Bioveta News

Information bulletin for veterinarians

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Bioveta, a. s.,

European leader in production of rabies vaccines, launches

RABADROP®

Milan HUŇADY, D.V.M.

VACCINE DEVELOPMENT MANAGER

RABADROP®

Bioveta, a.s. is currently launching a new vaccine for oral immunization against rabies - **RABADROP.** Development of this vaccine is based on many years of experience in development and production of LYSVULPEN vaccine.

In the Czech Republic, oral vaccination of foxes against rabies was performed for the first time in 1989 with the vaccine containing the SAD B19 strain. In 1991, Bioveta acquired the vaccine strain SAD Bern, prepared MSV for production and commenced production of the oral vaccine with this vaccine strain at the beginning of 1992. The autumn vaccination campaign in 1992 and all subsequent campaigns in the territory of the Czech Republic were performed only and exclusively with the vaccine against rabies under the trade name LYSVULPEN. The rabies has been eradicated from the entire territory of the Czech Republic, using this vaccine.

In the period from 1992 to 2017, more than 300 million doses of LYSVULPEN were produced and shipped to 19 countries in total (Belarus, Bosnia and Herzegovina, Bulgaria, Montenegro, Czech Republic, Georgia, Croatia, Kazakhstan, Kosovo, Lithuania, Latvia, Hungary, Moldova, Poland, Romania, Russia, Slovakia, Slovenia, Serbia). In these countries, this vaccine contributed significantly to eradication of rabies.

In connection with a favourable disease situation in Europe, requirements for the rabies virus vaccine strains used for oral vaccination of foxes and raccoon dogs have been changed recently, with the emphasis on a high degree of attenuation. This is why Bioveta, a. s. has prepared a new vaccine strain designated as **SAD Clone**.

Preparation of the new vaccine strain is based on the original SAD Bern vaccine strain. **By repeated passaging and cloning under various conditions of cultivation, the virus clone having altered properties and features has been obtained.**

Unlike the original SAD Bern strain, which retains a certain degree of residual pathogenicity after intracerebral administration in adult mice, the **new clone shows zero values in the so-called ICP test (intracerebral pathogenicity index). Genetic stability and presence of a genetic marker,** an important tool to distinguish the strain from other vaccination and field rabies virus strains, are other important features of the newly acquired strain.

The newly acquired rabies vaccine virus SAD Clone has been tested in laboratory studies on both the target and the non-target animal species. The obtained results of the safety and efficacy tests have demonstrated a high degree of safety while maintaining excellent immunogenic properties and features of the original strain.

Results of the tests created the basis for compilation of the documentation and for successful registration (marketing authorization) of the new vaccine for oral immunization of foxes and raccoon dogs against rabies named **RABADROP**.

Ms Lucie Střelcová testing the vaccine virus SAD Clone.

RABADROP®, currently the best vaccine against fox rabies

Milan Huňady, D.V.M.
Head of RABADROP Vaccine Development Team

How does the RABADROP vaccine strain differ from the LYSVULPEN one?

The SAD Clone vaccine virus contained in Rabadrop is absolutely apathogenic after intracerebral administration in adult mice, whilst the SAD Bern strain contained in Lysvulpen retains a certain degree of pathogenicity.

A relative genetic uniformity, compared to the "classic" vaccine strains (SAD Bern, SAD B19), showing a different degree of heterogeneity, is a specific feature of the SAD Clone virus.

Your observations, points of interest in the course of vaccine development?

The careful, meticulous work of the colleagues in virus cloning focused on selection of the clones with the gradually decreasing pathogenicity represented an important contribution to development of the new vaccine. Subsequent testing and selection of suitable candidates for further virus passaging is also worth mentioning.

Can you describe the main features of the production process? What has been innovated in it?

The new method of culturing the BHK-21 cell culture, which the vaccine virus is produced on, is the fundamental change. The suspension cultivation system in the bioreactors is used; it replaces the adherent method utilizing individual Roux bottles. The new system is more demanding on technical equipment, but at the same time enables significant increase in labour productivity.

How could it be identified that the animal has already been vaccinated?

The vaccinated animal has antibodies against rabies virus and it is also possible to detect presence of tetracycline in the bones or teeth of the animal.

Jiří Nezval, D.V.M.
Director of the Section of Production, Development and Product Innovation

What was the main impulse to develop a new vaccine?

The main impetus for development of a new vaccine was the competitive pressure, because LYSVULPEN was accused of residual pathogenicity after intracerebral administration to adult mice (in practice impossible to cause by oral vaccination), and some other, mostly false, information even from reputable workplaces dealing with rabies were wrongly attributed to it.

When we embarked on development of a more attenuated strain, originating from the rabies strain used in LYSVULPEN, **we have simultaneously increased convenience of use of the new vaccine by increasing thermal stability of the strain, and possibility of storing the new vaccine outside the freezer at 2-8°C for up to 90 days.**

Where will the RABADROP vaccine be used?

Wherever there is the need to control or eradicate rabies in wild animals. The fox and raccoon dog, as the dominant rabies vectors to other animals and humans, are protected against rabies after vaccination. This prevents subsequent transfer to domestic dogs, livestock and thus - indirectly through these domestic animals or directly by the infected fox – to the human. Approximately 60,000 people die of rabies in the world every year. This is an unimaginable amount for an European, but that is the reality.

The decentralized registration procedure of RABADROP has been completed recently in 14 countries of the European Union (BG, CZ, DE, EE, FI, GR, HR, HU, LT, LV, PL, RO, SI, SK), registration is in progress in Ukraine and will continue outside the European Union in the form of national registration procedures.

Will LYSVULPEN be sold even in the future?

I think so. Lysvulpen is very effective vaccine with great stimulation of the antibody response in the vaccinated foxes, raccoon dogs, but also other carnivores or omnivores who devour the bait with the vaccine. During the 30-year period of use, the vaccine has eliminated or rapidly reduced incidence of rabies in half of Europe. The Czech Republic has not reported a single case of rabies for over 10 years already.

How often should the aerial vaccine distribution be performed?

Basic vaccination has to be performed twice a year, in spring and autumn. This is usually sufficient and after several years of vaccination the vaccinated area will be healed. In addition to a high-quality vaccine, it is also necessary to ensure good organization of bait distribution and the whole organization of vaccination in general.

Is the RABADROP vaccine suitable for street dogs?

We do not have this information in SPC, because registration for dogs automatically excludes the vaccine from the MUMS type of registration (a simpler form of registration for minor animal species or minor scope of use), which means that we would have to prove – besides the tests of onset and duration of immunity – even the so called field trials of use of the vaccine in the street dogs in towns and villages. From the organizational point of view, this is impossible to perform in Europe. However, we have verified by the laboratory studies that the vaccine is well-tolerated by the laboratory dogs, is safe and produces a high antibody response (higher than 0.5 IU) sufficient for long-term protection.

A NEW CLONE OF VIRUS and its GENETIC STABILITY and UNIFORMITY guarantee high efficacy and safety of the oral rabies vaccine

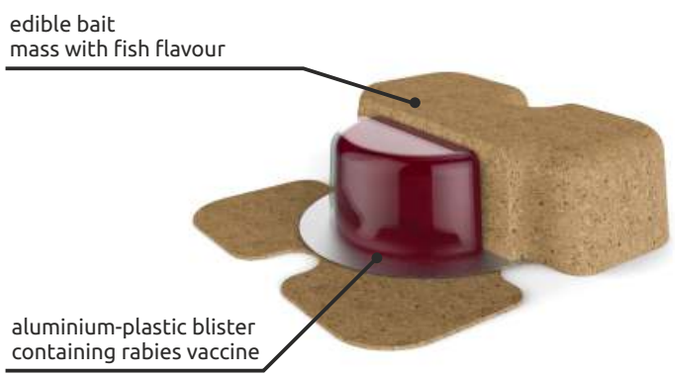
RABADROP®

*Name of this new vaccine is combination of the letters **RA** (rabies) -**BA** (bait) -**DROP** (rabies-bait-drop). Thus, **RABADROP** is the rabies vaccine for oral immunization of foxes and raccoon dogs which contains live, highly attenuated rabies virus in a stabilizing medium. The mixture of virus and stabilizing medium is filled in sealed blisters which are wrapped with bait mass. The resulting bait is distributed to the target area primarily by airplanes.*

The bait mass is attractive for the target species, and tetracycline is used as the indicator of vaccine uptake. One vaccine dose (one vaccine bait) contains 1.8×10^{6} to $1.8 \times 10^{8.5}$ $TCID_{50}$ of the vaccine virus. The target animals develop an immune response after the vaccine virus-containing baits are consumed. Once the animal bites the vaccine blister through, the vaccine virus comes into contact with the immunocompetent organs in the oral cavity and the nasopharynx. Specific antibodies against rabies are produced in the target animals and a protective immunity status against rabies virus develops.

EFFICACY

Laboratory studies of **RABADROP** vaccine efficacy have been designed to meet requirements of the Directive 2001/82/EC as amended by the Directive 2009/9/EC and requirements of the European Pharmacopoeia monograph 5.2.7. - Evaluation of efficacy of veterinary vaccines and immunosera, as well as requirements of the specific European Pharmacopoeia monograph 0746. The challenge viruses used in the efficacy studies are the field rabies virus isolates that have been isolated from a clinically ill fox in the Central Europe in the period prior to rabies eradication in the region. In a first step, a study was performed to determine the minimum protective virus dose in foxes. In the efficacy studies, the batch of vaccine containing the minimum virus titre per dose was used. Onset of immunity was determined by serological examination in the course of the immunity duration study. The challenge test confirmed that the antibody level ≥ 0.5 IU/ml protects against the rabies virus infection. The antibody level, considered protective in all vaccinated animals, was observed from days 10 to 365 after vaccination. All vaccinated animals were protected against clinical signs of the disease and mortality caused by the rabies virus for the time period of 12 months following vaccination.



SAFETY

All laboratory studies of RABADROP vaccine safety were performed in accordance with OECD Guidelines for Good Laboratory Practice, the General European Pharmacopoeia Safety Monograph 5.2.6, VICH 44 Guideline and the European Pharmacopoeia Monograph 0746. Bioveta, a.s. is the holder of the Good Laboratory Practice Certificate (GLP).

WITHIN THE SCOPE OF THE SAFETY TESTS 17 STUDIES WERE PERFORMED IN TOTAL

- administration of one vaccine dose to the target species (fox)
- administration of one vaccine dose to the target species (raccoon dog)
- repeated administration of one vaccine dose to the target species (fox)
- repeated administration of one vaccine dose to the target species (raccoon dog)
- administration of overdose (10-fold dose) to target animals (fox)
- administration of overdose (10-fold dose) to target animals (raccoon dog)
- administration of overdose in a dog
- administration of overdose in a cat
- administration of overdose in calves
- administration of overdose in wild rodents; in the study, the vaccine was administered orally to 10 rodent species *(Apodemus flavicollis, Apodemus sylvaticus, Mus musculus, Apodemus agrarius, Clethrionomys glareolus, Microtus arvalis, Micromys minutus, Apodemus uralensis, Cricetus cricetus, Rattus norvegicus)*
- administration of overdose in laboratory rodents (in this study, the vaccine was administered to laboratory mice by peroral, intramuscular and intracerebral route)
- stability study of the genetic marker
- spreading of vaccine strain from vaccinated to unvaccinated animals (5 studies: ● fox, ● raccoon dog, ● dog, ● wild rodents, ● laboratory rodents)

THE FOLLOWING HAS BEEN PROVEN WITHIN THE SCOPE OF THE SAFETY STUDIES

- **safety of the RABADROP vaccine for target animal species was confirmed after a single and repeated administration, as well as after administration of the 10-fold dose;**
- **safety was confirmed for the selected non-target animal species;**
- **stability of genetic marker of the vaccine virus was confirmed during 5 passages on the tissue cultures and in the brains of the sucking mice;**
- **vaccine virus spread from vaccinated to unvaccinated animals was not detected**

GENETIC UNIFORMITY

It has been established, based on development of new molecular genetic methods, that the attenuated vaccine strains of oral rabies vaccines used so far are not genetically uniform, but consist of more or less heterogeneous virus populations. The virus strain used in the RABADROP vaccine is derived from the original SAD Bern strain. By repeated passaging and cloning on tissue cultures, a higher degree of virus attenuation has been achieved while maintaining excellent immunogenic features. One of the important results of development of the new vaccine strain SAD Clone is, in addition to a higher degree of attenuation, also a relatively genetically uniform virus population. The virus has a stable genetic marker that permits to distinguish it from other rabies vaccine or field viruses. Stability of this genetic marker has been confirmed during 5 passages on tissue cultures and in the brains of the sucking mice.

MAIN ADVANTAGES OF THE NEW RABADROP VACCINE

SAD Clone – genetically uniform and stable strain

Zero pathogenicity to adult mouse after i. c. administration

New type of the bait mass

- higher impact resistance
- higher temperature stability

Vaccine storage

- can be stored for 90 days within the temperature range from +2°C to +8°C
- longer storage at the temperature of –20°C and lower

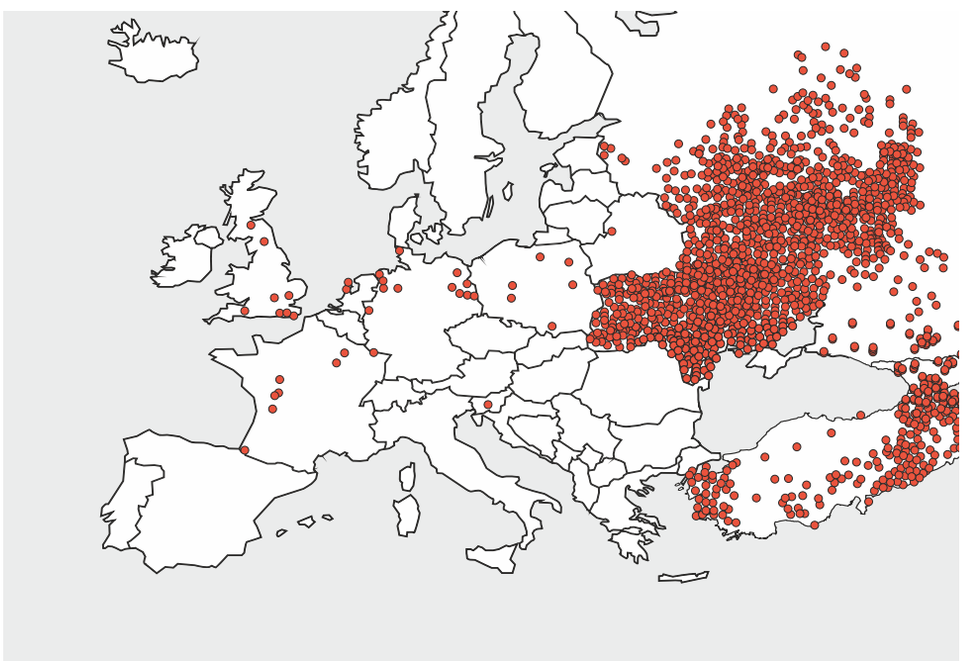
Rabies, a permanent global risk

In the Czech Republic, the last case of rabies was recorded in a wild fox in April 2002. In domestic animals, rabies was last detected in a cat in February 2001. The infection has practically been eradicated in the Czech Republic mainly thanks to the programme of oral vaccination of foxes against rabies realized in the period 1989 - 2009, in which Bioveta has been involved actively since 1992 with its unique LYSVULPEN product for oral vaccination of wild animals. Thanks to the favourable disease situation, the Czech Republic has been awarded the status of the rabies free country by the World Organization for Animal Health (OIE) in 2004. This situation still persists.

28th September World Rabies Day

On 28th September this year, same as in previous years, the "World Rabies Day" is commemorated. Rabies is no more an actual threat in the Czech Republic and many other European countries, but the possibility of introducing rabies in our territory is still real.

However, caution persists, vaccination of domestic animals, especially dogs, continues. All captive dogs, foxes and badgers must be vaccinated against rabies at the age of 3 to 6 months and revaccinated afterwards. The obligation to bring the animal which has injured a human to the veterinarian for examination on the day 1 and 5 after the injury is still valid. About 4,000 animals which injured humans are examined on average every year. However, not a single animal was found to be infected with rabies. In 2015, a rare case of rabies in a bat, found in Prague Rieger Gardens, was diagnosed. However, rabies of bats is considered a specific variant of the disease and therefore its occurrence has not affected the status of the rabies free state anyhow. Nevertheless, rabies still exists in some European countries. Infection occurs even in the countries not too far away from the Czech Republic. In Africa and Asia, in particular, tens of thousands of people die every year from rabies, most frequently children bitten by an infected dog. The European Commission has recently introduced its target to eradicate rabies throughout the European Union by 2030. Bioveta has been largely involved in this goal with its traditional LYSVULPEN product and now with the new RABADROP product, i.e. vaccines for oral vaccination of wildlife.

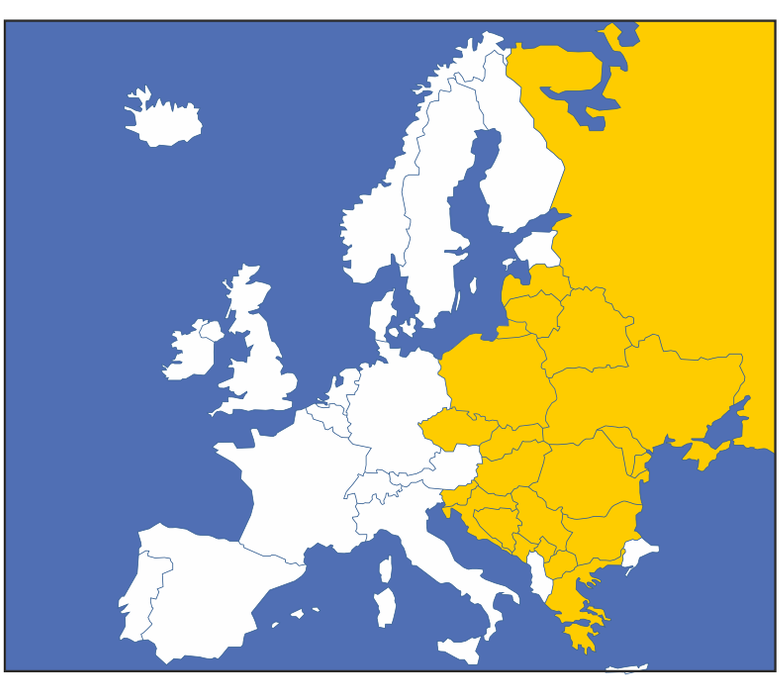


RABADROP® is continuation of the LYSVULPEN vaccine's very successful story

Development of the RABADROP vaccine, currently the best fox rabies vaccine, is based on the LYSVULPEN vaccine. During its 27 years on market, it has become the most successful product of the Bioveta company's 100-year history. It is possible to claim without exaggeration that the Lysvulpen vaccine has contributed materially to eradication of rabies in wild foxes in the Central and Eastern Europe.

During its existence, it has been used as a major component in the fight against rabies of foxes and raccoon dogs in 21 countries globally. The total amount of distributed doses exceeds 358 million. The countries that use the LYSVULPEN vaccine with a great success include Poland, Hungary, the former Yugoslavia, Greece and even the exotic Kazakhstan. Many of these countries (Bulgaria, Greece, Croatia) report zero occurrence of rabies in foxes, even if these countries (e.g. Latvia or Slovakia) are closely adjacent to the endemic areas. We must not forget the status of the "rabies free country" awarded by the World Organization for Animal Health, which the Czech Republic achieved in 2004 as the first Eastern European country. A large share of this primacy is undoubtedly attributable to the LYSVULPEN vaccine.

In the course of its existence, LYSVULPEN has been tested countless times whether it will stand up not only to the strict requirements of the European Union or the World Health Organization, but also to various geographical conditions. The bait melting point, virus stability at elevated temperature or other efficacy parameters belong among the routine tests that Lysvulpen undergoes day in, day out. Safety of the vaccine has been verified and checked in more than 40 animal species, including monkeys and seagulls. LYSVULPEN has successfully passed all these tests and has become a reliable tool in the fight against rabies in many European countries.



Fox and racoon dog were always the primary target species for LYSVULPEN. As rabies also affects other wildlife species, efficacy of Lysvulpen was gradually verified in wolves, jackals, and stray dogs. Use of the vaccine for oral vaccination of stray dogs against rabies is of great interest to the countries of the Southeast Asia and Northern Africa, where the packs of these dogs endanger the human population and especially children.

The current eradication programmes against rabies explicitly prefer use of the aerial distribution over the manual distribution. LYSVULPEN has been innovated several times during its life cycle to meet the demanding criteria for aerial distribution. Innovation of the bait had to ensure not only its strength to drop from the height of over hundred meters, but also its compatibility with the automatic distribution device for dropping the bait from the aircraft deck. The aerial distribution itself utilizes satellite guidance and software that allows online analysis of bait distribution as well as control of the distribution pattern.

All these achievements are reliably followed by the RABADROP vaccine of the new generation, and so there is nothing left but to quote words of John Davison Rockefeller: "Do not be afraid to give up the good to go for the great".

European countries using the LYSVULPEN vaccine to combat rabies

A NEW VACCINE AGAINST RABIES

RABADROP®

it does not need to be transported and stored at the temperature of –20°C, but within the temperature range from +2 to +8°C (for up to 90 days), which brings significant savings and user comfort for the customer, and at the same time the vaccine is less energy consuming and less expensive to store and distribute

ENVIRONMENTALLY FRIENDLY PRODUCT

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