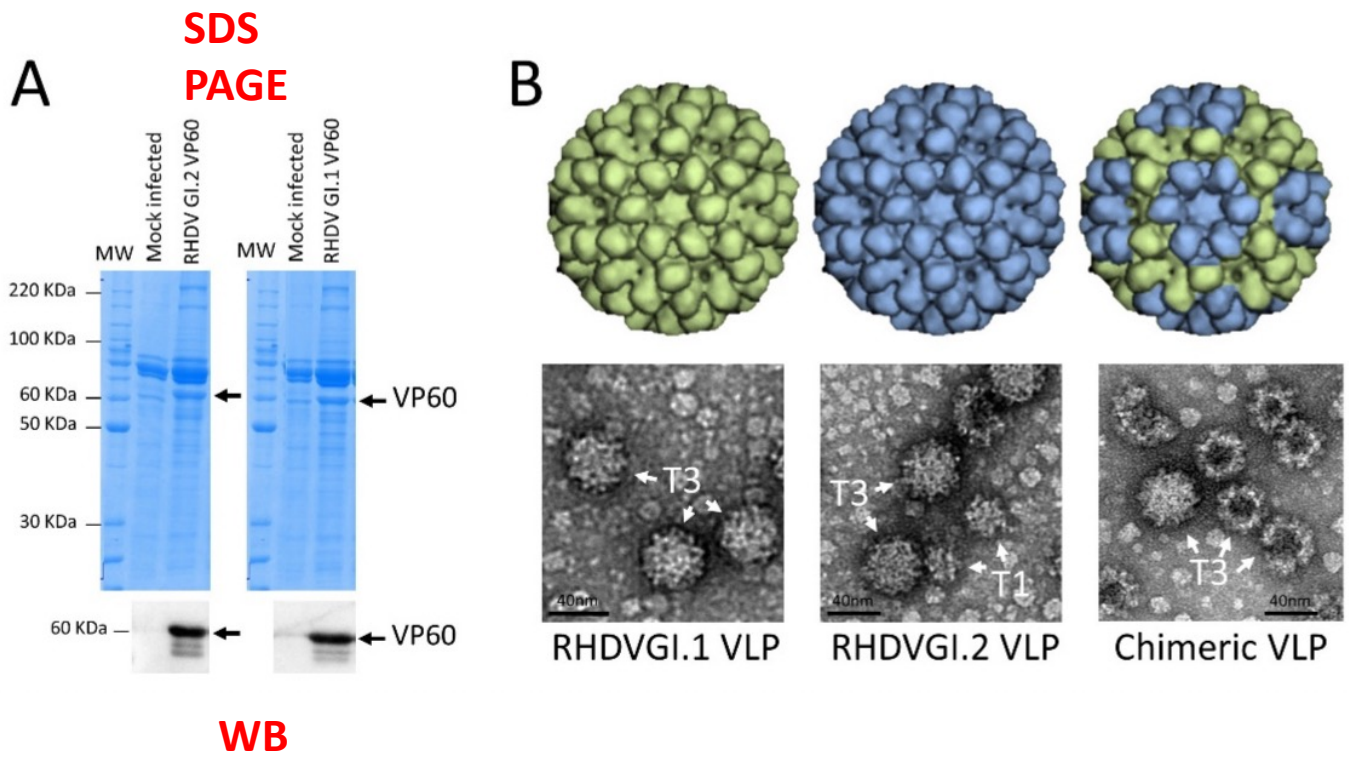


Figure 1. Expression of protein VP60 from RHDV GI.1 (isolate AST89) and RHDV GI.2 (isolate N11) in *T. ni pupae* by two different recombinant baculoviruses, and VLP formation by infecting the pupae with individual baculovirus or co-infecting with both vectors. (**A**) SDS-PAGE gels resolving the extracts from infected pupae with individual baculovirus and stained by Coomassie blue (**B**) A 3-D model of the hypothetic conformation of the resulting VLP after infection with individual recombinant baculovirus and the resulting VLPs after co-infecting with both vectors. Representative electron micrographs of purified VLPs resulting from the infection with individual baculovirus or after a co-infection (chimeric)

Chimeric VLPs Protect Rabbits against a Lethal Challenge with RHDV GI.1 and RHDV GI.2 viruses

Table 1. Experimental design.

Group	Age	Vaccination	Challenge
A	30 d	PBS	RHDVGI.2
B	30 d	20 µg Chimeric VLPs	RHDVGI.2
C	30 d	40 µg Chimeric VLPs	RHDVGI.2
D	30 d	5 µg RHDVGI.2 VLPs + 5 µg RHDVGI.1 VLPs	RHDVGI.2
E	60 d	PBS	RHDVGI.1
F	60 d	40 µg Chimeric VLPs	RHDVGI.1

Abbreviations: PBS Phosphate buffered saline; RHDV Rabbit haemorrhagic disease virus; VLP virus-like particle.

Challenge after 7d

Table 2. Rabbit protection results obtained with different vaccine formulations after challenge of vaccinated rabbits with the two RHDV serotypes.

Group	Vaccination	Virus Challenge	Mortality	Virus Genome Detection
A	PBS	RHDVGI.2	4/5	5/5
B	20 µg chimeric VLP	RHDVGI.2	1/5	1/5
C	40 µg chimeric VLP	RHDVGI.2	0/5	0/5
D	5 µg RHDVGI.1 VLP +5 µg RHDVGI.2 VLP	RHDVGI.2	0/5	0/5
E	PBS	RHDVGI.1	4/5	nd
F	40 µg chimeric VLP	RHDVGI.1	0/5	nd

Abbreviations: PBS Phosphate buffered saline; RHDV Rabbit haemorrhagic disease virus; VLP virus-like particle.

Antibodies anti-RHDV

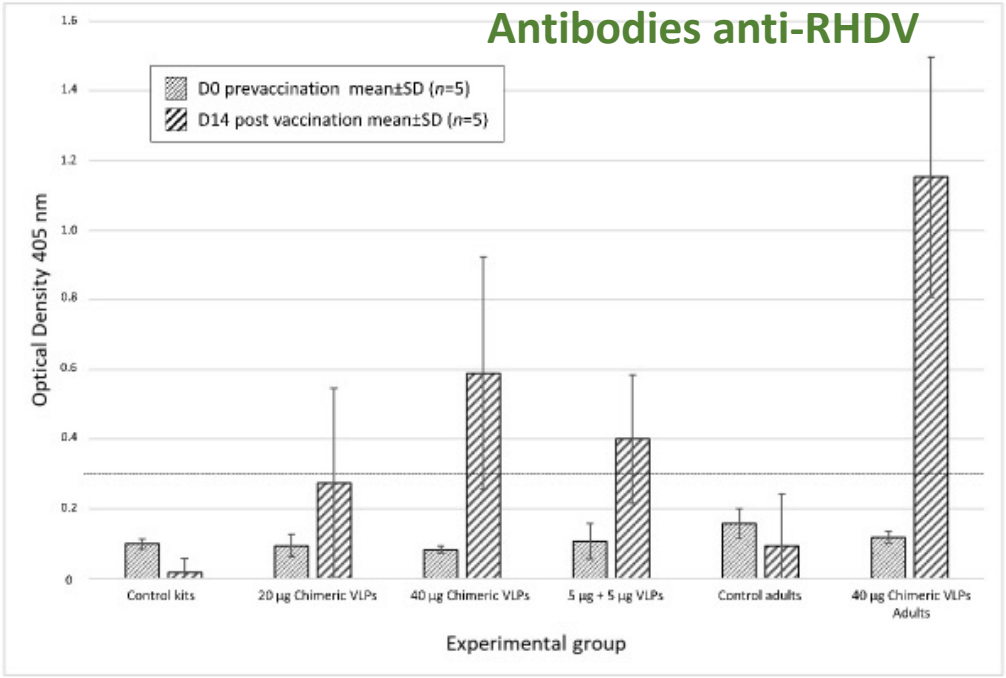


Figure 5. Detection of anti-RHDV antibodies in rabbit sera pre- (D0) and post vaccination (D 14) by ELISA. The graph shows optical density (450 nm) of sera versus mean ± SD (n = 5) from the four groups of rabbits challenged with RHDV GI.2 (kits of 30 d of age) and two groups (adults 60 d) challenged with GI.1. Sera were analysed using the commercial ELISA a cut-off value of 0.3 is indicated with the dotted line.

Surviving animals

Nuovo vaccino biotecnologico per RHDV/RHDV2

L'EMA ha autorizzato un vaccino basato sulle VLPs prodotte con la tecnologia CrisBio in pupae di *Trichoplusia ni*:

- Vaccino bivalente
- VP60 di RHDV, ceppo Ast89
- VP60 di RHDV2, ceppo N11
- Adiuvante, idrossido di alluminio
- Eccipienti, tiomersale

Grazie per l'attenzione!

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