ORIGINAL SCIENTIFIC WORKS | LUCRĂRI ORIGINALE

ROMVACBLUE–4, VACCINE AGAINST BLUETONGUE PREPARED USING A VIRUS STRAIN ISOLATED IN ROMANIA

ROMVACBLUE–4, VACCIN CONTRA BLUETONGUE PREPARAT CU O TULPINĂ DE VIRUS IZOLATĂ ÎN ROMANIA

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ABSTRACT | REZUMAT

Bluetongue Disease is a global viral disease caused by an *Orbivirus* reovirus*,* borne by hematophagous insects. The clinical symptoms are hyperthermia, shortness of breath, depression, swollen purple cyanotic tongue etc. In 2014, the disease appeared in our country as well. BTV virus serotype 4 was isolated by IDAH experts, and in 2015 was taken over by Romvac experts who adapted it on cell cultures, described it and prepared master and working seeds as well as the first vaccine batches during 2016. The vaccine is inactivated and recommended for active immunization of sheep, cattle and goats. All laboratory control assays foreseen by domestic and international rules were performed.

As far as vaccine safety and efficacy concern, 3 trials were carried out, of which 2 in the laboratory and 1 in the field in Călărași County. The trials involved unvaccinated BTV antibodies-free sheep, goats and cattle which were inoculated with Romvac vaccine and in parallel with a reference vaccine from the market. Unvaccinated animals were each time kept as control. The first inoculation was followed by booster vaccine after 21 days, then serological assays (ELISA) were performed on the animals after 7, 14, 21, 28, 35, 42, 49, 80 up to 450 days. Before and after vaccination, the animals were monitored for overall health status, body temperature variation and local postvaccinal reactions.

Main results:a) safety; all vaccinated animals proved good tolerance of the vaccine without local reactions or general symptoms that could be due to the vaccine care (nevertheless, small local edema and short-term fever up to 1.5°C were noticed); b) specific immune response; was as expected and every time almost identical to that induced by the reference vaccine; dynamics increased within 25-30% after 7 days, 65-75% after 14 days, 80-90% after 7 days from booster, more than 90% after 14 days from booster, being stable up to 450 days, far beyond the borderline (40%). The so-obtained results were as expected – significantly positive and almost identical to those obtained with the reference vaccine, sometimes even higher.

Keywords: Bluetongue, BT virus, replication, inactivated vaccines

Boala limbii albastre – Bluetongue este o virază globală produsă de un reovirus din genul *Orbivirus,* transmisă de insecte hematofage. Se manifestă clinic prin hipertermie, dispnee, depresie, limbă puternic tumefiată, de culoare violacee, cianozată etc.

În anul 2014, a apărut și în România. Virusul BTV serotipul 4 a fost izolat de specialiștii IDSA, iar, în 2015 a fost preluat de specialiștii companiei Romvac, care l-au adaptat pe culturi celulare, l-au caracterizat, au preparat tulpini matcă și de lucru și, în cursul anului 2016, au obținut primele serii de vaccin. Vaccinul, un produs inactivat, este recomandat pentru imunizarea activă a ovinelor, bovinelor și caprinelor. S-au efectuat toate controalele de laborator: puritate, inactivare, stabilitate etc., prevăzute de normele interne și internaționale.

In ceea ce privește siguranța și eficacitatea vaccinului, s-au montat 3 experimente: 2 în laborator și 1 în teren, în județul Călărași. S-au folosit oi, capre și bovine, nevaccinate, libere de anticorpi BTV, care au fost vaccinate cu vaccin Romvac, comparativ cu un vaccin comercial de referință. De fiecare dată, s-au menținut animale nevaccinate ca martor.

După prima inoculare, s-a aplicat un rapel, la 21 de zile, animalele fiind apoi testate serologic (ELISA) la 7, 14, 21, 28, 35, 42, 49, 80 până la 450 zile. S-au urmărit, de asemenea, starea generală a loturilor înainte și după vaccinare, variația temperaturii corporale și reacțiile post vaccinale locale.

Rezultate principale: a) siguranța; s-a dovedit că toate animalele la care s-a aplicat vaccinul au suportat bine inoculul,neobservându-se semne locale sau simptome generale care să poată fi atribuite acestuia (au existat, totuși, mici edeme locale și febră de până la 1,5°C, de scurtă durată); b) răspunsul imun specific; a fost, de asemenea, cel proiectat și, de fiecare dată aproape identic cu cel indus de vaccinul de referință; dinamica fiind crescătoare în intervalele 25-30% - la 7 zile, 65-75% - la 14 zile, 80-90% - la 7 zile de la rapel, peste 90% - la 14 zile de la rapel, rămânând în platou până la 450 de zile, cu mult peste borderline (40%). Rezultatele astfel obținute au fost la nivelul scopului propus - semnificativ pozitive și aproape identice cu cele ale vaccinului de referință, uneori superioare.

Cuvinte cheie: Bluetongue, virus BT, replicare, vaccinuri inactivate

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INTRODUCTION

BTV is a member of *Orbivirus* genus, *Reoviridae* family, one of the 22 acknowledged species and serogroups of the respective genus (Fig. 1, Fig. 2).

Bluetongue Disease is carried by hematophagous insects (*Culicoides immicola* – (Fig. 3) and the clinical symptoms are hyperthermia, dyspnoea, depression, highly swollen, purple, cyanotic tongue etc (5).

Vaccination against bluetongue disease is being performed in several countries to reduce animal loss, BTV spread and to allow safe transportation of animals. Live attenuated vaccines are cheap to manufacture, provide protective immunity after a single inoculation and have proven to be efficient in treating the clinical form of the disease. A major risk when using live attenuated vaccines is their capacity of spreading the virus via vehicles, possibly with reversion to virulence or genomic recombination of vaccine virus with wild virus strains. The frequency of these situations is still uncertain, yet the transmission of virus strains from attenuated vaccines via *Culicoides* vehicle has already been researched in Europe (5, 8).

Inactive vaccines are considered safer than modified live virus vaccines because they do not allow viral replication. Therefore, they are efficient in avoiding virus spread among susceptible species, being successfully used in field trials and recommended by EU authorities (2, 4).

In 2014, bluetongue disease appeared in Romania also. BTV serotype 4 was isolated by IDSA experts and in 2015 was taken over by Romvac experts who adapted it on cell cultures, described it, prepared master and working seeds and, during 2016, obtained the first vaccine batches.

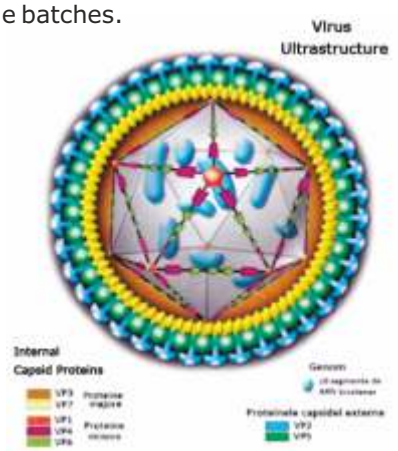

Fig. 1. BTV, double-chained RNA ultra-structure and capsid with 7 viral structural proteins (savoirspartages.cirad.fr)

Fig. 2. Protein VP7- a molecular model (fineartamerica.com)

Fig. 3. *Culicoides spp*.- one of the vehicles (thermofisher.com/bluetongue-virus-outbreak-in-france)

These works have resulted in ROMVACBLUE – 4 inactivated vaccine, a liquid injectable product used for active immunization of cattle against bluetongue disease virus (BTV) serotype 4, in order to prevent viral infection in sheep, cattle and goats and to reduce the intensity of clinical symptoms induced by BTV (Fig. 4).

The onset of immunity takes place 21-28 days after vaccination, depending on the reaction of each animal, and it lasts for 12 months. To maintain immunity, animals should be revaccinated as per the regulations of the sanitary and veterinary authorities.

Fig. 4. Inactivated vaccine against bluetongue disease virus serotype 4 manufactured by Romvac Company SA

MATERIALS AND METHODS

⏩ *BTV - 4 - R14* isolated by IDSA experts in 2014, was taken over by Romvac experts in 2015, who adapted it on cell cultures, described it and prepared master and working seeds;

⏩ *$BHK_{21}C_{13}$* cell line: from ECACC, PHLS, Centre for Applied Microbiology and Research, Porton, Salisbury Wilts SP40 JG – UK (ECACC Nr. 84100501). The main tissue for BHK_{21} (Baby Hamster Kidney) is baby hamster kidney (9), stored at the ECACC DE Inst. Pirbright – UK. Origin: ECACC No. 84100503 (Fig. 5 a);

⏩ *RK_{13}* cell line: from ECACC, PHLS, Centre for Applied Microbiology and Research, Porton, Salisbury Wilts SP40 JG–UK. ECACC No. 84100501. The main tissue for *RK_{13} (*Rabbit kidney*)* is rabbit kidney (Fig. 6 a);

⏩ VERO cell line was provided by the international authority ECACC (European Collection of Animal Cell Cultures, Health Protection Agency–Porton Down Salisbery, Wiltshire SP4 0JG England) where it was stored by the cell bank of WHO (World Health Organization), Geneva - Switzerland, at passage 134 (product of Merieux Institute – France – cells cultivated in the fermentor on Cytodex 1 microcarriers). It derives from original ATCC vials No.CCL-81 (Fig. 7 a);

⏩ *Earle`s Minimum Essential Medium (EMEM);*

⏩ Cell growth and virus replication systems: rotating (Bellco), static (Nunc™ Easy Flask™) and bioreactor (Sartorius);

⏩ Elisa ELX800 absorbance reader and ELISA ELx50 plate washer (BioTek Instruments Inc.);

⏩ Laboratory assays: virus infectivity on cell lines, seroneutralization (6), immunoenzymatic (ELISA kits – for determination of occurrence, intensity, dynamics and duration of immune response);

⏩ Vaccines: inactivated vaccine against bluetongue disease in ruminants prepared by Romvac Company from BTV - serotype 4 (ROMVACBLUE-4) and inactivated vaccine against bluetongue disease prepared with BTV - serotype 4, authorized in Romania (BLUEVAC, Spain);

⏩ Animals: sheep, goats, cattle.

⏩ Clinical studies: determination of safe vaccination and of vaccine efficacy in ruminants. The first step is testing the safe administration on a small number of animals,as per European Pharmacopoeia 8.0/01.2014 (1, 3) and OIE Manual 2014 (5). If the vaccine is safe, the second step follows; at this step, the specific immune response (efficacy) is determined after vaccination of ruminants under farm conditions, as per Pharmacopoeia 8.0/01.2014 (1).

RESULTS AND DISCUSSIONS

BTV-4-R14 was adapted and grown on $BHK_{21}C_{13}$, RK_{13}, VERO cell lines in static system, then in highly-productive systems (rotating and bioreactor), with a viral titer above $10^{7,5}CPID_{50/ml}$ (Fig. 5, Fig. 6, Fig. 7).

BTV4 - R14 viral suspension was inactivated using B - propiolactone and formaldehyde as inactivation agents, at concentrations much under the limit recommended in the literature (7).

Fig. 5. BHK_{21} cell monolayer:
a) normal (uninoculated) cells. b),c) specific BT-induced CPE, May-Grümvald-Giemsa staining

Fig. 6. RK_{13} cell monolayer:
a) normal (uninoculated) cells. b),c) specific BT-induced CPE, May-Grümvald-Giemsa staining

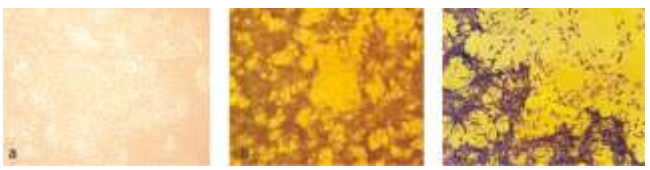

Fig. 7. VERO cell monolayer:
a) normal (uninoculated) cells. b),c) specific BT-induced CPE, May-Grümvald-Giemsa staining

Laboratory assays (purity, inactivation, stability etc.) and clinical assays (on unvaccinated, BTV antibodies-free sheep, goats, cattle) were conducted.

Elisa assays confirmed the presence, dynamics and level of specific serous BTV antibodies, thus establishing the onset and duration of specific immunity against bluetongue disease virus.

The safety assays revealed that:

➢ All vaccinated animals tolerated the vaccine very well. No significant local or general symptoms were noticed which could be attributed to the vaccine (yet there were noticed short-term small local edema and fever up to 1,5°C); (Fig. 8, Fig. 9, Fig. 10).

➢ The immune response of animals is appropriate – it is positive and after booster vaccination it reaches

the maximum serous antibody level. (Fig. 11, Fig. 12 Fig. 13).

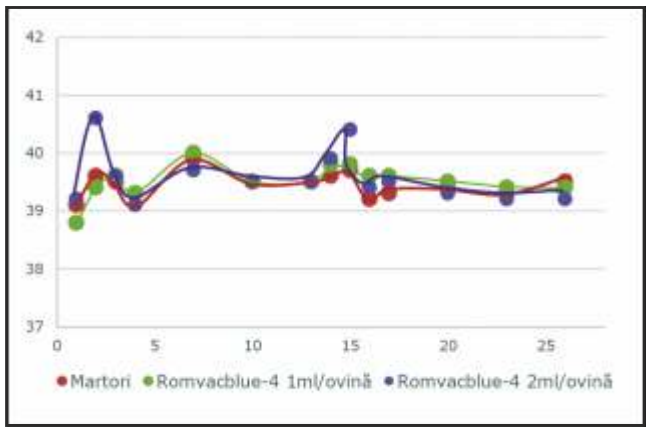

Fig. 8. Graphic representation of temperature monitoring for young sheep vaccinated with Romvacblue-4 – Gruiu Farm

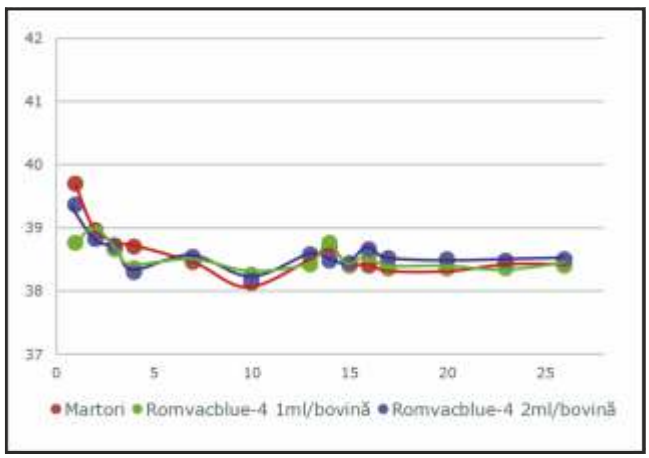

Fig. 9. Graphic representation of temperature monitoring for young cattle vaccinated with Romvacblue-4 – Gruiu Farm

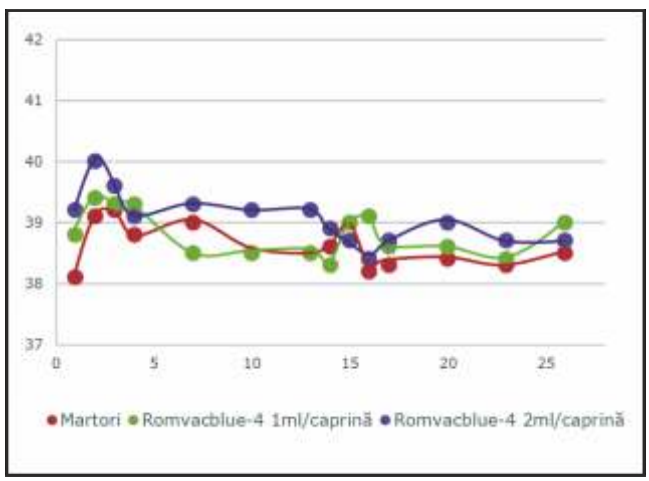

Fig. 10. Graphic representation of temperature monitoring for young goats vaccinated with Romvacblue-4 – Gruiu Farm

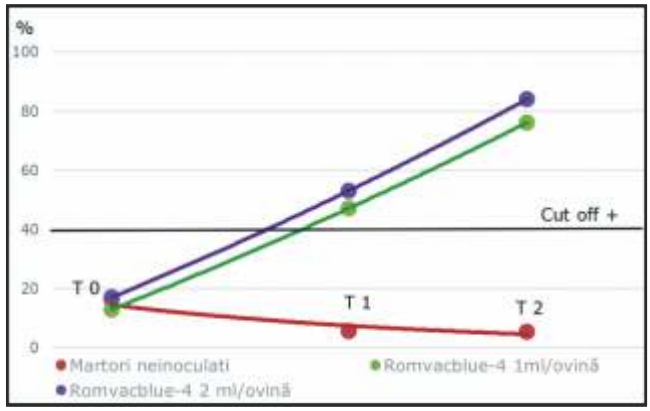

Fig. 11. Safe vaccination with Romvacblue-4 in young sheep – Gruiu Farm (T0 - immune response before vaccination, T1 - before booster, T2 - 2 weeks after booster)

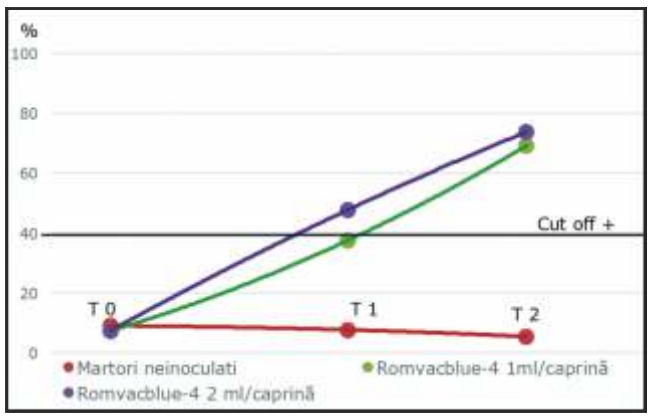

Fig. 12. Safe vaccination with Romvacblue-4 in young goats – Gruiu Farm (T0 - immune response before vaccination, T1 - before booster, T2 - 2 weeks after booster)

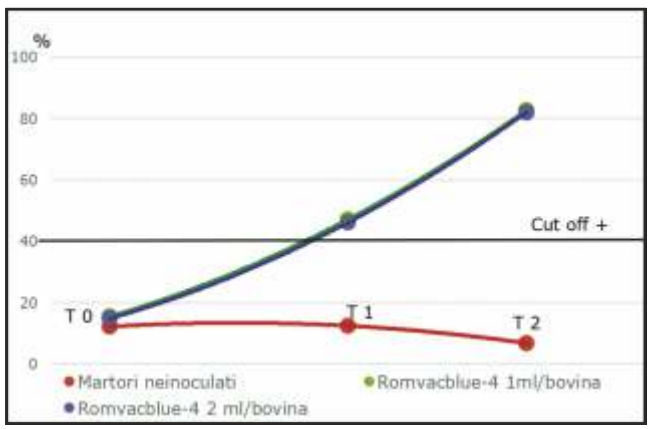

Fig. 13. Safe vaccination with Romvacblue-4 in young cattle – Gruiu Farm (T0 - immune response before vaccination, T1 - before booster, T2 - 2 weeks after booster)

The specific immune response was, of course, the expected one and each time identical with that in-

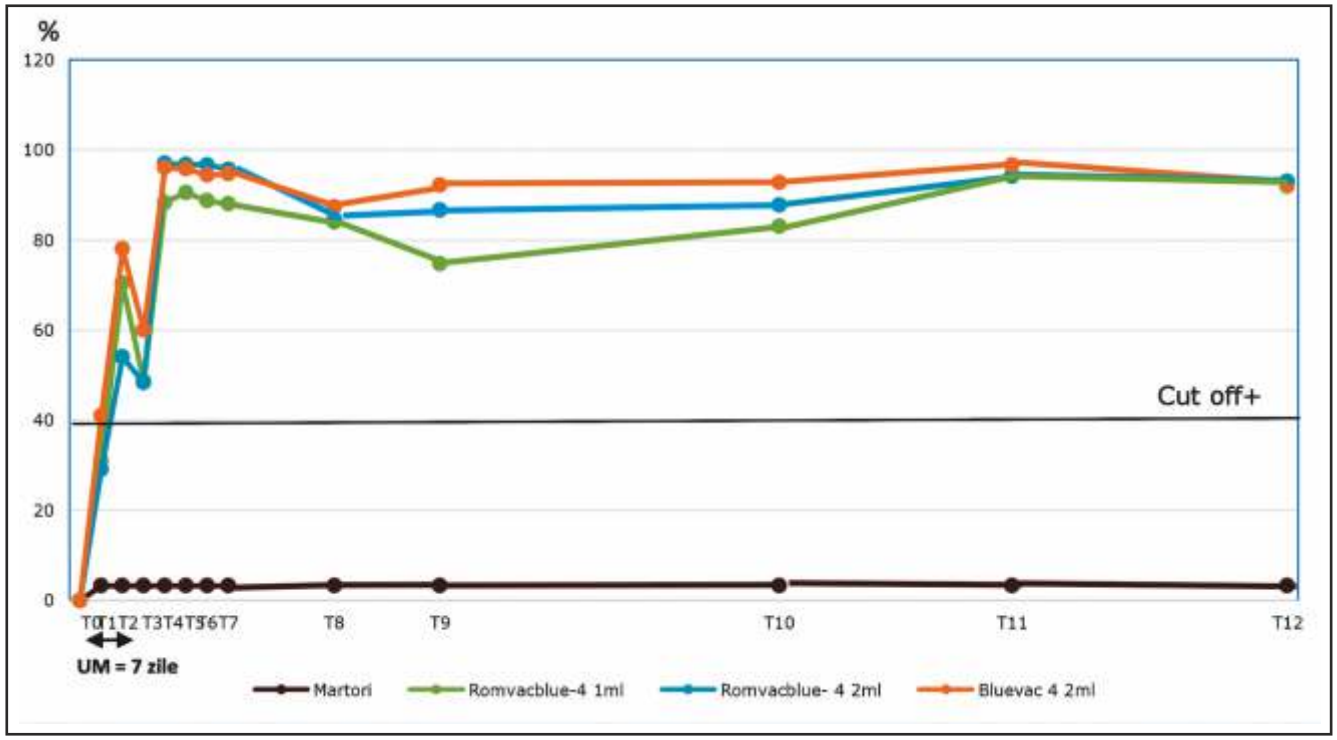

Fig. 14. Graphical representation of the Elisa - assayed immune response after vaccination with Romvacblue-4, compared with a commercial vaccine, Bluevac 4 (T0 – immune response before vaccination, T1 – 7 days after vaccination, T2 – 14 days after vaccination, T3 – on booster day, T4 – 7 days after booster, T5 – 14 days after booster, T6 – 21 days after booster, T7 - 1 month after booster, T8 – 2 months after booster, T9 – 3 months after booster, T10 – 7 months after booster, T11 – 1 year after vaccination, T12 – 1 year and 3 months after vaccination)

duced by the reference vaccine; dynamics increased by 25 - 30% - 7 days, 65 - 75% - 14 days, 80 - 90% - 7 days after booster and by more than 90% - 14 days after booster, staying like that up to 450 days, much over the borderline (40%) (Fig. 14).

CONCLUSIONS

Bluetongue virus serotype 4 isolated in Romania was taken and adapted on cell cultures determining the virus growth conditions, cell extraction, concentration of viral suspensions obtained and identification of virus by infectivity, seroneutralization and genetic assays. The master and working seeds were prepared, tested and stored at -80°C. The inactivation conditions were established (pH, temperature, time, beta-propiolactone and formaldehyde concentration), preparing the first micro batches of inactivated vaccine absorbed on aluminum hydroxide gel and mixed with saponin derivative (Vet-Sap) – Romvacblue-4.

The clinical trial results revealed that the vaccine was appropriate regarding safety and efficacy:

- during the observation period, none of the vaccinated animals showed local or general symptoms attributable to the vaccine;

- the specific immune response was the expected one – significantly positive, most often identical with the reference vaccine, sometimes even higher.

ACKNOWLEDGMENTS

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