CONJUNCTIVAL REV 1 VACCINATION OF ADULT SHEEP AND GOATS IN TRÁS-OS-MONTES, PORTUGAL *

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SUMMARY : *Rev 1 vaccination is considered indispensable for small ruminant's brucellosis control in areas with high disease prevalence, especially when production systems are extensive and farms have a low technical profile. Vaccination of young replacement animals, with subcutaneous full dose, was applied in Portugal since 1972 and extended in the eighties. The decrease in brucellosis prevalence in 1990-1992, the implementation of the eradication programme co-financed by the EU in 1991, and the lack of human resources led to the progressive and early abandon of vaccination in the country.*

As a result, in certain regions like Trás-os-Montes, a mountainous area in the interior-north of Portugal, brucellosis prevalence started to sharply increase from 1997, resulting in heavy losses for the farmers, and a high cost for the Government. In 2000, 40% of slaughtered animals at national level came from this region. Flock prevalence reached 43.0% and animal prevalence 8.9%. Under these circumstances a mass vaccination campaign appeared as the best option for the disease control, following extensive discussions among interested parties.

Mass vaccination started in February 2001. In the first year of activity 67% of population was covered. The main problems were the occurrence of abortion in goat flocks were pregnant females were vaccinated and the restrain of animal movement that was not well accepted by farmers.

A follow-up of a sample of flocks to access vaccination efficacy at days 30, 120, 240 and 365 after vaccination was undertaken with good results.

The spirit of dialog and confidence that was developed between farmers and the veterinary services created a good environment to keep the vaccination pressure, with the vaccination of all replacements, and the continuity of the programme until a better epidemiological situation is achieved.

RÉSUMÉ : *La vaccination au Rev 1 est considérée indispensable pour le contrôle de la brucellose des petits ruminants dans des secteurs à haute fréquence de maladie, particulièrement dans les systèmes de production extensive et les fermes à faible capacité technique. La vaccination des jeunes animaux de remplacement, avec une dose sous-cutanée a été appliquée au Portugal depuis 1972 et prolongée dans les années quatre-vingts. La diminution de la prévalence de la brucellose en 1990-1992, la mise en oeuvre du programme d'éradication co-financé par l'UE en 1991 et le manque de ressources humaines, ont mené à l'abandon progressif de la vaccination dans le pays. En conséquence, dans certaines régions comme Trás-os-Montes, un secteur montagneux au nord du Portugal, la fréquence de la brucellose a commencé à augmenter brusquement à partir de 1997, aboutissant à de lourdes pertes pour les fermiers et à un coût élevé pour le Gouvernement. En 2000, 40% des animaux abattus au niveau national venaient de cette région. La prévalence des troupeaux infectés a atteint 43,0% et la prévalence des animaux infectés 8,9%.*

Dans ces circonstances, une campagne de vaccination massive est apparue comme la meilleure option pour le contrôle de la maladie, après discussion entre les parties intéressées. La vaccination massive a commencé en février 2001. La première année, 67% de la population a été vaccinée.

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Les problèmes principaux étaient la présence d'avortements dans des troupeaux de chèvres vaccinées et la restriction des mouvements des animaux qui n'a pas été bien acceptée par les éleveurs. Un suivi d'un échantillon de troupeaux pour évaluer l'efficacité de la vaccination aux jours 30, 120, 240 et 365 après la vaccination a été entrepris avec de bons résultats. L'esprit de dialogue et la confiance qui a été développée entre les éleveurs et les services vétérinaires ont créé un bon environnement pour maintenir la pression de vaccination, avec vaccination de tous les animaux de remplacement et poursuite du programme jusqu'à ce qu'une meilleure situation épidémiologique soit atteinte.

I - INTRODUCTION

Vaccination is considered a powerful tool for small ruminant brucellosis control, and is recommended by the WHO as a measure to prevent the disease dissemination (by reducing the excretion of microorganisms from the infected animals, the contamination of the environment and the rates of infection of exposed animals) and to reduce human brucellosis.

The main aspects to consider for the implementation of a vaccination campaign, in order to properly immunise the animals while reducing interference with the diagnosis of infection, are related to the vaccine to be used, the animal classes to be vaccinated, the appropriate dose and the application route.

The production of vaccines against *B.melitensis* was attempted by the Mediterranean Fever Commission, in Malta in the fifties and also by researchers in France, Japan, China and the United States of America [Alton, 1990a]. Several vaccines were developed and used like the *B.melitensis* Rev 1, the *B.melitensis* H38, the *B.suis* S2, and also the *B.abortus* S19. The Rev 1 vaccine was considered the most effective for the prevention of animal brucellosis [WHO, 1998] and is also approved by the OIE. This vaccine was developed in 1957, by Elberg and Faunce in California, from a live, attenuated and non streptomycin-dependent strain of *B.melitensis*, produced in 1955 by Herzberg and Elberg [Alton and Elberg, 1967]. Several studies and the extensive field use of Rev 1 vaccine confirmed its good immunising behaviour against *B.melitensis* [Alton and Elberg, 1967; Elberg, 1981; Elberg, 1996; Blasco, 1997].
The efficacy of this vaccinal strain is based on its relatively high persistence in vaccinated animals. When applied by the standard method ($1x10^9$ cfu, subcutaneously) the vaccine induces an intensive serological response that frequently interfere with the classical serological tests used in the infection diagnosis. Rev 1, therefore, retains some virulence and can induce abortion in pregnant females. The microorganisms may be excreted from the vagina and in the milk [Alton, 1990b].

These problems led to the preferential selection of young animal's vaccination, between 3 to 6 months of age, as the target of vaccination, especially in campaigns combining test and slaughter with vaccination. The serological testing of these young animals can be implemented 1 year after subcutaneous vaccination [Alton, 1990b], with only a small proportion remaining positive to serology [Lore *et al.*, 1973; Pappous and Hontou, 1988]. In infected flocks, the isolation of young vaccinated females, until the immunity is established, is advised to increase the efficacy of the vaccine [Elberg, 1981].

The disadvantage of the strategy of vaccinating only young replacements is that it is difficult and expensive to find enough kids and lambs at the right age for vaccination to provide effective cover, especially where there is an extended lambing season [Alton, 1990b]. Furthermore there is no sufficient prove that the immunity is lifelong, as referred by some authors, and population protection obviously depends on the average age of culling of vaccinated animals [WHO, 1998]. Vaccination of all animals is therefore recommendable in many circumstances, to obtain a good protection of the population and to rapidly reduce prevalence [Alton, 1987; Garin-Bastuji, 1995; Blasco, 1997; WHO, 1998].

To minimise the inconvenients of adult animals vaccination explained above, especially the induction of abortion and the persistence of antibodies, the use of reduced doses of vaccine was tested, inclusively in Portugal [Salles Henriques *et al.*, 1992], but has not

received widespread acceptance [Blasco, 1997; WHO, 1998]. For the same purpose, alternative routes of application were also tested and it is now generally accepted that the conjunctival route is better than the subcutaneous route, for both young and adult vaccination, inducing a good immunity, minimising the long-term persistence of vaccinal antibodies and reducing the allergic response [Fensterbank *et al.*, 1985; Blasco, 1997; Garin-Bastuji *et al.*, 1998].

However, the problem of abortion of pregnant females is not completely solved and vaccination of pregnant females should be avoided, as well as the vaccination of females less than one month before mating. There must be also a correct identification of vaccinated animals, control of movements and an adjustment of the serological diagnosis when adult vaccination is applied.

II – REV 1 VACCINATION IN PORTUGAL

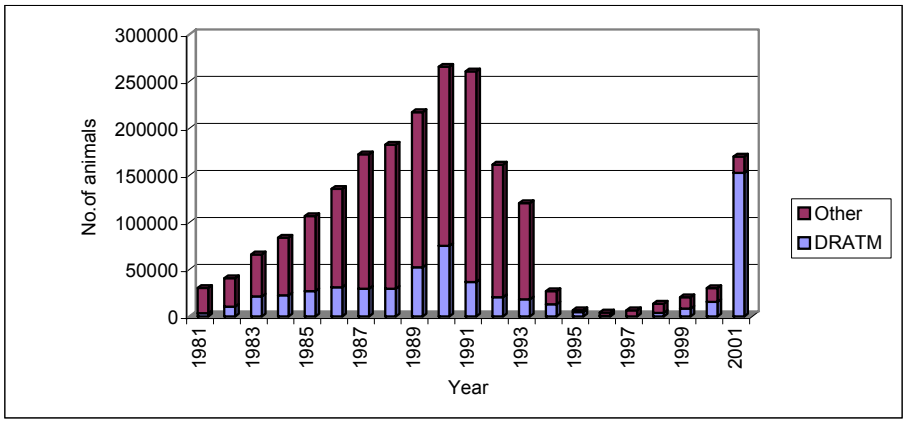
In 1971, following FAO/WHO Expert Committee recommendations, Rev 1 live attenuated vaccine, imported from Italy, was applied experimentally in 300 goats on a state farm with excellent results. In the next year, 1972, the vaccination of goats was made compulsory, but only in the most affected areas which included Leiria, Santarém and Lisbon [Corrêa de Sá *et al.*, 1990], in combination with the test and slaughter policy that had started in 1946 for the goat population and for sheep in contact with goats. Only young females were vaccinated, between 3-6 months of age and with 1-2 x 10^9 dose, by subcutaneous route.

Vaccination was not applied systematically, even in the areas mentioned above, until the late eighties when the accession of Portugal to the European Community in 1986, and the prospect of an open market in Europe in 1993, required an improvement of the sanitary status of Portuguese flocks.

The transfer to the private sector (especially producers' co-operatives and associations) of the responsibility for certain disease control programmes greatly increased the numbers of Rev 1 vaccinations of young animals (Graphic 1) which was applied together with the test and slaughter of serologically positive animals.

GRAPHIC 1

Small ruminants vaccinated with Rev 1 indicating those vaccinated in DRATM



An EC co-financed “mall Ruminant Brucellosis Eradication Plan” was approved in 1991 (DEC 91/217/EEC, 26th March) for a three-year period. This programme had the objective of certification of flocks and areas as “officially free of brucellosis” while keeping with the rules for the trade in small ruminants between EC member states. The recommended policy was the vaccination young females, between 3 and 6 months of age, in flocks with persistent infection and more than 2% positive animals (POR 233/91, 22nd March; POR 1051/91, 15th October). According to legislation, the introduction of replacement animals in non-indemne flocks was conditioned to Rev 1 vaccination of these animals. Even so, as observed in Graphic 1, vaccination was greatly reduced in 1992 and almost abandoned in the following years. The main reasons for this fact were related to the desire to achieve the officially free status of the farms, as required in EU legislation for the trading of animals, and also because the increasing of field work for test and slaughter made it very difficult to follow the vaccination campaign due to lack of human resources. Most production systems have poor seasonality of parturitions, which means that vaccination of young animals is a very labour intensive work. As an argument for abandoning vaccination, the persistence of serological reaction in vaccinated animals was also used, as well as the difficulties of isolating vaccinated animals, as advised.

In some of the regions as DRATM (Regional Directorate of Agriculture of Trás-os-Montes), test and slaughter was not sufficient to allow an improvement of the epidemiological situation and an increase of brucellosis was observed, especially after 1996.

In 1999 the “Brucellosis eradication evaluation board” recognised the urgent need to implement vaccination in areas with high disease incidence, as a complement to the test and slaughter policy.

In the year 2000, the General Directorate of Veterinary (DGV), after a EU Food and Veterinary Office inspection, reinforced the need to follow legislation concerning the application of Rev 1 in young replacements in non-indemne and indemne flocks in infected areas and the need of a good vaccination coverage along the years (at least 5 years). The re-introduction of animals in infected farms should be made only with vaccinated animals.

III – CONJUNCTIVAL REV 1 VACCINATION OF ADULT SHEEP AND GOATS IN TRÁS-OS-MONTES

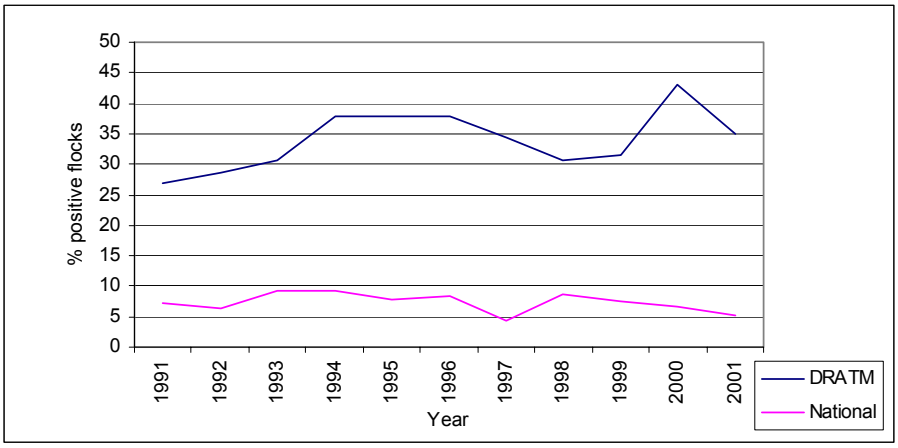
The small ruminants production system in DRATM, accounting for 15-20% of the sheep and goats population of the country, is of great importance to the local economy. This production system is semi-extensive, using marginal areas. Holdings are generally not isolated, and animals from a village are grazed together, with one shepherd, moving around the village in communal pastures. Animal movement is difficult to control and veterinary services have found very difficult to enrol the aged, poor and illiterate farmers into the eradication programme.

Small ruminant’s brucellosis is a zoonosis with a severe socio-economic impact and a high prevalence in DRATM. This region accounts for 38.5% of the notified human cases at national level. In fact the annual rate of reported cases in 2 000 was 0.5/10 000 inhabitants for the country while DRATM presented 4.4 notified cases/10 000 inhabitants.

This region also presented flock prevalences always above the national averages (Graphic 2), but even so, the vaccination of young animals was not systematically applied and decreased from 1992 being practically abandoned from 1995 (Graphic 3).

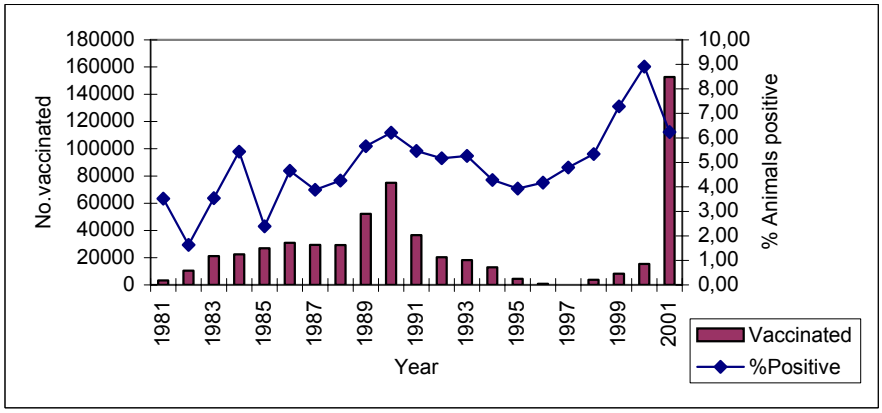
GRAPHIC 2

Percentage of brucellosis positive flocks in DRATM and at national level



GRAPHIC 3

Vaccination and animal prevalence in DRATM



The brucellosis prevalence at animal level started to increase in 1998, 6 years after vaccination has been stopped (Graphic 3), which correspond to the usual productive life of sheep and goats.

Some OPP (producer's organisations for animal health defence) restarted young animals vaccination in 1998. For example Moimenta da Beira, the OPP responsible for more than 1/3 of vaccinations in that year, had in 2000, 3.4% positive animals, in comparison with the 8.9% of DRATM.

In the year 2000, in DRATM, the prevalence of infected animals was 8.9% and the flock prevalence reached 43%. This region had 40% of the slaughtered sheep and goats under the brucellosis eradication programme in the country. This situation led to the reappraisal of the eradication campaign, along with the neighbouring regions (Entre-Douro e Minho and Beira Interior), the central veterinary services (DGV), the central laboratory (LNIV) and other specialists. With the view of rapidly reduce brucellosis prevalence, to protect public health and to maintain the small ruminant's production in DRATM, it was decided that a control programme should be immediately implemented and eradication should be considered as a long-term objective. New strategies were also selected: Rev 1 mass vaccination, using the conjunctival route, was considered the best option, combined with test and slaughter policy in indemne and isolated flocks with good level of management.

The choice of mass vaccination was mainly justified by (i) the production system characteristics (extensive system oriented to meat production; village-based management of flocks, intensive animal movement), (ii) the characteristics of the Rev 1 vaccine (a live vaccine inducing a good immunisation, the

possibility of reducing persistent titres and excretion using the conjunctival route), and (iii) the farmers perception that vaccination would be a valuable alternative to improve the situation.

Following the decision to implement a mass vaccination programme, several meetings were organised with the Veterinarians coordinating and working in the OPP, with the aim to discuss the objective of the programme, the criteria for vaccination and methodologies. There were also meetings with the Local Breeds Associations. Police forces were also involved in order to help the control of animal movement and animal identification.

A spirit of cooperation was achieved among farmers and veterinarians, with a very good participation of farmers.

The rules established were the following:

VACCINATION

- Vaccination of all flocks in the area, with the exception of B3 flocks perfectly closed (with their own pastures). These flocks will have a PIS (individual sanitary plan).
- Application of Rev 1 vaccine by conjunctival route, at a full dose ($1x10^9$ cfu).
- B3 flocks: vaccination of young replacement females or vaccination of all animals when the flock in a high risk area (in this case pregnant females 30-120 days are excluded and milk is not used for cheese production up to one month after vaccination).
- Non-B3 flocks: vaccination of all animals within a flock, excluding pregnant females 30-120 days.
- Blood-sampling of all animals before vaccination and slaughter of those serologically positive to the Rose Bengal Test.
- Restriction of animal movement at least up to 60 days post-vaccination.
- Beginning of adult animal's vaccination: February 2001. Duration: 1.5 to 2 years.

ANIMAL IDENTIFICATION

- In adult animals, replacement of the usual salmon-colour ear tag by a green ear tag, only if the salmon tag is wrongly placed or if the ID number is impossible to read.
- In adult and young animals: application of a green ear button with the year of vaccination and application of a tattoo (left ear or left groin fold) with the ID of the Region, County, month and year of vaccination.
- Registration of vaccinated animals in the Computer-based System PISA with the date of vaccination.

FOLLOW-UP OF VACCINATED ANIMALS

- Serological survey of adult vaccinated animals 24-30 months after vaccination.
- Serological survey of young vaccinated animals 12 months after vaccination.

PUBLIC INFORMATION ON THE VACCINATION CAMPAIGN

- Emission of an Official Edict announcing the new measures in place.

The vaccination programme is being implemented in the 13 OPP of DRATM (33 counties) since February 2001. Animals planned to be vaccinated were 85% of the existing animals (340 000), which means 289 000 small ruminants (the remaining animals belong to isolated flocks with good sanitary status or were already vaccinated when young).

Results obtained during the first year of vaccination were the following:

Total population	365 000
Vaccinated adult animals	142 065
Vaccinated young animals	25 810
Animals already vaccinated or from isolated flocks	28 847
Coverage of programme in the first year	196 722 (53.9%)

In the flocks already visited, another 46 781 (19%) animals were identified and not vaccinated (gestation, young age, etc.).

These flocks should be revisited for the vaccination of these animals, as well as all other flocks for the vaccination of young replacements. In the first year 243 503 animals were identified and registered, consisting of 67% of the population.

At the same time, the test and slaughter campaign is also under development, with 275 049 animals tested in 2001 and 17 156 animals to be slaughtered (6.2%). Efforts have been made to accelerate the removal of positive animals (32 days in average) and to rapidly compensate farmers. The situation found in the end of 2001 indicates a reduction of flock prevalence from 43.08% to 34.95% and a reduction of the percentage of positive animals from 8.91% to 6.24%.

The implementation of the mass vaccination campaign had some problems. The most important was the abortion of animals, especially goats. The risk of abortion of pregnant females vaccinated in the first four months of pregnancy is known to be high, diminishing at the end of pregnancy and at lactation. Several farmers decided not to wait and take the risk of vaccinating the pregnant females. The cases of abortions (around 1 750 abortions) were reported by farmers and a request was forwarded in the view of compensate these farmers. Reports also refer difficulties in some females having another pregnancy after abortion.

Animal movements restrain for 60 days after vaccination was also a measure difficult to be accepted by farmers.

IV – MASS VACCINATION FOLLOW-UP STUDY

The monitoring of the application of the vaccine, through the follow-up of a sample of six flocks (selected on the bases of the willingness of farmers to collaborate), from six different areas (with 607 animals) was carried out in order to evaluate the serological response at different time-periods after vaccination. Serological tests (Rose Bengal Test (RB) and Complement Fixation Test (CF)) were performed at days 30 and 120 post-vaccination. Two flocks were followed for a longer time, 240 and 365 days.

Table I indicates the serological results at 30 days after vaccination. Almost 88% of animals reacted to at least one of the two tests, therefore it was considered that the vaccination had the expected results. However, only 67% of animals reacted to both tests.

Data is also disaggregated between previously positive and negative flocks (considering day zero results; note that all positive animals at day zero were slaughtered). Previously negative flocks had a proportion of animals negative to both tests 30 days after vaccination that is five times bigger than positive flocks (Chi-square test indicates a significant difference between the two types of flocks).

TABLE I

Percentage of results in each category at day 30 after vaccination

%	Negative RB and CF	Positive RB and CF	Positive only RB	Positive only CF
Total n = 607	12.2	66.9	11.9	9.1
Negative flocks n = 355	18.3	60.3	12.7	8.7
Positive flocks n = 252	3.6	76.2	10.7	9.5
Sheep n = 536	13.8	63.4	12.5	10.3
Goat n = 71	0	93.0	7.0	0
Youngs n = 27	0	92.6	3.7	3.7
Adults n = 269	3.0	78.4	11.5	7.1

Comparing sheep and goats, significant differences were also found regarding the distribution of results. Goats were either positive to both tests or negative, while sheep had some results with only one test positive.

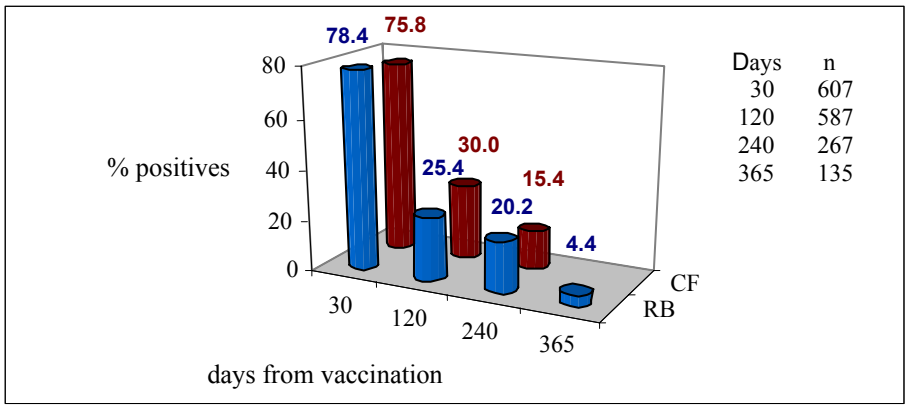
Between adult and young vaccinated animals, no significant difference was observed. However, the number of young animals in the sample is very small.

At flock level, the percentage of animals positive to at least one test at day 30 varied from 60.7% to 100%.

Graphic 4 shows the evolution of positive results to RB and CF in days 30, 120, 240 and 365 after vaccination.

GRAPHIC 4

Percentage of positive results to RB and CF, in several time periods after vaccination



Most animals were again negative four months after vaccination (70% in CF and 75% in RB), but at day 240, 20% of them were still RB positive. From the six animals found positive at day 365, five of them were previously negative (at day 240), indicating the presence of infection and not the persistence of vaccinal antibodies and pointing out the importance of a good information system to support decision making.

In goats the percentage of positive serological responses were higher than in sheep in day 30 but smaller at day 120. At this time RB was positive for only 4.5% of goats against 28.1% of sheep and CF was positive for 11.9% of goats and 32.3% of sheep.

V - CONCLUSION

The mass vaccination campaign will be completed by the end of 2002 and will be followed by young replacement animal's vaccination only, at least for another height years. The transference from the vaccination programme to test and slaughter was foreseen for 12 months after vaccination for young animals and 24-30 months after vaccination for adult animals. However, the time period between adult animals vaccination and the application of serological diagnosis was considered to be too long, after discussion with some EU experts, and it was decided that flocks could be tested as soon as one year after vaccination. Positive results will be evaluated with complementary tests as the Double Gel Diffusion Test with LPS as antigen.

A vaccination programme should also be supported by other measures in order to achieve a control of the disease and allow the implementation of eradication measures. Vaccination is being combined with test and slaughter of positive animals and all other necessary measures, like live animals and products movement control, timely compensation payment and farmers education, in order to achieve at a medium term a prevalence compatible with the implementation of eradication strategies.

A spirit of confidence and trust was developed in the last months, among all interested parties. With a campaign of vaccination lasting at least 5-7 years and with an intense cover of

the flocks, we expect to walk a big step towards control and, therefore, to eradication.

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