Contagious bovine pleuropneumonia vaccines: the need for improvements

Article *in* Revue scientifique et technique (International Office of Epizootics) · October 1995

DOI: 10.20506/rst.14.3.856 · Source: PubMed

Rev. sci. tech. Off. int. Epiz., 1995, **14** (3), 593-601

Contagious bovine pleuropneumonia vaccines: the need for improvements *

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Summary: *Contagious bovine pleuropneumonia (CBPP) vaccines are routinely used only in Africa. The vaccines are usually produced from one of two strains (T_1/44 and KH_3J), each of which has a streptomycin-resistant variant. The necessity for a 'master seed strain' is evident. At least one manufacturer in Africa produces a broth culture vaccine, while others produce a freeze-dried product. A standardised manufacturing protocol needs to be developed, together with in-process and final product quality control procedures. Some CBPP vaccine manufacturing procedures do not allow sufficient leeway for the execution of typical quality control practices. For example, it is difficult to perform batch testing on broth culture vaccine, as the vaccine is produced in its final container.*

Quality control test results from the Pan African Veterinary Vaccine Centre (PANVAC) are analysed in terms of causes of batch failure and indicators for process development. Taking potency as an example, most vaccine batches tested by PANVAC pass only at the limit of the OIE minimum requirement of 10^7 colony-forming units per dose. To improve the titre of the vaccine, it will be necessary to modify the manufacturing process, either by increasing mycoplasma yield during the culture phase or by minimising losses during downstream processes, especially freeze-drying.

Data on inactivated vaccines are scarce.

Duration of the immunity achieved with live CBPP vaccines is relatively short, in comparison with other live vaccines. Data may be required on the molecular basis of virulence and immunogenicity, as well as on the molecular immunology of CBPP, to enable the development of improved vaccines.

KEYWORDS: Contagious bovine pleuropneumonia – Mycoplasma – Quality control – Vaccines.

INTRODUCTION

Vaccines are widely recognised to be medicinal products. Consequently, the manufacture of vaccines must be subjected to quality assurance procedures and must adhere to the requirements of 'good manufacturing practices' (GMP). The Office

* This paper was presented at the Conference on foot and mouth disease, African horse sickness and contagious bovine pleuropneumonia, held in Gaborone (Botswana) from 20 to 23 April 1994.

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International des Epizooties (OIE) has prepared guidelines on the requirements for vaccines against contagious bovine pleuropneumonia (CBPP), setting standards for quality of vaccines (9). A manual to be published jointly by the Food and Agriculture Organisation of the United Nations (FAO) and the OIE will provide a practical guide to vaccine manufacturing in conformity with GMP requirements.

CBPP is an economically important disease of cattle, which is caused by *Mycoplasma mycoides* subsp. *mycoides* SC. Through a policy of rigorous restriction of cattle movement, slaughter and compensation, this disease has been eradicated in the United States of America and Europe. CBPP was also eradicated in Australia, through a combined policy involving restriction of animal movements, vaccination and slaughter (8). The disease remains endemic in Africa and is now considered the second most important epizootic of cattle (after rinderpest) on the African continent. For socio-cultural and economic reasons, application of the methods used to eradicate CBPP in other parts of the world may not be feasible in Africa. Hence, the only realistic way of controlling CBPP in Africa is by vaccination. As CBPP vaccination is employed only in Africa, the OIE requirements largely reflect African experience.

Since 1990, the Pan African Veterinary Vaccine Centre (PANVAC) has been concerned with the quality of CBPP vaccine, with a view to implementing the draft OIE requirements. Initially, a workshop was convened at the Central Veterinary Laboratory in Bamako (Mali), from 12 to 16 November 1990, for scientists directly involved in the production of CBPP vaccine in Africa (10). This workshop was conducted by FAO consultants, drawn mainly from the CIRAD-EMVT (Department of Husbandry and Veterinary Medicine of the Centre for International Co-operation in Agronomic Research for Developing Countries) and including the principal author of the OIE requirements. The present communication reflects the experience gained since the workshop was held.

TYPE OF VACCINE

Several forms of CBPP vaccines have been developed and used in the past. For instance, pastoral communities in Africa have used (and still use) the Willem's method of subcutaneous inoculation of infective lung fluids into unaffected cattle (12). Egg-based vaccines were used extensively in East Africa in the 1960s, but have since been abandoned due to the severe post-vaccination reactions (3, 11). Inactivated vaccines have also been tested experimentally, and most of the few reports in the literature suggest that such vaccines may not provide satisfactory protection (13, 6). At least one publication, however, reports successful protection of cattle using an inactivated, oil-adjuvanted CBPP vaccine (4).

Attenuated broth culture vaccines derived from serially-passaged isolates of *M. mycoides* subsp. *mycoides* – either in liquid form or lyophilised – are now the only vaccine types used in Africa. However, attenuation in CBPP vaccines seems to be particularly ill-defined. The FAO/OIE/Organisation of African Unity (OAU) Expert Consultation on CBPP (Paris, 15-20 March 1971) concluded that only the freeze-dried product was suited for CBPP vaccination campaigns, a recommendation reiterated in 1990 by the PANVAC workshop on CBPP vaccines in Bamako (10). Consequently, the following discussion relates to the experience of PANVAC with this product alone.

VACCINE SEED STRAINS

The attenuated seed strains currently in use in Africa are $T_1/44$, T_1-SR, KH_3J and KH_3J-SR.

T_1 was first isolated in Tanzania from a mild case of CBPP in the 1950s, and was subsequently passaged ten times in embryonated eggs. This attenuated strain was used extensively in East Africa as a vaccine against CBPP. Such egg-based vaccines have since been abandoned, as they provoked lung lesions. The agent was further passaged in eggs to the 44th level (to produce the $T_1/44$ strain). Broth culture vaccines were prepared from this strain, and were used either in liquid form or as freeze-dried product. $T_1/44$ still has some residual virulence in some taurine breeds of cattle, but provides good protection (for at least one year) in vaccinated cattle. A streptomycin-resistant variant (T_1-SR) was developed by workers at CIRAD-EMVT. This was produced by passaging the $T_1/44$ strain three times in the presence of increasing concentrations of streptomycin. T_1-SR is reported to cause less severe (minimum) post-vaccinal reactions than the $T_1/44$ parent strain, while maintaining the same level of immunogenicity.

KH_3 was first isolated in the Juba region of southern Sudan. This strain was passaged 88 times, and vaccines prepared from this attenuated strain have been used in West and Central Africa, in particular. A streptomycin-resistant variant (KH_3J-SR) has also been developed. KH_3J and KH_3J-SR do not cause any reactions in vaccinated animals, irrespective of the route or the volume of inocula used. However, these strains confer only poor immunity (protection), lasting for six months.

Thus the factors to be considered in choosing a seed strain for CBPP vaccination are that the strain should be well characterised, to ensure '**standardised specificity**', and that the seed strain should be incapable of causing unacceptable post-vaccinal reactions. The PANVAC workshop on CBPP vaccine in Bamako (10) recommended the adoption of T_1-SR as the standard seed strain for CBPP vaccine manufacture in Africa. This recommendation was based on the following premises:

- T_1-SR causes less severe (minimum) post-vaccinal reactions than $T_1/44$ but gives similar protection.

- T_1-SR titre yields are between 10 and 1,000 times greater in media containing streptomycin.

- Streptomycin resistance could be used as a marker for the identity of T_1-SR.

This workshop also urged PANVAC to establish, in collaboration with EMVT (which is also the FAO/OIE International Reference Laboratory for CBPP), a tested T_1-SR seed bank for Africa. This has been achieved, and a stock of seed at the 51st passage is held at PANVAC and EMVT. This seed lot has undergone the prescribed *in vitro* tests and innocuity tests in laboratory animals at PANVAC and EMVT, but it has not yet been tested for potency and safety in cattle (9).

Before the establishment of a functional seed lot system which conforms to conventional practices, the following issues must be resolved:

- To date, there are no universal criteria for a CBPP vaccine master seed strain, nor is there any universally-accepted strain which might be used as an international master seed.

– There appears to be an undefined but slender distinction between attenuation and loss of immunogenicity. For example, KH_3J is the most attenuated strain but also the least immunogenic. It remains unclear whether virulence and immunogenicity have common or shared genes. A study of the molecular basis for virulence and immunogenicity would be valuable in generating vaccine strains of determined attenuation without impaired immunogenicity.

– The OIE norms prescribe a maximum limit of three passages between the seed lot and the vaccine. This restriction invalidates any attempts to operate a seed lot system, as such a system would involve a minimum of eight passages between the FAO reference master seed and the vaccine.

– Dyson and Smith (2) reported that sub-culturing *M. mycoides* in broth readily attenuated virulence, while the effect of sub-culturing on immunogenicity did not seem to have been well documented.

– The tests for identity also seem to be imprecise, particularly with regard to the differentiation of attenuated and virulent strains, and the differentiation of the various vaccine strains. In this regard, streptomycin resistance could be a useful marker for T_1-SR. Observations at PANVAC, however, indicate that streptomycin resistance is readily acquired. *Acholeplasma* and *Mycoplasma* strains which are resistant to streptomycin have been detected as contaminants of rinderpest and CBPP vaccines. Furthermore, CBPP vaccines submitted from two laboratories and labelled as T_1/44 have been found to be partially resistant to streptomycin (J. Litamoi, unpublished findings).

– Identity testing at PANVAC of vaccines from six CBPP vaccine producers in Africa (using the growth inhibition test [i.e. 1,500-150,000 viable mycoplasmas per 6 cm diameter Petri dish] with PG_1 antiserum supplied by EMVT) has shown that T_1-SR vaccines, the PANVAC T_1-SR seed and the EMVT T_1-SR master seed contain breakthrough colonies: to date, no such colonies have been detected in T_1/44 and PG_1. Such breakthrough colonies have been cloned by triple endpoint dilution, and preliminary tests (agar gel immunodiffusion, colony morphology and size) indicate that these clones may be *M. mycoides*. Thus the T_1-SR strain appears to contain a sub-population of *M. mycoides* which might be antigenically different from the parent stock. Together with other observations, this seems to indicate the need for a detailed antigenic analysis of CBPP vaccine strains, employing monoclonal antibody probes.

– Although it is claimed that T_1/44 and T_1-SR have similar immunogenic properties, the authors have been unable to trace published data which provide a quantitative comparison. Doutre *et al.* (1) demonstrated that cattle vaccinated in Senegal with T_1-SR vaccine – as either a monovalent or bivalent rinderpest/CBPP freeze-dried preparation – were fully protected against contact challenge nine months after vaccination. In these experiments, the authors employed a dose of 10^8 viable mycoplasmas per dose. In another experiment – conducted jointly by the Kenya Veterinary Vaccine Production Institute (KEVEVAPI) and PANVAC – a 10^7 dose of T_1-SR (titrated after vaccination) failed to elicit a detectable complement fixation (CF) antibody response (unpublished findings). The results of experiments in Senegal and Kenya may be less discrepant than they appear at first. The Kenyan experiment employed the 'field dose,' while the dose used in Senegal was ten times the field dose. The CF titres obtained in Senegal were generally recorded after forty days, and were relatively low (mainly 1/10 to 1/20) in comparison to post-challenge titres (greater than 1/640). Challenge tests have not been performed in Kenya to validate the immunogenicity of T_1-SR. Therefore more experiments in cattle are required, to support the recommendation to adopt T_1-SR as the universal CBPP reference vaccine strain.

VACCINAL DOSE

The OIE requirements for CBPP vaccines stipulate that 'the minimum vaccinal dose for cattle is 10^7 viable mycoplasmas' (9).

Local transport conditions may lead to a reduction of vaccine virulence during transit, and it is therefore recommended that production laboratories supply vaccines having titres of at least 10^8 mycoplasmas per dose.

The method prescribed for titration is the tube method (7). Comparative titrations at PANVAC have shown the microtitre technique to have comparable sensitivity, and this has therefore been selected as the method of choice. The minimal requirement for titre per vaccine dose is considered as the pass mark.

The data from PANVAC indicate that, at present, the recommendation for 10^8 mycoplasmas per dose is not readily attained by most vaccine producers.

Between 1991 and the end of 1993, the PANVAC laboratory at Debre Zeit, Addis Ababa (Ethiopia), performed a total of 214 titrations.

On the evidence of the results, summarised in Table I, the average package for CBPP vaccine should not exceed 50 doses.

TABLE I

Results of contagious bovine pleuropneumonia vaccine titration by the Pan African Veterinary Vaccine Centre between 1991 and 1993

Year	No. of tests	Mean	Standard error	Minimum	Maximum
1991	27	7.5	0.47	2.8	9.7
1992	41	8.7	0.156	6.4	10.8
1993	146	8.9	0.056	6.5	10.3
Total	214 *	8.7 **	0.082 ***	2.8	10.8

* represents 69 tests using combined rinderpest/CBPP vaccine and 145 using monovalent CBPP vaccine: of the total, 6 tests were with KH_3J strain, 48 with $T_1/44$, and 160 with T_1-SR

** median = 9.0

*** standard deviation = 1.194

The average titre obtained for vaccines tested during 1991 was significantly lower than for those tested in 1992 and 1993 ($P < 0.03$), while between 1992 and 1993 there was no significant rise in mycoplasma titre per vial ($P = 0.216$). The pass rate improved substantially between 1991 and 1993 (33%, 47% and 80% in 1991, 1992 and 1993, respectively). The mean log. titre per dose for vaccines (monovalent preparation) which passed PANVAC quality control in 1991, 1992 and 1993 was 7.17, 7.95 and 7.39, respectively. The pass rate for the combined rinderpest/CBPP vaccine was 79% with a mean log. titre per dose of 7.28 for the CBPP component.

One-way analysis of variance of the data revealed no significant variation in titre which could be attributed either to the vaccine strain (i.e. T_1-SR, $T_1/44$ or KH_3J [$P = 0.421$]) or to whether the vaccine was freeze-dried as monovalent CBPP or as combined CBPP/rinderpest ($P = 0.13$). However, a significant variation between producers emerged ($P = 0.001$), with producer B-7 averaging the lowest titre (mean = 7.46 log. mycoplasmas per vial) (Table II).

TABLE II

Results of contagious bovine pleuropneumonia vaccine titration by the Pan African Veterinary Vaccine Centre according to producer

Producer code	No. of titrations	Mean titre per vial	Standard error	Range
A-8	12	9.08	0.055	8.8-9.4
B-7	8	7.46	0.096	7.1-7.8
DAM-5	40	8.74	0.15	6.4-10.8
H-4	18	8.84	0.126	7.6-9.7
M-8	121	8.69	0.125	2.8-9.8
T-4	12	9.42	0.170	8.5-10.3

CONCLUSION

After initial problems, most producers of freeze-dried CBPP vaccine in Africa are now able to manufacture a product of consistent quality. Bacterial and fungal contamination is no longer a serious problem. The use of laminar air-flow cabinets and closer adherence to elements of GMP have helped to reduce this problem. Inadequate sterilisation of skim milk was found to be a common source of contamination in some production laboratories. This has been corrected by adherence to guidelines issued by PANVAC. The most critical issue faced by vaccine producers is the titre requirement for the vaccine dose.

As the results obtained by PANVAC demonstrate, no manufacturer in Africa seems consistently able to supply vaccine in accordance with the recommendation of 10^8 mycoplasmas per dose (9), i.e. a minimum of $10^{9.7}$ or 10^{10} mycoplasmas per vial of 50 or 100 doses, respectively. To correct this anomaly, either the minimum requirements will need to be altered or a detailed programme of investigation on product improvement will be required, which would result in an increase in the yield of mycoplasma present in the bulk before freeze-drying and/or reduce losses during downstream processing.

The minimum requirement of 10^7 mycoplasmas per dose is based on the work of Gilbert and Windsor (5); this work appears not to have been repeated by others, presumably due to the expense involved in such experiments and the expected imprecision of results. Laboratory animal models do not seem to have been used for such studies, although Smith (14) showed that mice can parallel cattle in their susceptibility to *M. mycoides*. The data in the paper by Gilbert and Windsor (5) could be interpreted as suggesting that the immunising dose of the $T_1/44$ strain is less than the figure of 10^7 colony-forming units (CFU) inferred by the authors, as relatively wide (2 logs) dilution intervals were used, thus indicating a median protective dose of between 10^5 and 10^7 CFU. Secondly, the challenge tests were performed six months post-vaccination, rather than at the time when peak immune response could be expected, which is the usual moment for potency assessment of vaccines. The Gilbert and Windsor experiments (5) appear to have been intended to assess duration of immunity rather than potency of the vaccine. Therefore, animal experiments seem to be necessary to determine the minimum immunising dose. In the interim, an arbitrary value of $10^{6.5}$ mycoplasmas per dose may be

a more practical pass mark for vaccine potency than the current level of 10^7. Consideration also needs to be given to whether the immunising dose established for the $T_1/44$ strain is strictly valid for other strains or variants.

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NÉCESSITÉ D'AMÉLIORATION DES VACCINS DE LA PÉRIPNEUMONIE CONTAGIEUSE BOVINE. – M.M. Rweyemamu, J. Litamoi, V. Palya et D. Sylla.

Résumé : *L'Afrique est la seule région au monde où l'on pratique la vaccination systématique contre la péripneumonie contagieuse bovine. Les vaccins sont le plus souvent produits à partir de l'une des deux souches ($T_1/44$ et KH_3J), résistantes à la streptomycine. Il faut, bien sûr dans un premier temps, établir un « lot de semence » de ces souches. Un laboratoire africain au moins fabrique le vaccin en milieu liquide tandis que les autres offrent un produit lyophilisé. Il convient de mettre en place un protocole de fabrication normalisé ainsi que des procédures de contrôle de la fabrication et de la qualité du produit final. Certains procédés de fabrication du vaccin contre la péripneumonie contagieuse bovine ne permettent pas d'effectuer les contrôles de qualité classiques. Par exemple, les vaccins obtenus par culture en milieu liquide et produits directement dans leur conditionnement final peuvent difficilement être contrôlés lot par lot.*

*Les résultats des contrôles de qualité effectués par le Centre panafricain de vaccins vétérinaires (*Pan African Veterinary Vaccine Centre*: PANVAC) ont été analysés pour rechercher les causes de défaut sur des lots entiers et définir les paramètres de production. Par exemple, la plupart des lots de vaccins examinés par le PANVAC atteignent à peine le seuil minimum d'activité fixé par l'OIE, à savoir 10^7 unités formant colonie par dose. Pour améliorer le titre du vaccin, il faudra modifier le procédé de fabrication en augmentant la production de mycoplasmes pendant la phase de culture ou en réduisant au minimum les pertes au cours des étapes ultérieures, notamment au cours de la lyophilisation.*

Les données sur les vaccins à mycoplasmes tués sont peu nombreuses.

Les vaccins à mycoplasmes vivants contre la péripneumonie contagieuse bovine confèrent une immunité relativement plus courte que les autres vaccins à germes vivants. Il faudra peut-être d'autres informations sur les bases moléculaires de la virulence et de l'immunogénicité, ainsi que sur l'immunologie moléculaire de la péripneumonie contagieuse bovine, pour améliorer ces vaccins.

MOTS-CLÉS : Contrôle de qualité – Mycoplasme – Péripneumonie contagieuse bovine – Vaccins.

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VACUNAS CONTRA LA PLEURONEUMONÍA CONTAGIOSA BOVINA: LA NECESIDAD DE MEJORAS. – M.M. Rweyemamu, J. Litamoi, V. Palya y D. Sylla.

Resumen: *Las vacunas contra la pleuroneumonía contagiosa bovina no se aplican de forma rutinaria más que en África. Estas vacunas se elaboran por lo general a partir de una u otra de dos cepas, $T_1/44$ y KH_3J, cada una de las cuales*

posee una variante resistente a la estreptomicina. La necesidad de una «cepa de referencia» es evidente. Por lo menos uno de los fabricantes de África produce una vacuna en medio de cultivo líquido, mientras que otros elaboran un producto liofilizado. Es preciso el desarrollo de un protocolo de fabricación estandarizado, así como el de métodos de control tanto del proceso interno como de la calidad del producto final. Algunos de los procedimientos de fabricación de la vacuna contra la pleuroneumonía contagiosa bovina dificultan la correcta aplicación de los métodos habituales de control de calidad. Por ejemplo, resulta difícil efectuar controles de lote en el caso de la vacuna en medio de cultivo líquido, pues ésta se produce dentro de lo que va a ser su envase final.

*Los resultados de las pruebas de control de calidad efectuadas por el Centro Panafricano de Vacunas Veterinarias (*Pan African Veterinary Vaccine Centre*: PANVAC) son objeto de análisis, con vistas a identificar las causas de la presencia de lotes defectuosos y definir indicadores para el desarrollo del proceso de fabricación. Tomando como ejemplo la potencia, la mayor parte de los lotes examinados por el PANVAC apenas alcanzan el mínimo de 10^7 unidades formadoras de colonias por dosis establecido por la OIE. Para mejorar el título de la vacuna será preciso modificar el proceso de fabricación, ya sea incrementando el rendimiento de mycoplasma en la fase de cultivo o minimizando las pérdidas en las etapas subsiguientes, en especial durante el liofilizado.*

Los datos sobre vacunas inactivadas son escasos.

En comparación con otras vacunas vivas, la inmunidad que confieren las vacunas contra la pleuroneumonía contagiosa bovina es relativamente breve. Tal vez el desarrollo de vacunas más eficaces requiera datos sobre la base molecular de la virulencia y la inmunogenicidad, así como sobre la inmunología molecular de la enfermedad.

PALABRAS CLAVE: Control de calidad – Mycoplasma – Pleuroneumonía contagiosa bovina – Vacunas.

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