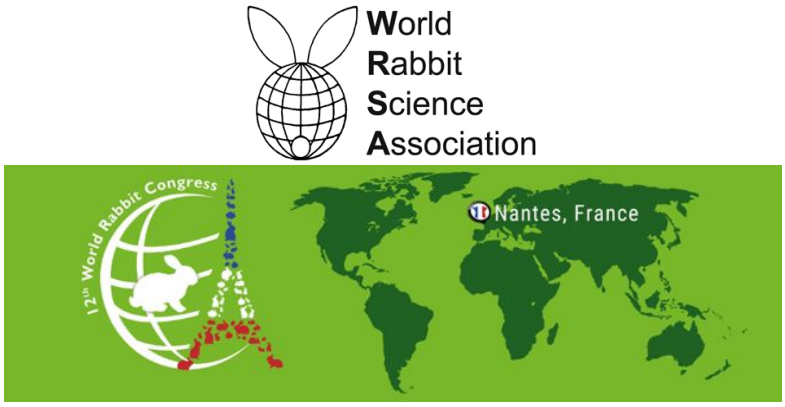

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Full text of the communication
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CONCENTRATION OF ANTIBODIES AND IMMUNOGLOBULINS IN DOES AND THEIR OFFSPRING VACCINATED AGAINST *RHDV-2*

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ABSTRACT

Rabbit haemorrhagic disease virus 2 (RHDV-2) is a highly infectious disease and causes significant mortality in rabbit farms, especially during fattening. Despite vaccination of all reproductive does, new cases of fattening RHDV-2 are reported in French rabbit farms, which raise the question of humoral immunity transmission from females to their kits. Serological monitoring was carried out in a farm on 30 primiparous does vaccinated at 10 weeks of age against RHDV-2, and on 2 of their kits. Four months after the first vaccination, 3 out of 30 does didn't have RHDV-2 Antibodies (Ab). After a booster vaccination of does (D25), geometric mean titration (GMT) was increased with a wider range of cELISA RHDV-2 Ab titers (from 80 to 1280 at D25 and from 80 to 20480 at D36). At weaning (D36), the kits with the highest GMT of cELISA RHDV-2 Ab (GMT = 1712) came from does with high titers (GMT = 465) at D25, but those rabbits didn't have Ab at D59, 14 days after they have been vaccinated. Conversely, kits with low cELISA RHDV-2 titers (GMT = 160) at D36 were from does with very low titers at D25, and they had the highest titers (GMT = 160) at D59. These results indicate that there is a large individual variation in the humoral immune response of does to vaccination, that there is an effective transfer of maternal Ab to kits, and that vaccination of reproductive does seems to inhibit the early development of young rabbit humoral immunity.

Key words : Rabbit haemorrhagic disease, RHDV-2, humoral immune response

INTRODUCTION

Since 2010, Rabbit haemorrhagic disease virus is due to a variant virus called RHDV-2, different in its genetic and clinical profile from the classic RHDV virus (Boucher & Nouaille, 2013). Since 2013, French breeders have specific RHDV-2 vaccine, and they apply vaccination according to a preventive plan on reproductive animals only, because its cost is too high for a systematic vaccination of meat rabbits. However, this practice, in addition to strengthening biosecurity on farms, has not prevented the emergence of high fattening mortalities since 2016 (Le Gall-Reculé & Boucher, 2017). This raises the question of the factors that influence the effectiveness of vaccination, and in particular of the modalities of maternal Ab transmission to the offsprings. Indeed, several studies, in other species, show that maternal Ab can inhibit vaccination (Fablet & Renson, 2016). The aim of this work is therefore to deepen the knowledge of humoral immune situation of does and their kits in a vaccinated farm by monitoring a cohort within a herd without clinical expression of Rabbit Haemorrhagic Disease.

MATERIALS AND METHODS

Experimental design

The protocol is shown in figure 1. In a farm of 600 primiparous does, 30 does randomly selected had a serological monitoring. These does were bred identically (housing, feeding), and were all vaccinated at 10 weeks by a subcutaneous mono-injection of a vaccine containing the RHDV-2 strain in oil-adjuvant

(ERAVAC®). The 30 does had a blood test 25 days after parturition (D25), which was 17 weeks after their first vaccination. On the same day (D25), 24 does had a vaccination with the same vaccine (ERAVAC®), and 6 control does, were not vaccinated, in order to check the absence of circulating RHDV-2 in the farm during the trial. A blood test was carried out again on the does, 5 days (D30) and 11 days (D36) after vaccination.
For each of the 30 does, 2 kits were randomly chosen from the litter and identified. Blood from these 60 kits were collected at Birth + 36d (D36) at the same time as their mother. At 45 days of age (D45), they were vaccinated with ERAVAC®, and had a blood sample before vaccination. A final sample was taken 14 days after vaccination (D59). In addition, in order to check the absence of circulating virus in the farm, at D59, 1 additional rabbit per unvaccinated female, and not vaccinated at D45, had a blood sample.

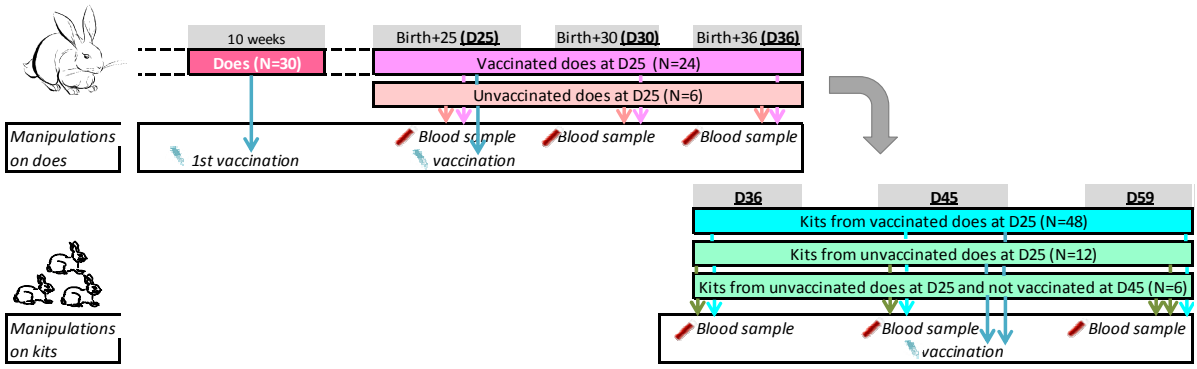


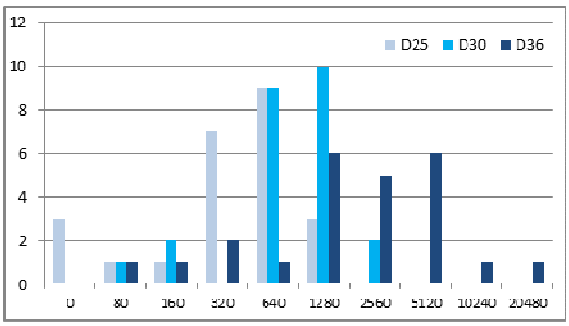
Figure 1: Experimental plan (blood samples and vaccinations dates)

Analyses
Serums obtained by centrifugation were stored at -20°C until shipment in refrigerated transport to the reference laboratory of the International Epizootic Office for RHDV in Brescia, Italy.
All serums were analyzed by cELISA technique using anti-RHDV-2 monoclonal antibodies. This competitive ELISA analysis detects all kinds of immunoglobulins to the variant RHDV-2 virus.
All the rabbit serums at D59 were analyzed to titrate the IgM-2 by a direct reverse ELISA technique. IgMs are the first antibodies to be formed after contact with an antigen.
IgG-2 titrations were carried out randomly on young rabbits at vaccination at weaning D36 (n=46), at D45 before vaccination and at D59 (n=30). IgGs form after IgMs, with higher levels and over longer durations.

RESULTS AND DISCUSSION

All does (N=30)
At D25, before vaccination, the GMT of cELISA RHDV-2 Antibodies (Ab) of does was 458.4 (Table 1). Among the 30 does, 3 does (10%) didn't show humoral immunity. At D30 (5 days after booster vaccination) and D36 (11 days after booster vaccination), GMT was increased and all the does showed positive Ab titers. At D36 there was a wider range of cELISA RHDV-2 Ab titers (from 80 to 20480) compared to D25 (from 80 to 1280) and D30 (from 80 to 2560) (Figure 2). So, there is a variable individual humoral immune response to vaccination.

Control does (unvaccinated animals during the trial) (N=6 does and 6 young rabbits)
The 6 unvaccinated does had similar cELISA RHDV-2 Ab titers between D25 (GMT = 403.2) and D36 (GMT = 452.5). In addition, at D59, the 6 kits not vaccinated were negative in cELISA RHDV-2 Ab. These results confirm the absence of circulating RHDV-2 in the farm during the trial.
Table 1: Geometric Mean (GMT) of cELISA



RHDV-2 Antibodies of does

	Vaccinated does	Unvaccinated does	All does
cELISA RHDV-2 Ab	(N=24)	(N=6)	(N=30)
GMT D25	475.5	403.2	458.4
GMT D30	783.4	640.0	752.4
GMT D36	1810.2	452.5	1371.9
multiplier factor D25—D36	*3.8*	*1.1*	*3.0*

Figure 2: Number of does as a function of cELISA RHDV-2 AB titers on the 3 sampling dates

Vaccinated does at D25 (N=24)

At D25, which was 17 weeks after the primary vaccination, GMT of cELISA RHDV-2 Ab of the vaccinated does was 475.5. But among these 24 does, 3 does (12%) didn't have cELISA RHDV-2 Ab. The explanation can be found in the vaccine's marketing authorization : the non-development of Ab can result from bad vaccination practices, or from the presence of factors limiting the immune capacity of animals.

At D30 and D36, GMT of the cELISA RHDV-2 Ab was respectively 783.4 and 1810.2 : these increasing Ab titers mean a positive response to vaccination.

Kits

At D36 (weaning), GMT of cELISA RHDV-2 Ab of all kits was 13.7 (Table 2), but 20 kits out of 60 (33%) didn't have any Ab.

From Figure 3, it seems that at weaning the more the doe showed RHDV-2 Ab, then, the more her kits showed RHDV-2 Ab too. So, in this farm, transmission of maternal Ab to the kits was effective ; this transmission can occur during gestation and lactation (Krehenbuhl & Sordat, 1979; Jacquier, 2014).

At D45 (just before vaccination of kits), GMT of cELISA RHDV-2 Ab was 10.4, but only 16 rabbits out of 60 (27%) still had RHDV-2 Ab.

At D59, 2 weeks after their vaccination, only 58 rabbits (instead of 60 rabbits) were taken, due to the death of 2 rabbits : only 10 rabbits out of 58 (17%) had cELISA RHDV-2 Ab (figure 3), but with a higher GMT compared to D45. We can notice that 14 days after vaccination GMT of cELISA RHDV-2 Ab was increased by 3.8 which is the same evolution than females 11 days after vaccination.

At D59, 4 rabbits had a very high cELISA RHDV-2 Ab titers (GMT=160) and high IgM-2 (GMT=10280). These 4 rabbits came from females without Ab at D25 ; moreover, at D36, they didn't have cELISA RHDV-2 Ab and they had very low level of IgG-2 (2 rabbits were negative and 2 rabbits had titers of 160, while GMT of all rabbits was 2079).

On the contrary, 48 rabbits out of 58 (83%) didn't have cELISA RHDV-2 Ab at D59 and low level of IgM-2 (GMT=1778). These rabbits were all from females with positive titers of RHDV-2 Ab at D25 (GMT = 465) and had positive Ab titers at D36 (GMT = 13.5, 73% of positive rabbits) and higher levels of IgG-2 (GMT = 1413).

Table 2: Geometric Mean (GMT) of cELISA RHDV-2, IgG-2 and IgM-2 of kits

All kits (N=60)	Ac cELISA RHDV-2	IgG-2	IgM-2
GMT D36	13.7	1280 (N=46)	
GMT D45	10.4	560 (N=30)	
GMT D59	40	2079 (N=30)	1778 (N=58)
multiplier factor D45—D59	*3.8*	*3.7*	

It therefore seems that rabbits from does with a high titer of cELISA RHDV-2 Ab at weaning didn't develop quickly their own humoral immunity after vaccination, having still maternal Ab. Conversely,

rabbits from does with low Ab titers at weaning seem to develop their own immunity more quickly (Figure 4).

AB titers of females at D25 \ AB titers of kits at D36	N	D	10	20	40	80	160
N	100%	0%	0%	0%	0%	0%	0%
80	100%	0%	0%	0%	0%	0%	0%
160	0%	0%	50%	50%	0%	0%	0%
320	32%	14%	45%	9%	0%	0%	0%
640	9%	0%	41%	45%	5%	0%	0%
1280	0%	0%	50%	50%	0%	0%	0%

Figure 3 : Link between kits antibodies level at D36 and their mothers' Ab titers at D25

AB titers of females at D25 \ AB titers of kits at D59	N	D	10	20	40	80	160
N	0%	0%	0%	0%	33%	0%	67%
80	50%	0%	50%	0%	0%	0%	0%
160	100%	0%	0%	0%	0%	0%	0%
320	100%	0%	0%	0%	0%	0%	0%
640	86%	14%	0%	0%	0%	0%	0%
1280	100%	0%	0%	0%	0%	0%	0%

Figure 4 : Link between kits antibodies level at D59 and their mothers' Ab titers at D25

CONCLUSIONS

It appears in this study that, first, there is a huge variability in immune response after RHDV-2 vaccination, on reproductive females. Concerning young rabbits, the quickest immunized after vaccination are those which have the smallest maternal antibodies titers at weaning and at vaccination, and they also came from females with the lowest titers. Conversely, when rabbits did not show cELISA RHDV-2 Ab 14 days after vaccination, they came from does with the highest GMT of RHDV-2 Ab at D25. However, antibody titers reflect an immune capacity, they cannot be correlated with a level of protection against a viral challenge. But rabbits without antibodies are not protected.

These results highlight the added-value of serological monitoring to improve vaccination protocols and good vaccination practices. Indeed, in French rabbit farms, it is common to observe primiparous mortalities (sometimes significant) in a vaccinated herd : immune breakdown factors, together with the first parturition, could explain these observations.

The interest of hyper-immunization of does and its impact on the development of immunity of young animals needs further studies. Better maternal Ab transmission to the rabbit and inherent potentially protection should be checked ; it could be reasoned in terms of cost / benefit for the breeders.

ACKNOWLEDGEMENTS

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Concentration of antibodies and immunoglobulins on does and their offspring vaccinated against RHDV-2

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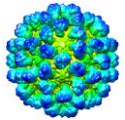
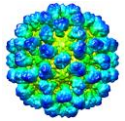
World Rabbit Congres
Nantes, November 2021

Introduction



- Since 2010, Rabbit haemorrhagic disease virus is due to a variant (**RHDV-2), different in its genetic and clinical profile** (Boucher & Nouaille, 2013).

Introduction



- Since 2010, Rabbit haemorrhagic disease virus is due to a variant (**RHDV-2), different in its genetic and clinical profile** (Boucher & Nouaille, 2013).

- Since 2013 reproductive animals are vaccinated with a **specific vaccin against RHDV-2.**

- But : emergence of **high fattening mortalities since 2016**
  (Le Gall-Reculé & Boucher, 2017).

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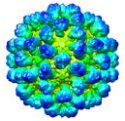

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- Since 2013 reproductive animals are vaccinated with a **specific vaccin against RHDV-2.**

- But : emergence of **high fattening mortalities since 2016**
  (Le Gall-Reculé & Boucher, 2017).

→Which factors influence the effectiveness of vaccination ?

→What are the modalities of maternal Ab transmission to the kits (no rabbit reference before our study) ?

Materials and methods

- Farm of 600 primiparous does : serological monitoring of 30 does randomly selected. Identical breeding (housing, feeding)

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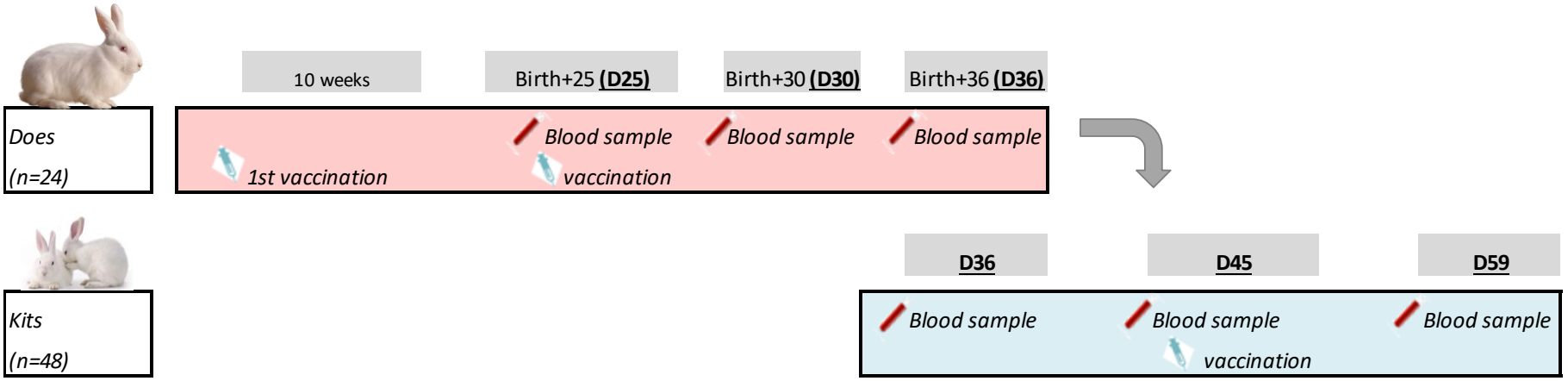


Materials and methods

- Farm of 600 primiparous does : serological monitoring of 30 does randomly selected. Identical breeding (housing, feeding)

Does (n=24)

10 weeks — Birth+25 **(D25)** — Birth+30 **(D30)** — Birth+36 **(D36)**

1st vaccination — *vaccination* — *Blood sample* — *Blood sample* — *Blood sample*

Kits (n=48)

D36 — **D45** — **D59**

Blood sample — *vaccination* — *Blood sample* — *Blood sample*

Validation of the absence of virus circulating with unvaccinated animals

Materials and methods

- Farm of 600 primiparous does : serological monitoring of 30 does randomly selected. Identical breeding (housing, feeding)

Does (n=24)

10 weeks — 1st vaccination

Birth+25 **(D25)** — Blood sample — vaccination

Birth+30 **(D30)** — Blood sample

Birth+36 **(D36)** — Blood sample

Validation of the absence of virus circulating with unvaccinated animals

Kits (n=48)

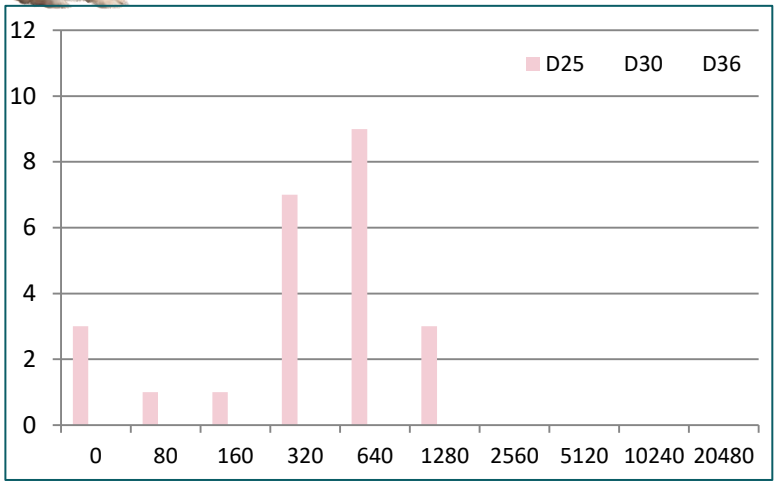
D36 — Blood sample

D45 — Blood sample — vaccination

D59 — Blood sample

- Analysis : RHDV-2 Ab and IgG-2 on all females and kits at each age, and IgM-2 on kits

Immunity of the primiparous does

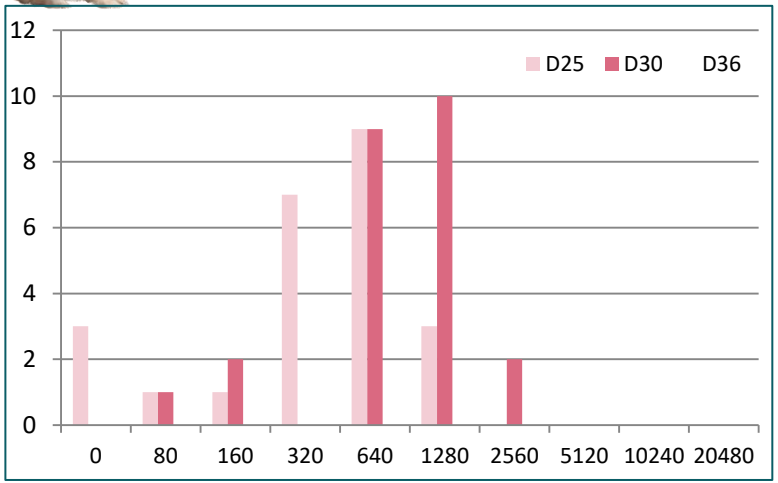


- GMT (only on positive females)
  - **D25 = 476** (CV 30%)

Number of does as a function of cELISA RHDV-2 Ab titers on the 3 sampling dates

→ At D25, **3/24 does (12%) don't have Ab** (bad vaccination practices ? factors limiting the immune capacity of the animals ?)

Immunity of the primiparous does

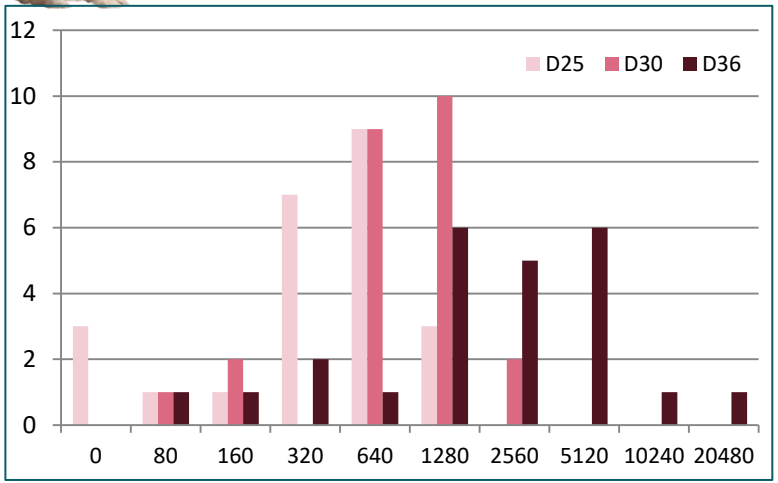


- GMT (only on positive females)
  - **D25 = 476** (CV 30%)
  - **D30 = 783** (CV 37%)

Number of does as a function of cELISA RHDV-2 Ab titers on the 3 sampling dates

→ At D25, **3/24 does (12%) don't have Ab** (bad vaccination practices ? factors limiting the immune capacity of the animals ?)

Immunity of the primiparous does



- GMT (only on positive females)
  - **D25 = 476** (CV 30%)
  - **D30 = 783** (CV 37%)
  - **D36 (weaning) = 1810** (CV 62%)

Number of does as a function of cELISA RHDV-2 Ab titers on the 3 sampling dates

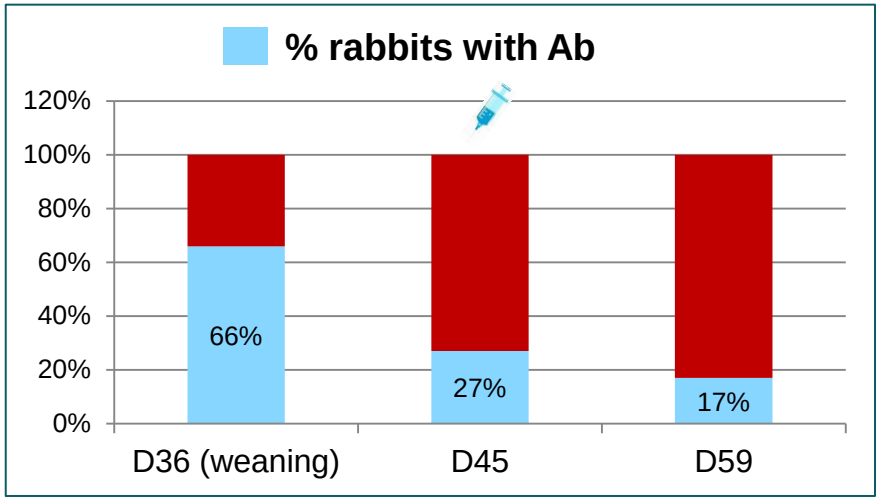
→ At D25, **3/24 does (12%) don't have Ab** (bad vaccination practices ? factors limiting the immune capacity of the animals ?)

→ Development of Ab following vaccination (11 days after vaccination, **geometric mean of titers * 3.8**)

→ **Strong dispersion** of titers according to individuals

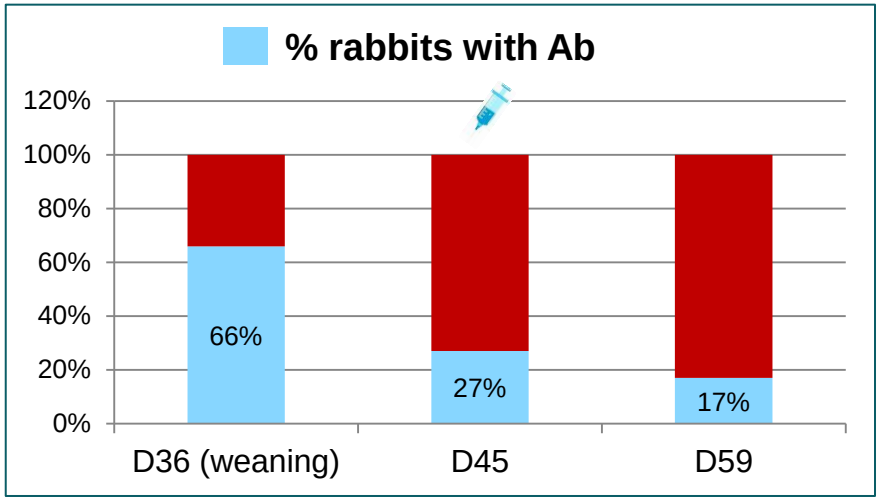
Feedia By TECHNA

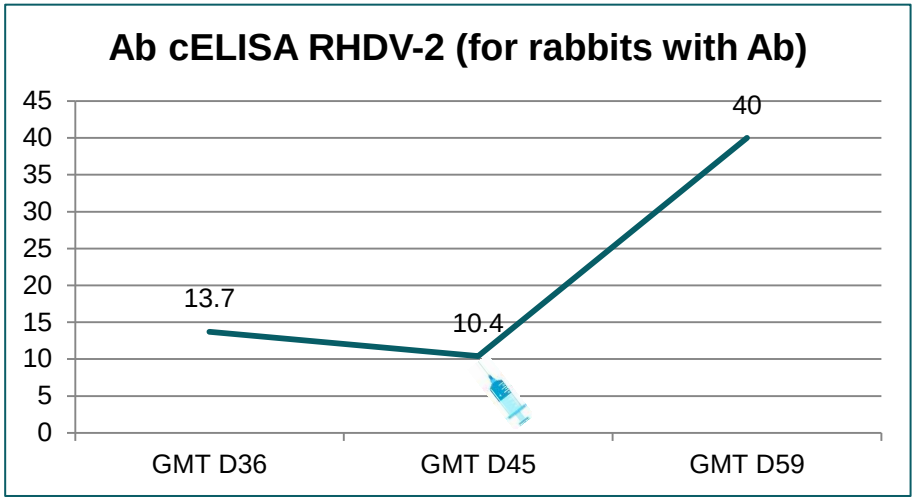
Immunity of their kits



- **66% of young rabbits have maternal antibodies** at D36 (weaning)
- The pourcentage of rabbits with antibodies **decreases with age, despite vaccination**

Immunity of their kits





- **66% of young rabbits have maternal antibodies** at D36 (weaning)
- The pourcentage of rabbits with antibodies **decreases with age, despite vaccination**
- For the rabbits which have a response to the vaccine, **the geometric means is *3.8,** 14 days after the vaccination

Feedia By TECHNA

link between rabbits' immunity and their mother

AB titers of females at D25	AB titers of kits at D36						
	N	**D**	**10**	**20**	**40**	**80**	**160**
N	100%	0%	0%	0%	0%	0%	0%
80	100%	0%	0%	0%	0%	0%	0%
160	0%	0%	50%	50%	0%	0%	0%
320	32%	14%	45%	9%	0%	0%	0%
640	9%	0%	41%	45%	5%	0%	0%
1280	0%	0%	50%	50%	0%	0%	0%

Ab of kits at D36 VS Ab of females at D25

→ At weaning : **higher titers on kits from high titers mothers** and inversely

link between rabbits' immunity and their mother

AB titers of females at D25	AB titers of kits at D36						
	N	**D**	**10**	**20**	**40**	**80**	**160**
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1280	0%	0%	50%	50%	0%	0%	0%

Ab of kits at D36 VS Ab of females at D25

AB titers of females at D25	AB titers of kits at D59						
	N	**D**	**10**	**20**	**40**	**80**	**160**
N	0%	0%	0%	0%	33%	0%	67%
80	50%	0%	50%	0%	0%	0%	0%
160	100%	0%	0%	0%	0%	0%	0%
320	100%	0%	0%	0%	0%	0%	0%
640	86%	14%	0%	0%	0%	0%	0%
1280	100%	0%	0%	0%	0%	0%	0%

Ab of kits at D59 VS Ab of females at D25

- At weaning : **higher titers on kits from high titers mothers** and inversely
- Vaccination + 14 days : **young rabbits** which **developed antibodies didn't have Ab at weaning** and come from **mothers with low levels**.

link between rabbits' immunity and their mother

AB titers of females at D25	AB titers of kits at D36						
	N	**D**	**10**	**20**	**40**	**80**	**160**
N	100%	0%	0%	0%	0%	0%	0%
80	100%	0%	0%	0%	0%	0%	0%
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Ab of kits at D36 VS Ab of females at D25

AB titers of females at D25	AB titers of kits at D59						
	N	**D**	**10**	**20**	**40**	**80**	**160**
N	0%	0%	0%	0%	33%	0%	67%
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320	100%	0%	0%	0%	0%	0%	0%
640	86%	14%	0%	0%	0%	0%	0%
1280	100%	0%	0%	0%	0%	0%	0%

Ab of kits at D59 VS Ab of females at D25

IgG-2 D45 (at vaccination)	IgM-2 D59 (14 days after vaccination)					
	N	**320**	**640**	**1280**	**2560**	**10240**
N	0%	0%	0%	0%	50%	50%
160	100%	0%	0%	0%	0%	0%
320	33%	0%	67%	0%	0%	0%
640	67%	5%	14%	14%	0%	0%

IgM-2 of kits at D59 VS IgG-2 of kits at D45

- At weaning : **higher titers on kits from high titers mothers** and inversely
- Vaccination + 14 days : **young rabbits** which **developed antibodies didn't have Ab at weaning** and come from **mothers with low levels**.
- **Same response with Immunoglobulins** (high IgM-2 at D59 = low IgG-2 at D45)

Conclusions

- Tendency : 1 breeding
- Immune dynamics in a herd:
  - 12% of primiparous does don’t have detectable Ab at D25
  - Huge variability in immune response
  - 66% of young rabbits have Maternal Ab at weaning
  - Quick development of Ig on rabbits with less Ab at weaning (without AOM)

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- Further studies are needed :
  - To know the interest of a hyper-immunization of does
  - To better understand the maternal Ab transmission and its protection on young rabbits

Feedia
By TECHNA

Thank you for your attention

Great thanks to : GAEC Rolland, UNEAL team and SELAS CVE team for their perseverance and their stubbornness for the blood sampling…

TECHNA
More than Nutrition