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SAFETY AND EFFICACY OF THE "BORGHI" STRAIN VACCINE AGAINST MYXOMATOSIS

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INTRODUCTION

Myxomatosis was reported in Italy for the first time in the Summer of 1955 close the French border (Fioretti, 1985). Since then it spread all over our Country and nowadays it has become as endemic. In spite of the few reported cases it is believed that its epidemiology is quite different. A number of small breedings escape veterinary surveillance and often at the borders preventive measures fail when hares are introduced from hunters' associations in small cages (Cancellotti et al. 1987). Yet, wild outbreaks are rather difficult to get under control as contagion is insect-borne. If sanitary controls are feasible in intensive breedings they are disregarded in small rural ones that result as the main reservoirs of the virus. The situation has worsened when clinical modifications of the natural Myxomatosis have occurred: virus dermotropism seems to have lowered in favour of a more marked pneumotropism. Vaccination is then the best measure to control the disease. Aim of the paper is laboratory and field checks of a new vaccinal strain that developed at the Istituto Zooprofilattico Sperimentale delle Venezie e del Trentino, Padua, Italy (Cancellotti, 1985) is to be commercially available shortly.

MATERIALS AND METHODS

1. Modified "Borghi" strain live vaccine, lyophilized, supplied by the Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy, its viral titre being $10^{5.6}$ $TCID_{50}$/vaccinal dose. The main features of the vaccinal virus were as follows: it induced syncitya only in cell cultures, with no lysis and cell detachment; given intravenously (i.v.), intradermally (i.d.), and intratesticularly it was re-isolated from rab-

bit organs 72 to 120 hrs following infection when temperature raised. Serial passages of the virus, repeated for 5 consecutive times prevented strain re-virulentation. It always induced a mild temperature rise between 72 to 120 hrs following injection. Therefore, Borghi strain was stabile; induced experimental immunity started rapidly and serological tests revealed antibodies for 4 to 8 weeks following vaccination; i.d. vaccinal dose was equivalent to $10^{5.3}$ $TCID_{50}$ on RK_{13} cells.

2 Modified commercial live vaccine, lyophilized, from McKercher and Saito (1964) modified strain, with a titre of $10^{3.5}$ $TCID_{50}$/vaccinal dose.

3. Myxomatosis wild virus, "TE" strain, isolated from a natural outbreak of the disease. The virus was titred $10^{3.7}$ ID_{50}/ 0.2 ml when given 8-week rabbits i.d. It induced dermotropic signs of the disease and killed over 80% of the experimentally infected animals.
4. Rabbits of albino New Zealand breed were used in lab trials. In field also California breed subjects and fattening crossbreds were employed.
5. Virus titrations were carried out in microtiter plates according to the current techniques employing RK_{13}-line rabbit kidney cells. Antibody titration was performed with serum neutralization conditioned by guinea-pig complement according to Cancellotti (1985).

RESULTS

1. Safety of the Borghi strain vaccine was tested subcutaneously (s.c.) and i.d. with dermojet of 1, 10, and 100 vaccine doses to 2 Kg rabbits. Pregnant does were also treated s.c. and i.d. with 1 and 10 vaccinal doses during the first and the second half of gestation. All animals were from a disease-free breeding and had never been vaccinated before. The test, reported in Table 1 was as follows: animals tolerated vaccine injection even if at high doses and showed no clinical signs except for a temperature rise 3 to 5 days p.i.; a local mild inflammation reaction at the injection site was seen that disappeared within 7 days. A local oedema was present only in animals given 100 doses. Tested pregnant does showed general and local reactions similar to the previously described ones that disappeared within 7 days. Deliveries took place regularly. No differences in the number of healthy newborn bunnies in comparison with the average of the untreated animals were ever observed.

The efficacy of Borghi vaccine was evaluated in 2 Kg white New Zealand

ANIMALS	INOCULATION		GENERAL REACTION	LOCAL REACTION	REGRESSION OF REACTIONS
No	Route	Doses			
5 weight 2 Kgs	s.c.	1	Temperature rise	Mild inflammation	About 7 days
5 " 2 "	s.c.	10	" "	" "	" 7 "
5 " 2 "	s.c.	100	" "	Oedema	" 7 "
5 " 2 "	i.d.	1	" "	Mild inflammation	" 7 "
5 " 2 "	i.d.	10	" "	" "	" 7 "
5 " 2 "	i.d.	100	" "	Oedema	" 7 "
3 <2-week gestation	s.c.	1	" "	Mild inflammation	" 7 "
3 <2- " "	s.c.	10	" "	" "	" 7 "
3 <2- " "	i.d.	1	" "	" "	" 7 "
3 <2- " "	i.d.	10	" "	" "	" 7 "
3 >2- " "	s.c.	1	" "	" "	" 7 "
3 >2- " "	s.c.	10	" "	" "	" 7 "
3 >2- " "	i.d.	1	" "	" "	" 7 "
3 >2- " "	i.d.	10	" "	" "	" 7 "

Tab. 1 - Safety of "Borghi" vaccine in young adults and pregnant does.

rabbit groups compared with another homologous vaccine of the commerce. As to evaluate the immunizing activity of vaccines, groups of 5 rabbits were vaccinated s.c. and i.d., with scalar doses of both products. Four rabbit groups were given Borghi vaccine s.c. the doses being 10^0; 10^{-1}; 10^{-2}; 10^{-3}, respectively. Four further groups were given the same doses i.d. Eight rabbit groups were given commercial vaccine following the same schedule. Three weeks p.i. the above animals and 2 unvaccinated control groups were infected i.d. with 3,000 ID_{50} of the Myxoma virulent "TE" strain. Results are reported in Fig. 1; 10 unvaccinated control died of Myxomatosis 10 to 14 days following injection; s.c. vaccinated rabbits showed 147 PD_{50}/vaccinal dose (PD_{50} = 50% protective dose), while with i.d. injection the same vaccine showed 68 PD_{50} vaccinal dose; the commercially available vaccine showed a markedly lower efficacy. Subcutaneously, it ensured 32 PD_{50}/vaccinal dose, while i.d. it gave 68 PD_{50}/vaccinal dose.

3. Field test lasted 2-years in an agricultural Co-operative and in two

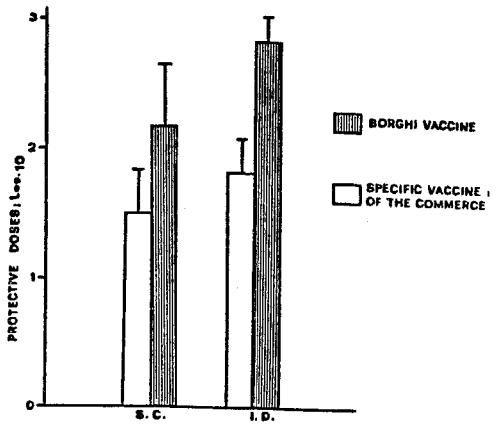

Fig. 1 - Immunizing activity of the Borghi strain in comparison with the kind of inoculation and other specific vaccine commercially available.

intensive breedings. Breedings were all located in at risk areas of Central Italy where the disease had frequently appeared in the last 5 years both with dermotropic and respiratory signs; prophylaxis was performed every 6 months, early in Spring and Autumn. Fattening rabbits were vaccinated when 4-week age i.d. at weaning. On the whole 30,750 rabbits were treated of which 20,700 were fattening subjects in at high risk breedings.

Controls lasted 12 weeks as to check any possible anomalous reaction in each treated animal. No reactions different from those already described in safety tests were seen. Emphasis is deserved to the results of vaccinations of pregnant does as in treated breedings no inconveniences were ever met. These data are similar to those recorded in our agricultural Co-operation of Northern Italy where Borghi vaccine was employed extensively (Fruscalzo and Sabbion, 1986).

In breedings located in at risk areas where the vaccine had been used for prophylactic purposes on all animals no sign of the disease was ever seen. So far we have no convincing argument to warrant the efficacy of the product, even if in other untreated breedings of the same area a high mortality rate for Myxomatosis was recorded in the same period.

Emergency vaccinations performed with Borghi vaccine involved 3 diseased breedings. As a whole 862 does, 854 suckling bunnies, 4,800 fattening rabbits were treated. In such cases the efficacy of the

vaccine was definitely proved. In breedings safety tests of the Borghi vaccine were in progress, so only a part of the animals were treated in order to check possible differences of productivity between vaccinated and unvaccinated animals. The disease had been probably insect-borne and diagnosed with some delay, when percentages of clinically diseased animals were rather high: nearly 5%, 10% and 30% in the first, second and third breeding, respectively. Vaccination was performed i.d. on animals present in the breeding, leaving aside the ones vaccinated less than 6 months before. Control groups to prove the spreading of the wild virus were also formed.

The results, reported in Fig. 2, are as follows: losses (dead and discarded) among unvaccinated animals ranged 58%; losses of animals vaccinated previously to wild virus bursting ranged 0,5% with slight differences among different breedings; average losses in the breeding where emergency vaccinations had been performed when animals number was 5% reached 1.2% with peaks of 6.0% in the groups of young; in the breeding where symptomatology involved nearly 30% animals, emergency vaccination reduced losses in comparison with unvaccinated controls; however, losses were marked in various groups with a final average of 32% of the total animal number.

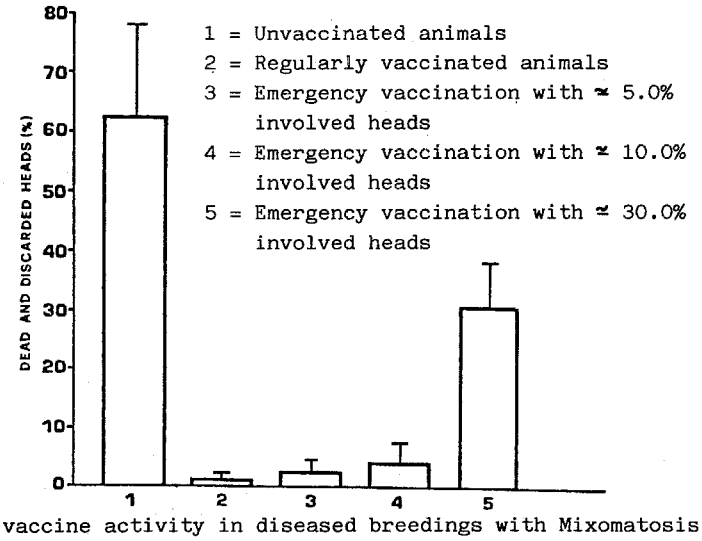

Fig. 2 - Borghi vaccine activity in diseased breedings with Mixomatosis different rates.

Worth noticing that emergency vaccination performed in the three breedings stopped disease spreading within 3 to 5 days and induced remission of skin lesions in most rabbits showing lesions at an early stage.

CONCLUSIONS

Both safety and stability of the Borghi vaccine are of a rather good level. Both in lab and field trials this vaccinal strain never induced local and general severe reactions following s.c. and i.d. injection. Only with doses 100 times higher than the vaccinal one a small oedema appeared locally, whose regression took place within 7 days leaving no traces. Injection of does at different gestational times and 3-week old bunnies with non parenteral immunity gave satisfactory results: in does deliveries were normal and no interference with subsequent reproductive cycles was ever recorded in does, growing rate was normal and unwanted reactions never took place in bunnies.

The efficacy of the Borghi vaccine is good and however, protection ensured by the s.c. and i.d. administered vaccinal dose is higher than the one from the commercial homologous vaccine and lasts 6 months at least. In field the vaccine had a strong action mainly when emergency vaccinations were practised in already diseased breedings. If symptomatology is clear only in 5 to 20% animals, vaccination stops virus spreading within 3 to 5 days. Yet, it induces remission of the clinical signs within 2 to 3 weeks in animals with an early symptomatology. As a consequence losses from natural diasease are markedly reduced.

ACKNOWLEDGEMENTS

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REFERENCES

CANCELLOTTI F.M., 1985 - Caratteristiche dello stipite vaccinale Borghi. Rivista di Coniglicoltura, 22 (3), 24-31

CANCELLOTTI F.M., FACCHIN E., GALLAZZI D., 1987 - Methods of prophylaxis of Myxomatosis in Italy. In AUXILIA T. - Rabbit production systems including welfare; C.E.C. Directorate - General for Agriculture, EUR 10983 EN, pagg. 105-114.

FIORETTI A., 1985 - Il problema della Mixomatosi in Italia. Rivista di Coniglicoltura, 21 (10), 47-56.

FRUSCALZO G., SABBION P., 1986 - Mixomatosi vaccinale con il Borghi. Rivista di Coniglicoltura, 23 (6), 17-19.

McKERCHER D.G., SAITO J., 1964 - An attenuated live-virus for Myxomatosis. Nature (London), 202, 933-934.

SUMMARY

Both safety and efficacy of the attenuated live vaccine against Myxomatosis, prepared with an homologous strain Borghi have been proved in laboratory tests. Such a vaccine has also been used successfully in intensive farms where infection had naturally spread. Vaccination of over 20-day animals, using dermojet, has not resulted in adverse reactions and has had no effect on productive and reproductive cycles.

Seroneutralizing antibodies have remained for at 60 days in vaccinated animals, their protection against the naturally acquired disease has been ensured for 6 months.

In farms with Myxomatosis in progress the emergency vaccination with Borghi strain has prevented the virus from spreading within 5 days.

Regression of the whole clinical signs in animals at the early onset of symptoms of the disease has taken 2 to 3 weeks. Obviously, severe syndromes with an advanced decay of the general conditions have not been involved.

RIASSUNTO

Sono state dimostrate in laboratorio l'innocuità e l'efficacia del vaccino vivo attenuato contro la Mixomatosi, allestito con ceppo Borghi, omologo. Tale vaccino è stato anche utilizzato con successo in allevamenti intensivi in cui l'infezione si era diffusa naturalmente. La vaccinazione, effettuata con dermojet in soggetti di età superiore ai 20 giorni non procura reazioni indesiderate e non incide negativamente sul ciclo produttivo e

riproduttivo. Gli animali vaccinati presentano anticorpi sieroneutralizzanti per almeno 2 mesi e per almeno 6 mesi risultano protetti nei confronti della malattia naturale. In allevamenti con Mixomatosi in atto, la vaccinazione d'urgenza con ceppo Borghi blocca la diffusione del virus entro 5 giorni. La regressione del quadro clinico, nei soggetti ai primi sintomi della malattia, avviene entro 23 settimane; naturalmente non vengono influenzate sindromi gravi, con forte compromissione dello stato generale.

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Both safety and efficacy of the attenuated live vaccine against Myxomatosis, prepared with a homologous strain "Borghi" have been proved in laboratory tests.
Such a vaccine has been also used successfully in intensive farms where infection had naturally spread. Vaccination of animals over 20-days, by using dermojet, does not result in adverse reactions and has no effect on productive and reproductive cycles.
Sero-neutralizing antibodies remain for at least 180 days in vaccinated animals thus ensuring their protection against the naturally acquired disease.
In farms with Myxomatosis in progress the emergency vaccination with strain "Borghi" prevents the virus from spreading within 5 days.
Regression of the whole clinical signs in animals at the early onset of symptoms of the disease takes 2 to 3 weeks. Obviously severe syndromes with an advanced decay of the general conditions are not involved.

INNOCUITA' ED EFFICACIA DEL VACCINO CONTRO LA MIXOMATOSI CEPPO "BORGHI"

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