Development of vaccines against peste des petits ruminants: CIRAD's achievements and future challenges

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Summary
CIRAD, France, in collaboration with the Pirbright Institute, United Kingdom, was the first laboratory to develop a live attenuated vaccine against peste des petits ruminants (PPR). This vaccine available in the 1980s was shown to provide a life-long immunity after a single injection [5] and is now widely used in sheep and goats. New vaccines are in development, more specifically thermotolerant vaccines for use in tropical conditions and DIVA (differentiating infected from vaccinated animals) vaccines to gain virus-free status more easily and rapidly. This paper reviews most of the current and recently developed vaccines at CIRAD and emphasises their adequate delivery and utilisation in the field, for successfully decreasing the incidence of PPR.

Keywords
CIRAD – peste des petits ruminants (PPR) – DIVA vaccine – thermotolerant vaccine.

Introduction

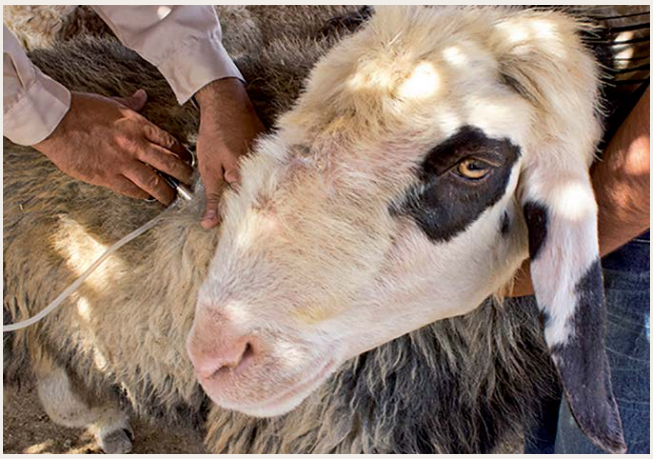
Peste des petits ruminants (PPR) constitutes one of the major hurdles to the improvement of small-ruminant production directly affecting animal keepers. Its huge impact on small-ruminant production has led to the development of a global control and eradication strategy [8]. Vaccination is the key, acknowledged tool used to control and eradicate PPR. CIRAD has been greatly involved in the development of vaccines since the 1980s as exemplified by the achievement of the first PPR vaccine by attenuation of the Nigeria 75-1 isolate [7]. As with all members of the *Paramyxoviridae* family, PPRV is heat-sensitive requiring an effective cold chain in hot climates. The drawback of this thermosensitivity has been partly overcome by the use of stable freeze-drying formulations resulting in enhanced stability during manufacturing, shipping and storage [20] or the use of thermostable capripox vaccine backbone for inserting PPR transgenes by homologous recombination to prepare multivalent and thermotolerant vaccines [2, 3, 6]. Furthermore, CIRAD team is undertaking major efforts towards developing new generation vaccines against PPR that lead to DIVA and antiviral strategies expected for successful PPR control and eradication.

Conventional attenuated PPRV vaccines

Effective live attenuated PPR virus vaccines are now widely available. One of the most currently produced vaccines is a live attenuated strain obtained at CIRAD in collaboration with the Pirbright Institute. The wild PPRV strain from which the vaccine is derived was isolated in Nigeria in 1975 [22] and attenuation obtained after 74 successive passages on Vero cell culture [7]. Soon after, field trials on nearly 100,000 animals were implemented by CIRAD, to demonstrate the efficacy of this vaccine which is also devoid of residual side effects such as abortion in pregnant animals. The vaccine confers clinical protection against all PPRV lineages and also prevents transmission of the challenge virus to in-contact animals. Protective antibodies generated after a single injection persist for at least three years, which is most of the time the economic life of a small ruminant. These results demonstrated the potential of this vaccine if used globally for the eradication of PPR. Several vaccine manufacturing facilities worldwide (Table I) received the seed strain directly from CIRAD or through AU-PANVAC based in Debre Zeit, Ethiopia, in the case of African laboratories. All now have the capacity to produce and deliver millions of PPR vaccine doses. Strict quality controls on the seed stock delivered by CIRAD are undertaken on a regular basis, including the certification of absence of pestivirus and mycoplasma contamination. In addition, by massive parallel sequencing using next generation sequencing technology, the seed of this vaccine was recently shown to be free of adventitious pathogens. To ensure the quality of these veterinary vaccines for safe use during vaccination campaigns in Africa, AU-PANVAC has an outstanding role, not

Table I
List of manufacturing facilities producing the peste des petits ruminants vaccine strain Nigeria 75-1

Country	Laboratory	City
Botswana	Botswana Vaccine Institute	Gaborone
Cameroon	Laboratoire National Vétérinaire (LANAVET)	Garoua
Chad	Institut de Recherches en Élevage pour le Développement (IRED)	Farcha N'Djamena
China (People's Rep. of)	Tiankang Biopharmacuetical	Urumqi
Egypt	Veterinary Serum and Vaccine Research Institute	Cairo
Ethiopia	National Veterinary Institute	Debre Zeit
Iran	Razi Institute	Karaj
Iraq	Al-Kindi Co.	Baghdad
Israel	Abic	Beit Shemesh
Jordan	Bio-Industries Center (JOVAC)	Amman
Kenya	Kenya Veterinary Vaccines Production Institute (KEVEVAPI)	Nairobi
Mali	Laboratoire Central Vétérinaire	Bamako
Morocco	Biopharma	Rabat
Nepal	Biological Products Division, Veterinary Complex	Tripureshwor, Kathmandu
Niger	Direction des Laboratoires Vétérinaires (LABOCEL)	Niamey
Nigeria	National Veterinary Research Institute	Vom, Plateau State
Pakistan	Intervac (PVT) Ltd.	Lahore
Saudi Arabia	Veterinary Vaccine Production Centre	Riyadh
Senegal	Laboratoire National de l'Elevage et de Recherches Vétérinaires (LNERV), Institut Sénégalais de Recherches Agricoles (ISRA)	Dakar Hann
South Africa	Onderstepoort Biological Products Ltd.	Onderstepoort
Sudan	Central Veterinary Research Laboratory	Soba Kartoum
Turkey	Central Veterinary Control and Research	Etlik Ankara
Turkey	Vetal Veterinary Vaccines	Adiyaman
Turkey	Dollvet	Cadde Merkez Sanliurfa



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only on the quality assurance of PPR vaccines, but also in strengthening Africa's capacity-building in veterinary vaccine development production. In addition to the PPR vaccine strain Nigeria 75-1, other live attenuated strains, such as Sungri 96, more recently developed by other laboratories – particularly in India (e.g. MSD Animal Health [Merck], Indian Immunologicals Ltd., Hester Biosciences Ltd.) –, Arasur 87 or Coimbatore 97 vaccines, successfully passed potency tests in sheep and goats according to the OIE guidelines [17].

Vaccines acceptable for a DIVA strategy

PPRV expresses two glycoproteins on its outer envelope, the haemagglutinin (H) and the fusion (F) proteins which are vital for cell attachment and penetration. These two proteins are also key antigens for inducing protective host immune response. The nucleoprotein (N) while unable to confer protective immunity, is a strong and early inducer of serum antibodies, and thus may serve as a negative mark in DIVA vaccines when combined with the serological diagnostic tests targeting this protein [13]. For better protection, a DIVA vaccine should be designed with at least H or F proteins. Such a DIVA vaccine should be linked to companion diagnostic tests capable of detecting both the marker and the specific glycoproteins to attest to induced immune response and therefore the guarantee of DIVA vaccination. DIVA vaccines and companion diagnostic tests would be ideal tools for emergency vaccination in case of the occurrence of an outbreak in a PPR-free country or zone and in the eradication phase of the disease since it would accelerate the process.

Different strategies are on-going for the development of DIVA vaccines, but they can be summarised as follows: the modification of the current attenuated vaccine by reverse genetics or the production of recombinant viruses expressing the H and/or F proteins.

Using reverse genetics technology, the PPR vaccine strain Nigeria 75-1 was modified to express marker proteins in order to differentiate vaccinated from infected animals, whilst maintaining characteristics of the original vaccine. For the moment the ideal marker for a commercial vaccine is not yet available. However, recent prototype vaccines have been developed bearing a fluorescent protein [10, 15]. The advantage of the reverse genetics strategy lies in process scale-up for steep rise in the production of the resulting DIVA vaccine since it is not expected to require a different manufacturing scheme than the parental PPR vaccine.

In the second strategy, most studies have dealt with recombinant PPR-capripoxvirus vaccines using the KS1 strain [2, 6]. KS1 recombinant vaccines expressing either H- or F-PPRV demonstrated protective efficacy when administered in a single subcutaneous shot in previously unexposed animals. Doses as low as 0.1 Particle Forming Units (KS1 expressing F-PPRV) proved to protect goats against challenges involving both virulent PPRV and capripoxvirus strains. More recently, a study demonstrated full protection against capripox challenge of animals with prior exposure to PPRV secondarily vaccinated with a recombinant KS1-PPR recombinant vaccine, demonstrating that pre-immunity against PPRV does not interfere with the immunogenicity of a recombinant PPR-capripoxvirus vaccine [3]. The same study showed partial protection against PPRV in animals with prior exposure to capripox virus secondarily vaccinated with a KS1-PPR recombinant vaccine. Researches are ongoing in order to develop new generation recombinants, integrating the DIVA strategy with an improved immunogenicity, safety and an increased efficacy in pre-immune animals.

Combined vaccines for multiple disease protection

CIRAD pioneered the use of combined vaccines against the viruses causing sheep pox, goat pox and PPR, the most obvious and simplest way of achieving protection against these diseases and particularly economically justifiable. Vaccines used in combination have been evaluated in sheep and goats [14] and proved to be safe and immunogenic as demonstrated by others [4, 9]. In addition, the vaccine strain was unable to spread from vaccinated animals to in-contact animals [7]. Different recombinant vaccines expressing a unique valence can be delivered in combination to confer protection against several different diseases. This implies that the vaccines, at least in small-scale experiments, do not interfere with the immunogenicity of the other. Broader studies performed in field conditions are now awaited to

confirm these results. The newly launched PPR eradication campaign is a great opportunity to target multiple diseases at the same time as previously undertaken, for example during the Pan-African Rinderpest Campaign by combining vaccines against rinderpest and contagious bovine pleuropneumonia (CBPP). Regional specificities will define which diseases to tackle to maximise the impact of vaccination and applied laboratory and field studies should be undertaken to verify whether the sheep or goats could be inoculated with monovalent vaccines against two or three diseases at the same time or whether bi- or trivalent vaccines using conventional vaccine strains (such as the bivalent rinderpest-CBPP vaccine for cattle) could be developed. Alternative strategies are currently being developed to generate a recombinant capripoxvirus vaccine with multiple valences (e.g. PPR and Rift Valley fever).

Thermotolerant vaccines

Given the enormous challenges associated with the distribution of PPR vaccine for the global eradication of PPR that are faced by the majority of developing countries, the key issue is the thermal stability of the vaccines. The thermostable capripox-based vaccines may offer significant contribution to this objective. The main focus of conventional attenuated PPRV strains is to improve stability by extending the virus half-life at high temperatures compatible with tropical conditions in order to maintain a viral titre higher than the minimal requested vaccine dose. Improvements have been made in terms of lyophilisation formulations. Collaborative work between CIRAD and Instituto de Biologia Experimental e Tecnológica (IBET, Portugal), under the framework of a project carried out at National Veterinary Institute (NVI) in Ethiopia clearly demonstrated the ability to produce higher quality PPR vaccines by replacing Weybridge medium by a Tris/Trehalose formulation [21]. Lyophilised vaccine half-life at 37 and 45 °C could be extended by up to 2.5 and 2 days, respectively. These results corresponded to previously published results [20] and demonstrated that improved formulation procedure could be rapidly and successfully transferred to a vaccine-producing laboratory.

Vaccination strategy and vaccine delivery

Mass vaccination of all animals in the target population is the only efficient vaccination strategy for PPR eradication. The target immunity threshold of 80% of small ruminants is generally considered appropriate in vaccination programmes to stop the spread of disease. But some field and experimental work has shown that it could be lower. More research should be carried out to achieve a better estimate of this threshold while allowing for the complex relationships between small-ruminant population dynamics, post-vaccination immunity coverage, and PPRV transmission (FAO/OIE). With this in mind, partial vaccination of the population, for example targeting small middle-aged stock [12] may reduce the virus load in circulation but comes with two major drawbacks: first, PPRV will continue circulating in the general population; second, the wild PPRV will have more opportunities to adapt and produce escape mutants. A similar situation probably occurred in the past with rinderpest virus, causing the emergence of so-called RPV lineage II and complicating the eradication process. Vaccination campaigns must also be coordinated at national and regional levels to ensure good vaccination coverage of all small ruminant populations. Practical implementation of vaccination campaigns is of great importance. This includes the acquisition of vaccine submitted to rigorous quality control, maintaining the cold chain (at least until a thermotolerant vaccine is made widely available), adequate means for the vaccination teams, as well as careful preparation of the campaigns. Successful mass vaccination in developing countries is a very challenging objective and calls for effective Veterinary Services, which involve public and private veterinarians. The effectiveness of delivery systems for animal health services particularly for vaccination is crucial and in this regard the participation of private veterinary para-professionals and representatives of producers' and farmers' communities (animal health workers) can be very effective in reaching small ruminants in difficult areas (e.g. remote or insecure areas) or when the animal population density is very low such as in smallholder village production systems in crop-based humid zones. This partnership requires effective implementation of appropriate legislation and veterinary supervision.

Treatment against PPR

Preventive measures are the main strategies for reducing the impact of PPRV in the world. Today, despite constantly improving vaccine strategies, vaccination of small ruminants against the disease remains problematic and in practice, is not applied continually during inter-epidemic waves. In addition, given the necessary delay of several days to mount an efficient immune response to induce sufficient

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protection, vaccine cannot be used to stop an outbreak immediately. Effective and rapid control of PPR is foreseeable using cheap antiviral compounds. Antivirals based on synthetic short interfering RNAs (siRNAs), a new class of molecules with a significant potential for therapeutic applications could be good candidates since they can be delivered in viral vectors and biologically synthesised in the treated animals. A breakthrough has occurred in the last decade for the design options of siRNA to prevent the *in vitro* selective degradation of viral RNA. Although siRNA as potential emergency tools against PPR are not yet available, efficient *in vitro* activity making for more than 90% inhibition of PPRV replication was reached when targeting the N gene [11, 18, 19]. Excellent knock-down of PPRV replication brings us closer to a therapeutic application for the future. Such antiviral therapeutics could complement vaccination to limit the clinical impact of PPR quickly in emergency situations in the context of new introduction into free zones or of re-emergence in endemic areas. New therapies to treat infection may therefore be of particular interest.

Conclusion and outlook

PPR control is a priority for poverty alleviation. Because of the fast turnover of small ruminant populations, and insufficient investment or political commitment for improving their health and productivity, PPR control will not be an easy task. Post-vaccination evaluation of PPR clinical incidence and immune status of target populations will be needed to monitor the progress of PPR control. The development of marker vaccines will certainly facilitate the final control phase by preserving the ability to monitor the epidemiological situation during vaccination campaigns while contributing to secure international trade in animals and animal products. Full and reliable genome sequence knowledge of the PPR vaccine strain Nigeria 75-1 is the basis for the development of a DIVA vaccine. A double mark strategy, both recognised by small ruminants is ongoing at CIRAD to allow differentiation of vaccinated from infected and from vaccinated/infected animals. Multivalent capripox recombinant vaccines used differentially to protect ruminants against two or more diseases, depending on the local situation, is a strategy currently underway. In addition to the available PPR-capripox vaccine, recombinant capripoxvirus vaccines targeting bluetongue and Rift Valley fever have proved their efficacy [1, 16], a stepping stone toward second generation of tri- or quadri-valent recombinants. Ongoing trials with PPR-capripox vaccine will determine the duration of immunity in field conditions. Following the example of the work carried on the vaccinia virus, improvement of the capripox backbone is foreseen by subtracting neutralising epitopes and expanding the number of antigens expressed to comply with DIVA strategy and enhanced immunogenicity. Antiviral therapy still has an economically unbearable cost, but some cheap biological molecules could have an economic interest combined with vaccination in eradicating the disease.

Such innovative concepts and developments have been achieved thanks to the continuing collaboration with Southern partners, notably African countries. This collaborative work for science-based and integrated control programmes against PPR will be supported by strengthening the capacity of the team working on that subject with, for example, the recruitment of technicians and experts, notably in virology and in epidemiology-modelling as well as the development of the collaborative projects with the socio-economists working on animal health at CIRAD and in partners' institutions.

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