Commission of the European Communities

INFORMATION ON AGRICULTURE

Veterinary vaccines

– A COMPARATIVE ANALYSIS OF REGULATIONS IN THE MEMBER STATES FOR THREE MAJOR DISEASES

No 17

August 1976

VETERINARY VACCINES

A comparative analysis of regulations in the Member States for three major diseases

Series: "Information on Agriculture" Nº 17

The vaccines and vaccination programmes in this study are concerned with three different diseases: Foot- and -Mouth Disease, Swine Fever and Newcastle Disease in the three most important domestic species for food production (cattle, pigs and poultry), and they represent the whole spectrum of differences and controversies encountered.

A brief description of the main problems of vaccine production and vaccination is presented as a general introduction to the comparative study of rules and regulations. There are few differences in the rules for most vaccines and they are rarely specific to the individual vaccines.

For each of the three vaccines under study an introductory summary of information on the virus, the disease and the principle types of vaccines are given.

The study is only published in English.

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Manuscript finished in March 1976

August 1976

COMMISSION OF THE EUROPEAN COMMUNITIES
DIRECTORATE-GENERAL FOR AGRICULTURE
Directorate: Agricultural Economics – Division: "Balance-sheets, Studies, Statistical Information"

F O R E W O R D

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This volume sets out a comparative analysis of rules and regulations in the EEC countries for three different diseases : Foot- and-Mouth Disease, Swine Fever and Newcastle Disease in the three most important species for food production (cattle, pigs and poultry).

The following divisions also assisted in the work : "Statistics, Balance-sheets, General Studies" and "Harmonization of laws, regulations and administrative provisions relating to veterinary matters and zootechnics".

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This study does not necessarily reflect the views of the Commission of the European Communities in this sphere and in no way commits the Commission as to its future position on the subject.

Original language : English

I N T R O D U C T I O N

Vaccination programmes are especially in the veterinary field sometimes very contraversial subjects. The vaccines and vaccination programmes under study are concerned about three different diseases in the three for food production most important domestique species, and they represent the whole spectrum of differences and contraversies encountered. Thus it seems reasonable to conclude, that if decisions are reached concerning these three types of vaccines, they may at the same type provide guidelines for other veterinary vaccines in the Member States.

As a general introduction to the comparative study on rules and regulations it has been attempted to give a brief description of the main problems of vaccine production and vaccination. The general rules for the control of sterility, toxicity and such problems are not mentioned in the study, not because they are unimportant, on the contrary, but because they are hardly subject to any conflicting views or usages. In addition there are little differences in the rules for most vaccines and rarely specific for the individual vaccines.

For each of the three vaccines under study an introductory summary of information on the virus, the disease and the principle types of vaccines are given.

Ebba Lund

1. THE GENERAL PROBLEMS OF ACTIVE IMMUNIZATION AGAINST VIRUS DISEASES

Active immunization against infectious diseases has been practiced long before knowledge of the nature of bacteria and viruses was available. Empirically it was found that by transfering infection from one individual (man or animal) to the other at a stage or during a period, where the disease seemed to run a mild course or where the individual seemed more resistent to the disease or its consequence, one might obtain immunization. This immunization might be obtained without disease or at least without any serious symptoms. Even to day such methods are employed. The obvious risks of starting an epidemic by spreading the infection further from the intentionally inoculated animals or of having wrongly interpreted the virulence of the strain so that even the incubated individuals become seriously ill cannot, however, be eliminated.

More systematic attempts to produce vaccines started in the Western world with Jenner, who in 1796 vaccinated against smallpox by the use of cowpox virus produced in calves. Pasteur, who applied vaccination somewhat more scientifically, named the process vaccination after vacca (cow) thereby acknowledging the importance of Jenner's work.

Vaccines against viral diseases are especially important, because virtually no therapy against virus infections exists, but with the encreasing tendency towards resistance against antibiotics, it seems possible that vaccinations against bacterial and protozooan diseases will also become more important than at present.

Vaccines should be safe and efficient, should preferably be effective for long periods after vaccination with a minimum of boosters, and complications should be few. Vaccines, which ideally fulfill these criteria, hardly exist, al-

though great progress has been made. The first necessary condition for producing a vaccine is that a method for the isolation and cultivation of the organism in question exists. This is not always the case. In other situations it may be possible theoretically to produce a vaccine, but practical and economic considerations may make vaccinations quite unrealistic. It is not reasonably to consider vaccination programmes for man or animals that include several hundreds of virus strains.

Because of rapid genetic variations in some virus strains it will become very difficult to carry out true profylactic vaccination programmes. It is very inefficient to vaccinate against last year's virus strain if this year's strain is so different that there is no cross-immunization possible between the strains. This type of problem may arise in connection with e.g. influenza of man and foot and mouth disease.

In some cases vaccination may be less efficient, because even the natural infection causes only transient protection. With present day knowledge it is, however, quite difficult to distinguish between an immunity of short duration and the result of a situation, where many diffirent serotypes of a virus exist, such that the same disease may repeatedly become manifest in the same individual but caused by different types of virus.

In the production of microorganisms to be used for vaccines it is not enough that the harvested material is antigenically potent. Both pathogenic and non-pathogenic organisms contain several antigens that have nothing to do with the provocation of or protection against disease. It is possible to cultivate bacteria, and possibly also viruses, in such a way that they may multiply, but have lost the ability to stimulate protective immunological reactions, whether the immunization is due to the formation of immune-globulins and/or functions through "cell-media-

ted" immunity. Thus it is not enough that a product is antigenically potent, it must contain the right antigens. Consequently although it is very important and useful to have a number of serological and other controling methods for the evaluation of the potency of a vaccine, ultimately the only true evaluation remains the evaluation of the protective capacity of a vaccine, estimated through a realistic challenge experiment employing the animals of the type that should be protected. All sorts of approximative evaluations may be obtained by emplcying methods giving information about antigenicity and other characteristics, but such methods can only serve as substitutes and even this only under very specific circumstances, which must be evaluated most carefully.

In a few cases (e.g. diphtheria and tetanus) the disease is almost solely due to the production of a single toxin. In such cases the vaccine production is a relatively simple matter, as it is possible by treatment with e.g. formalin to detoxify the toxin without destroying its essential antigenicity. Unfortunately, in by far the most of the infections, virulence of the pathogen and consequently also the protection of the host animal is governed by a number of factors, some of which seem difficult to identify and give their proper relative importance. A satisfactory vaccine therefore contains a mixture of components, some of which are perhaps not necessary, but they are at least apparently not harmful.

A virus in principle consists of a strand of nucleic acid containing the infectious material surrounded by a proteinaceous material of more or less complicated structure that sometimes contains other substances (lipids and carbohydrates). The antigenicity and specificity towards the host cell are a property of these outer parts. Consequently one may in principle operate with three different types of vaccine-preparations:

1) An inactivated (or "killed") vaccine, which is a sus-

pension of virulent, active virus, which by some means (usually chemical) has been rendered inactive (is "killed") to such a degree, that viable, infective virus-particles are no longer demonstrable.

2) A "live," attenuated virus vaccine, which contains a suspension of infective virus particles that are not disease-producing if used properly.

3) Extract vaccines where the virus suspension is fractionated chemically in such a way, that the nucleic acid component is not present in the vaccine neither as infectious nor as inactivated molecules. The vaccine contains, however, the proteins and should have full antigenicity.

1. 1. K i l l e d v i r u s-v a c c i n e s

A virus suspension is produced through the harvest of a material from animals, embryonated eggs or some kind of cell cultures which previously have been inoculated with the virus. This raw suspension is separated in a suitable degree from cellular components etc, and it is checked, that the amount of virus present is large enough so that the suspension after inactivation contains a sufficient amount of antigen to be useful as a vaccine. The inactivation may be carried out by means of formalin, beta-propiolactone or other chemical means, or physical methods, such as UV radiation, may be employed. The reaction is carried out, at a certain temperature and employing a certain amount of the compound or treatment, so that it may be deduced from previous experiments that the virus suspension is inactivated. On the other hand the reaction cannot be allowed to continue for so long that the antigenic capacity is diminished in such a way, that the product is not useful as a vaccine.

For a certain period in the history of the development of inactivated virus vaccines disagreable accidents took place, because such virus strains that were especially virulent were chosen as vaccine strains on the assumption that in

this way the best antigens were also obtained. As virulence and antigenicity are not properties that are correlated, all that was obtained was a vaccine that was potentially more dangerous, than if a less virulent strain had been employed. This was because a possible residues of active virus left, after the inactivation period were still of the most virulent type.

The testing of a vaccine is considerably more work-consuming and exacting than the production of the vaccine. The test work contains several important steps.

The vaccine is tested in a specified way to show that it can immunize suitable experimental animals (potency test). The control for different contaminants is a relatively simple matter as long as bacteria and fungi are thought of but the controls that must be carried out on the cellular material for contaminating viruses remain a complicated matter.

Innocuity tests for residuals of active virus in vaccines may present a statistical problem: It is not enough that a random sample of the proper size from a certain batch is without demonstrable amounts of active virus. Only if the whole production has been found equally satisfactory, may the single batch be accepted. It is statistically quite possible that a single batch out of an unsatisfactory production may pass the accepted tests and still not be acceptable, because of the probability that this batch also contains an unacceptable amount of virus. This problem was quite serious in the early years of polio vaccines.

Even if a vaccine has passed the different tests it is still possible that it may contain a small residue of active virus, which is under the threshold of the sensitivity of the test for innocuity. If the whole herd is vaccinated simultaneously this will not cause problems, but if single animals are vaccinated in non-immune herds the virus may after a number of animal passages be pro-

pagated in such amounts that overt disease is produced and further transferred. Corresponding problems may arise through unsuitable storage or wrong dosing of the vaccine so that more infectious virus is present in a vaccine dose than was intended by the producer.

When a vaccine has passed all tests required for showing that it is properly inactivated and contains sufficient amounts of antigen, the most important test must follow: Is it a vaccine, i.e. does it protect the animals against disease? Not too long ago it was generally accepted that if antibody production could be provoked, then protection would follow by necessity. It is now accepted, that "immunity" is a heterogenious capacity, and that a vaccine may contain good antigens, but give poor protection. On the other hand immunity may be present on a cellular level, so that an individual may be protected against a viral disease without having demonstrable amounts of antibodies.

It is usually accepted, that an efficient inactivated vaccine may protect, but that the protection is of limited duration. The period depends among other things on the amount of antigen inoculated. It is also accepted, that even a modest residual immunity may be boosted by a natural infection or revaccination.

Apart from the problems connected with the possibility of not having a sufficiently inactivated vaccine various side effect of undesirable nature may be encountered. These side effects are usually due to sensitization, so that hypersensibility reactions may occur in connection with revaccination. Individual animals may also show allergic reactions even at primary vaccinations because of reactions against components of the vaccine.

1. 2. L i v e v a c c i n e s

Live vaccines have advantages over inactivated ones, because by multiplication and spread of the virus in the organism a complete series of protection-provoking antigens may be formed, antigens which perhaps are not

formed in the same way outside the whole animal body. There must be specific reasons why live vaccines are most effective in a number of cases where cellular immunity seems to play an especially important role. This is found for tuberculosis, brucellosis and a number of viruses. Present day knowledge of immunology is not sufficient to explain the mechanisms involved.

The old smallpox vaccine by Jenner and all other pox vaccines are live vaccines. These vaccines are examples of original field strains, which have been very useful as live vaccines without any special manipulations. In most cases live, avirulent strains are obtained by attenuation through passages in a foreign host, so that infectivity is preserved, but virulence is lost for the normal host. The methods employed in this work are totally empirical.

The necessary controls for live virus vaccines are usually still more complicated than the controls for the inactivated vaccines. Controls must be established to make sure that the virus strain of the vaccine does not modify itself to the point of not being infectious for the normal host. The vaccine must also be so controlled that the strain through further passages in the foreign does not revert in the direction of the original, virulent virus strain. The purity of the vaccine must be controlled so that the vaccine neither contains bacteria nor viruses released from the cellular material. After that the protective capacity of the vaccine must be tested. As the virus, in a number of cases may spread from the vaccinated individuals it is necessary as far as possible to get an idea of the genetic stability of the virus strain, so that spread through several individuals of the natural host does not result in the reversal of the attenuated strain to a more virulent form. In some cases (e.g. rabies vaccines) it has been found, that a virus strain may be quite sufficiently attenuated for use in one species (e.g. dogs), whereas another species (e.g. cattle) may get the disease. If a vaccine contains a strain of virus, which may infect several wild and domestic

species, then careful consideration must be given to whether it is advisable to let loose a virus into a reservoir of animals with the possible result that a disease is spread rather than just getting an extended immunization. Such a disease may become next to impossible to control.

A special problem is encountered in connection with vaccination of pregnant animals or vaccination with vaccines containing viruses, which may spread from vaccinated individuals to pregnant animals. Any virus infection, even such natural infections that normally remain subclinical or with minor symptoms may be serious for the early embryo. Therefore even an attenuated virus strain, which normally gives symptoms of infection only in the rare case and therefore may be employed as a vaccine, may give problems in connection with pregnancies. Malformations or embryonic death, generalized congenital or neonatal infections may result depending on the time during pregnancy, when the infection occurs.

An important advantage in the use of a live vaccine is that the natural infection is copied to a much higher degree, than seems possible in connection with the use of an inactivated vaccine with the result, that the chance of acquiring a long lasting and general, cell-bound as well as humoral immunity is increased considerably.

The practical and economic advantages connected with the use of a live vaccine are of course considerable in all such cases, where oral administration or a spray preparation may be employed.

Live attenuated virus vaccines are often made with virus-types that are quite labile, i.e. then rather easily loose their infectivity (e.g. herpesviruses, paramyxoviruses) and therefore unsuitable handling and storage after the vaccine has left the producer may damage the quality of the live, attenuated vaccine: If the virus becomes inactivated or partly inactivated then no active virus or an inadequate dose to "take" is employed when vaccinating and no immunization is obtained at all.

Also in connection with the use of live vaccine hypersensitivity reactions may occur, e.g. against substrate components from the preparation, but the problems seldom become serious as has been the case with some inactivated vaccines.

By chosing the proper dose of a proper virus strain for the vaccine a 100% take could theoretically be expected. In practical situations interference phenomenons may, however, disturb the situation, because natural, but inapparent infections with other viruses may interfere, so that no take is obtained with the vaccine infection. For this reason revaccinations even with a live vaccine may be desirable.

1. 3. Extract-vaccines

If a culture fraction can be obtained which contains the protection-provoking antigens and no allergens, a very satisfactory vaccine may be obtained from such a fraction. It has been possible to produce such vaccines against e.g. pertussis and other bacterial infections, and a number of experimental virus vaccines have been made. From a control point of view an ideal situation arises, if the antigens and the infectious nucleic acid of the virus particles are separated from each other. Unfortunately the results obtained with e.g. measles vaccines have been far from promising. In spite of the fact that these vaccines have been good antigens, stimulating the production of antibodies, no production against natural infection was found. On the contrary, the average measles case in the vaccinated children was aggravated compared with the average case of non-vaccinated children. The same has sometimes happened, when inactivated vaccines have been employed.

2. THE CONTROL OF THE VIRUS DISEASES OF THE STUDY

For centuries the control of virus diseases of domestic animals has been of mutual interest to a number of countries, but a common policy does not exist, not even within the EEC. There are permanent threats in all three types of infections, but the ultimate control may not be achieved in the same way in all the cases. The proper procedure may depend not only on the virus, the animal in question and the epidemic situation, but also on the local economic, social conditions, traditions within the agriculture and many other things.

In principle there are only two ways of getting proper control: [1] general vaccination programmes or [2] eradication. The goal should be eradication, because in the long run this seems the only efficient way, but vaccinations may prove to be less costly and most valuable for years to come. With the proper vaccines the incidence rate of disease may be significantly reduced. Irrespective of the ultimate course to be followed joint efforts, improved diagnostic methods, standardization of procedures, the setting up of reference laboratories etc are the necessary minimum requirements to be satisfied to approach the ultimate goal of control.

3. FOOT AND MOUTH DISEASE VACCINE

3. 1. I n t r o d u c t i o n

3. 1. 1. Foot and Mouth Disease Virus

The virus of Foot and Mouth Disease was the first animal virus to be recognized as a filtrable agent by Loeffler and Frosch in 1897. The attempts to vaccinate against the disease were the immediate background for the discovery, which came a few years after the first description of a "filtrable agent", the Tobacco Mosaic Disease in plants.

The size of the FMD virus is around 25 nm, and the virus consists of an inner part of infective nucleic acid of RNA-type surrounded by a very densely packed shell of protein subunits symmetrically arranged around the nucleic acid strand. The virus belongs to the rhinoviruses of the picornavirus group ("the small RNA"-viruses) and is a stable virus resistent towards physical inactivation such as heat treatment, especially in the presence of high concentrations of organic material. The virus is easily destroyed both at high and low pH values.

There are 7 serological main types of FMD virus known: The A, O and C types, which are usually included in the trivalent vaccines employed in Europe. These three types are widely spread. The South African strains, SAT 1, 2 and 3 were found only in Africa until 1962, when SAT 1 spread to the Middle East and the European Turkey in 1962. Within the individual major groups new subgroups are occurring. This makes the inclusion of new subtypes in the vaccines necessary from time to time. The Institute at Pirbright is a World Reference Laboratory for the typing of strains.

3. 1. 2. Foot and Mouth Disease (Synonum: Aphtous fever)

A huge number of publications have appeared on the subject, Brooksby (1958) and Bachrach (1968) have published literature reviews.

The disease in cattle is usually not fatal, but leads to loss of condition and consequent economic loss. Fever and vesicular eruptions on mouth, tonque, muzzle, hooves and udder are characteristic symptoms. Sometimes lameness occurs. This symptom is prominent in pigs. Sheep, goat, deer, antilopes and other ruminants as well as pigs may become infected. A number of small laboratory animals may become infected experimentally.

The disease is extremely contagious by direct and indirect contact as well as by airborne dissemination. Uncooked meat and garbage may be a source of infection. The infection may be persistant for several months, and some animals may become cronic carriers. Australia, New Zealand, U.S.A. and Canada are free from the disease. The infection is endemic in parts of Continental Europe, in Asia, Africa and in South America.

3. 1. 3. Control

Where the disease is not endemic a policy of quarantine and slaughter may be an efficient and economically satisfactory means of control. However, in some areas the slaughter policies are supplemented with the use of inactivated vaccines. In endemic areas the use of formalin or acetylethylenimine inactivated vaccine with proper adjuvantia is commonly used. The raw material for a vaccine is either epithelia from tongues of infected cattle or some sort of cell cultures prepared from primary tissues or from established cell lines.

A number of countries employing compulsory vaccinations have strict rules about accepting only imports of vacci-

nated cattle in order to protect the local cattle. If the import is from a FMD free area this requirement may not be very useful as a safeguard. It might even be possible, that the vaccinated cattle occasionally represent a certain risk not present in the unvaccinated cattle. Accidents have occurred when the vaccine had not been properly prepared or tested. Import from areas with compulsory vaccination, which may mask or alter a FMD virus infection may, on the other hand, constitute real risk, but only if FMD is present in the area.

Attenuated live virus vaccines have been prepared by passages in mice, cell cultures or in eggs. The results of such vaccinations have varied in field trials and such vaccines are in general considered too dangerous for general use because of the wide host range of the virus.

F.M.D.V. Review References:

Brooksby, J.B. (1958) Adv. Virus Res. 5,1.
Bachrach, H.L. (1968) Ann. Rev. Microbiol. 22, 201.

3. 2. Legislation and regulations for FMD vaccines for cattle in the different Member States. A review of the relevant documents

Belgium

Arrêté royal du 3 avril 1965 relatif á la lutte contre la fiévre aphteuse.

Denmark

Apotekerloven af Juli 1962 (The Drug Act of July 1962). Important exceptions are granted for products from the State institutes. New legislation in preparation (Lov om lægemidler), but no essential principles seem changed.

From the Ministry of Agriculture there is Lov om vaccination mod mund- og klovesyge (Act on vaccination against foot- and mouth disease) March 1960 and Bekendtgørelse om vaccination mod mund- og klovesyge (Order on vaccination against FMD) Nov. 1960.

France

Contrôle officiel des vaccins antiaphteux (Arrêté du 8 juin 1965 J. Off. 29 juin 1965).

Arrêté portant modification de l'arrêté du 8 juin 1965 relatif au contrôle officiel des vaccins antiaphteux (J.O. 31 décembre 1965).

Arrêté du 2 juin 1971 relatif au contrôle officiel des vaccins antiaphteux.

Germany

3. Verordnung zum Schutz gegen die MKS von 29.1.71 einschliesslich der Anlage über die Impfstoffe.
Verordnung über Sera und Impfstoffe nach § 170 des Viehseuchegesetzes vom 27.2.73.
Ausführungshinweise zur Verordnung vom 27.2.73.
Richtlinie für die staatliche Prüfung von Maul- und Klauenseuche-Vakzinen (Juni 1973).

The paragraph 7 was modified on July 2, 1974. (The rules of the 4. rev. of the pharmacopea commission is not agreed upon by the Germans).

Holland

The legislation is the responsability of the Veterinary Department. Contrary to all other veterinary vaccines the FMD vaccine is produced and controled by Centraal Diergeneeskundig Institute in a special department in Lelystad.

Ireland

The Therapeutic Substances Act of 1932 has no veterinary equivalent.

Animal Remedies Act 1956.

Diseases of Animals (Disinfection) Order of 1931.

Italy

L. Ravaioli, Z. Orfei and M. Granieri: Controllo dei sieri, dei vaccini e dei prodotti diagnostici per uso veterinario Istituto superiore di sanita. Laboratori di Veterinaria. Roma 1964.

Ordinanza Ministeriale 7 luglio 1972. Profilassi vaccinaleobbligatoria dell'afta epizootica.

Normes por le contrôle du vaccin antiaphteux en Italie. Bull. Off. int. Epiz. (1972), 77, 1143-1147.

Declared intensions to follow the European Pharmacoposia Commission PA/PH/Exp. 15V/T (70)12. 4th revision of 12 April 1973, also Legge a 23 Giugno 1970, n 503 Ordinamento degli Istituto Zooprofilattico sperimentale. 11 Marzo 1974, n 101 Modifica delle legge 23 Giugno 1970, n 503 sull' ordinamento degli istituti Zooprofilattico sperimentale.

By this law the institutes of Torino, Brescia, Padova, Perugia, Roma, Teramo, Portici (Napoli), Foggia, Palermo and Sassari are research institutes doing general diagno-

stic work under the supervision of the Ministry of Publ. Health for particular problems. - Local problems are dealt with by the regional Public Health Author.The division of responsability is not always well defined.

Luxemburg

Follow Belgian rules.

U.K.

Medicines Acts 1968 and 1971.
The Medical Act 1969.
Guide to the Licensing System, prepared by the Dept. of Health and Social Security on behalf of the Health and Agriculture Departments of U.K., 1972.
Notes on applications for Product Licenses for veterinary medical products, prepared by the Ministry of Agriculture, Fisheries and Food on behalf of the Health and Agriculture Departments of the United Kingdom.

All vaccines are controlled under the Medicines Act. Regulations can be laid down for individual vaccines, but have not been for FMD.

3. 3. P r o d u c t i c n

3. 3. 1. Identification of the Producer

Belgium

Only the State Laboratory (Institut national de Recherches vétérinaires) may produce. All other products are forbidden including imports.

Denmark

The State Vet. Institute for Virus Research produces the vaccine and handles the viruses in question. The ministry of Agriculture may decide on imports of vaccines.

France

Any producer who, after application to the ministry of agriculture and on the base also of tests of a preliminary lot of vaccine, has obtained an authorization to produce a vaccine may do so. The rules and the criteria for the production and vaccine are specified in details in the legislation and the rules are such that as a matter of fact few institutes could manage to produce.

Germany

A producer who has obtained a permission according to the legislation (§ 7 and 8). Each batch must, however, be controled and released by the Authorized State Institute. There are at present 3 producers: Behringwerke, the German Wellcome and Bayer AG.

Holland

Only the State Laboratory, the Centraal Diergeneeskundig Institut has a governmental license to produce.

Ireland

There is no Irish production.
Imports would be by permission of the Department of Health

(after consultation with the epartment of Agriculture), but a license has never been issued.

Italy

The Institutes (Public institute directly under the ministry) authorized by the State. At present 4 such institutes are in principle producing, but in very different amounts.

Luxemburg

No production.

U.K.

At the Animal Virus Res. Inst., Pirbright, a stock of vaccine is held. The only licensed producer is situated at this institute, and no one else is allowed to handle the virus. The vaccine has never been used.

Summary:
The situation in the individual Member States is summarized in table 1. As may be seen three categories exist: No production (Ireland), production by licensed private firms (France and Germany) or by a State Laboratory (Belgium, Denmark, Holland, Italy and U.K.).

Table 1. Foot- and Mouth Disease Vaccine for Cattle

Schematic presentation of FMD vaccine use

Country	Producer	Controling authority	Rules for vaccination
Belgium	The State (Institut National de Recherche Vétérinaire)	The producer (the State)	Compulsory once a year
Denmark	The State (The State Vet. Institute for Virus Research)	The producer (the State)	Only in emergencies or by special permission
France	Licensed private firms	Two Institutes of the State (Alfort and Lyon) according to a distribution scheme by the Lyon-Inst.	Compulsory once a year
Germany	Licensed private firms	The State (Bundesforschungsanstalt der Viruskrankheiten)	Compulsory once a year
Holland	The State (Centraal Diergeneeskundig Institutt)	The producer (the State)	Compulsory once a year
Ireland	No production		Forbidden except by special permission by the ministry (has never been given)
Italy	Publ. Inst. directly under the Ministry	The State through two controllers either 1) Istituto Superiore di Sanita or 2) Direzione Generale Vet. Ministero della Sanita	Compulsory once a year
Luxemburg follows Belgium			
U.K.	In principle licensed private firm	The State (The Animal Virus Res. Inst. Pirbright)	Forbidden except in emergencies (vaccine never used)

3. 3. 2. Licenses for Establishment

Belgium

As only the Institut National de Recherches vétérinaire may produce there is no special license system.

Denmark

Although no legislation specifies this,the Vet. Auth. actually through their right to distribute the vaccines employed, control production so that only the Veterinary State Institute for Virus Research produces vaccine.

France

Legislation prescribes that after approval of premises etc and tests carried out on a preliminary lot, an authorization to produce may be obtained from the State. The rules are quite detailed. The product must be tested in bovines and have a K-value (see later) of $\geq 10^{1.2}$.

Germany

Although the legislation concerning production control since 1973 prescribes that the sample taking and testing is done by the State, it is still up to the Regional (Länder-) Government to issue establishment licenses.

Holland

No special license as the State Lab. is the only one permitted to produce.

Ireland

No production of vaccine.

Italy

The Publ. Inst. are selected and authorized by the Ministry, but the Regional Publ. Health Authorities have influence, at present Istituto zooprofilathico sperimental della Lombardia e dell' Emilia (Brescia) and the

corresponding institutes in Teramo, Perugia, Padova and Torino are authorized, but most of the vaccine is produced in Brescia.

U. K.

Licenses are issued for veterinary products by the Ministry of Agriculture, Fisheries and Food. The FMD vaccine production is the responsibility of Pirbright, but the general principles laid down in the Licensing system must be followed, and it is in fact the Wellcome Foundation, who produces.

3. 3. 3. Licenses for Release

Belgium

No special license as only the State lab. produces.

Denmark

The same as for Belgium. The Vet. Authorities are de facto releasing through their distribution of the vaccine.

France

All lots of vaccine whether produced in France or imported (through a permission given to a French laboratory) are tested in the finished state and on minimum lots of 50,000 doses of trivalent vaccines. The producer must request, that samples be taken. The producer of a batch of vaccine may at his own responsability release the vaccine, if he has not received a test result after 50 days. The controling lot may permit the use of the vaccine before the results are obtained from the tests, if the protocols of the producer show, that the vaccine is better than the quality required in the official test (this applies only to the period Jan. 1 to April 30).

Germany

The authorized State Institute releases the individual

production batches. According to the legislation the Authorized Lab. must decide if the vaccine is actually tested by authorized Institute, or if the tests carried out by the producer according to protocols are sufficient, but the official testing as such is compulsory. The rules are commented and further elaborated in the "Ausführungshinweise". There are a number of rules concerning the rooms etc., also about who is sampling and what samples are taken and kept, the protocols of production and other relevant subjects. If considered necessary because of the epidemiological situation, the Ministry of Agriculture may deviate from the normal control rules.

Holland

Same as for Belgium and Denmark; no special licence required and the State Lab produces the vaccine.

Ireland

The Vet. Auth. may decide on imports and this would in principle correspond to a release license.

Italy

Batches of one million doses of trivalent vaccines are licensed by the Istituto Superiore di Sanita after the prescribed tests have been carried out.

U. K.

The vaccine would be released by Pirbright, but has actually never been used.

3. 4. Controlling Authorities

Belgium

The producer (the State Laboratory).

Denmark

The producer (the State Lab.).

France

Two State Institutes: Le laboratoire central de contrôle et de recherches du service vétérinaire à Alfort et le laboratoire de virologie animal à Lyon. In Lyon a scheme is decided according to which the individual samples are distributed between the two laboratories.

Germany

The State through the Bundesforschungsanstalt der Viruskrankheiten in Tübingen.

Holland

The producer (the State Laboratory).

Ireland

No production or control.

Italy

In principle The State, but through different organisations than the producing institute. Some tests such as serum neutralization tests may be carried out in the Istituto Superiore di Sanita in Roma, but the challenge tests are carried out in the producing insitute under close supervision by one representative from Direzione Generale Veterinaria Ministero della Sanitá. The Torino institutes have their cattle tests carried out in Brescia, the Institute of Padova in Teramo.

U. K.

On behalf of the Ministry of Agriculture, Fisheries and Food the A.V.R.I., Pirbright, is testing the U.K. strategic reserve.

3. 5. The Controls and Standard required

3. 5. 1. General Rules

Belgium

No specific recommendations, bu the control is made according to the OIE and FAO standards.

Denmark

No general rules stated.

France

Rules are given both for the tests that should be carried out by the producer and for the official testing. A number of physical-chemical and sterility tests etc. are also stated. The rules for sampling are very concise and detailed.

The regulations concerning official testing of FMD vaccines in France (Order 8 June 1965 with annex and interpretation) were published by F. Lucam et al. (Le Contrôle officiel français des vaccins antiaphteux) in the OIE Bulletin vol. 65, No. 3/4, pp. 385-418, 1966. The Order 8 June 1966 was modified by Order 2 June 1971 (Journal Officiel No. 5590, 10 June 1971).

Details of testing procedures and statistical interpretation of results as applied in Lyon are given in "Le contrôle des vaccins antiaphteux inactivés" published by J. Fontaine et al. in Revue Méd. Vét. vol. 122, pp. 289-321, 1971.

Germany

A number of rules for sampling, sample size, sample storage etc. are given. The specifications for purity tests are given. The in-plant tests carried out by the producer are apparently not stated in the legislation except through the

general rules for vaccine production. These rules are very detailed. So far the Germans do not agree with the tests suggested in the Pharmacopoeia Commission. It is specifically stated, that the vaccine may be produced in calves, in Frankel cultures or in cell cultures from calf kidney or BKK cells.

Holland

The vaccine is produced in Frankel cultures. The protocol for the testing stays within the producing department. No general rules are stated.

Ireland

No production.

Italy

The required vaccine standards as to sterility and purity, container quality and labeling are expressed in the rules from 1964.

The rules from 1964 differ from the "Norme pour le contrôle-" 1972. The controls and standards officially required by the State are the ones of the publication from 1964, but both the control lab. of Istituto Superiore di Sanita, Roma, and the producing institutes are in general doing better than that, and the "Norme"-publication of 1972 is followed.

U. K.

No general rules are stated for the official acceptance tests or for the batch testing by the producer.

3. 5. 2. Innocuity Tests

Belgium

Testing is carried out on three groups of three cattle

each. The first group receives the vaccine contained in one bottle at the beginning of the bottling process, whereas the second receives the vaccine from one bottle in the middle and the third group receives the vaccine of the last bottle.

In each group, one animal receives one dose of vaccine by intramuscular injection and one dose intradermolingually.

The other two animals are vaccinated regularly with one dose of vaccine injected into the dewlap. The animals are kept under observation for three weeks.

Denmark

A. Tests in mice: 8 litters of baby-mice (2-4 days old) are inoculated intraperitoneally. Dose: 0.1 ml per baby-mouse. This examination is passed if none of the baby-mice dies within the first six days.

B. Tests in cattle: Three susceptible heifers, of which the body-temperature is controlled, are inoculated intradermolingually. Each animal is given 2 ml of the vaccine injected at 10 different sites in the middle third of the tongue. Every second day the body temperature is measured and two weeks after the inoculation a careful inspection of mouth and feet is made. If wounds, scars or other abnormalities are not found on the tongue, gingiva, palate as well as between and around the hooves, the vaccine is declared safe.

France

The vaccines must be incapable of producing FMD irrespective of the way it is used, and may not produce any pathological condition, when correctly used. The finished product is tested employing heifers from areas of Brittany. The vaccine may not produce vesicles where cattle have not been vaccinated. The vaccine must not produce vesicles or any other important pathological manifestations.

The tests by the producing institute for monovalent vaccines (dose 1 ml) on all batches are: Tests on primary cells of pig kidney for cytopathic effect; tests on cattle with the completed vaccine (Method of Henderson); injection (intradermolingual) of at least 2 ml of vaccine, distributed over 20 points of the tongue, of 2 or 3 cattle; subcutaneous inoculation of 5 ml of vaccine (3 doses).

Trivalent vaccines (dose 5 ml). The mixtures of trivalent vaccines are prepared from monovalent vaccines tested on cells and cattle. An innocuity test in cattle is carried out on all batches according to the same method as the one carried out with monovalent vaccines, however, trebling the injected doses.

The tests are carried out by the official controller. Only the batches of trivalent or monovalent vaccines which are ready for sale are tested officially. The vaccine is tested in calf kidney cells for cytopathic effect and is tested in cattle by a method similar to that applied by the producing institute.

Germany

Testing for innocuity and potency are - with the exception of serological tests - carried out in the plant of the producer or in some other place considered suitable by the testing institute. The tests must be carried out in the presence of the official controller.

Innocuity testing is carried out for infectious virus and tolerance.

The test for infectious virus is carried out in cattle (maximum age 2 years), which do not show any signs of disease, and which are free from neutralizing antibodies against FMD virus. It is assumed, that cattle are neither affected by nor immunized against FMD, if the neutralization titre is smaller than 1:4.

The vaccine is tested in 3 heifers by intradermolingual inoculation employing 5 ml of vaccine and also 0.1 ml

inocula of a concentrated (1:60) eluate from 3 l of the vaccine. After 4 days a 4-fold dose of the concentrated eluate is given subcutaneously (at least 20 ml). In addition the vaccine is tested in cell cultures in a prescribed way. Only if neither animals nor cell cultures react to the inoculation, the vaccine may be accepted. The vaccine must be rejected if the general reaction in one or two animals vaccinated in the potency test is so strong, that the health is considerably impaired, or when local reactions at the point of vaccination in one or more animals exceed the usual pattern, and such findings are confirmed in subsequent trial vaccinations in the same number of animals.

Holland

The innocuity of each batch is ascertained by injection into Irish steers. The number of animals depend on batch size (5 steers for a 2.000 l batch). Each animal receives about 5 ml of vaccine by means of multiple intradermolingual injection. After three days they receive an additional 20 ml intramuscularly. The animals are observed for 10 days. Temperature is measured twice daily. Thereafter, they are autopsied. Tongue, mouth, feet and rumen receive special attention.

Italy

The in plant innocuity test is done in mice for the weekly batches. The official testing is done according to the French rules by intradermolingual inoculation of 0.1 ml of vaccine each into 20 different sites on the tongue of 15 months old cattle. Four days later, if no lesions have been observed, the vaccine is injected into the normal route, but as a treble dose into the same animals.

U. K.

Initial tests are made by the manufacturers on the inactivated unformulated antigens, using a tissue culture technique. Six aliquots of 25 ml of each antigen suspension are tested for the presence of infective virus in Roux bottle cultures of BHK 21 cells. If cytopathic effects are not observed, the same volume of each culture medium

is further subcultured into two fresh cultures. In all, a total of three subcultures is made.

Antigen suspensions which have passed the tissue culture test are blended and vaccine is formulated by the incorporation of adjuvants. The innocuity test in cattle is made on the final product. Three cattle (Devon or Devon x Shorthorn steers, 18-24 months of age) are inoculated with the vaccine by intradermal injection at multiple sites on the tongue. Rectal temperatures are taken and the tongues and feet of the cattle are examined periodically for a total of 10 days. Provided no lesions associated with virus growth are observed, the vaccine batch is accepted as innocuous.

3. 5. 3. Potency Tests

Belgium

Six cattle are vaccinated per dose. Requirements: total absence of generalization lesions during the 15 days following challenge (10,000 bovine ID_5 intradermolingually). The activity of the vaccine is evaluated by serological methods.

Denmark

a) 20 guinea pigs are given each 1 ml of vaccine subcutaneously. Three weeks after vaccination a challenge dose of 10^3 guinea pig ID_{50} of virulent virus is given. Generalized disease should be prevented in the vaccinated animals with typical disease in the controls. If 10 or more of the vaccinated animals are free from secondary lesions the vaccine has passed the test.

b) 4 heifers are vaccinated subcutaneously using 10 ml of vaccine. After 3 weeks blood samples are drawn, and the serum titre determinated in cell cultures and in mice against 1500 ID_{50}.

The vaccine is registered according to the serum titre obtained in the following way:

Titre in cell cultures - - mice	≥ 32 ≥ 128	8- 32 32-128	< 8 < 32
Vaccine quality	I class	II class	not to be employed

Challenge experiments on the 4 vaccinated heifers are carried out 3 weeks after vaccination employing a concentration of 10^4 mouse ID_{50}/ml of virus. Two heifers are tested with intradermolingual inoculation of a 0.25 ml virus and two by rubbing their tongues with a virus drenched pad of cotton. The animals are kept under observation for two weeks. The virulent virus is controlled by titrations in mice, not in cattle, in order to minimize the spread of virulent virus. The challenged animals should remain completely free from generalized infection (secondary lesions).

France

Potency tests carried out by the producing institute (for monovalent vaccines): After a satisfactory innocuity test the protective dose 50% is established in guinea pig (PD_{50}) for all batches of vaccine. The vaccine dilutions are made in buffer, without adjuvant.

For each series of monovalent vaccines (say 4 to 6 batches), produced under the same conditions, the PD_{50} (cattle PD_{50}) is established. 15 cattle are vaccinated, i.e.: 5 with one vaccine dose, 5 with 1/4 of the dose and 5 with 1/16 of the dose, the dilutions being made with buffer without adjuvant.

Three weeks later, 10000 I.U. of fully virulent virus are inoculated in the vaccinated animals by the intradermolingual route in two points. After 5 to 6 days the animals are slaughtered; the primary and generalized lesions will then be registered. The potency in cattle (number of cattle PD_{50}) referred to one dose of vaccine, is then calculated according to the Probits method. Only those vaccines which have a PD >5 are used for the preparation of trivalent vaccines.

The antibodies of cattle vaccinated with 1 full dose are then titrated by serum-neutralization

Trivalent vaccines

For each series of vaccines (4 to 8 batches) prepared with tested monovalent vaccines, the PD_{50} is established for one batch. The method is identical to that employed for monovalent vaccines, the only difference being: that the volume of the dose is trebled.

The antibodies of the animals are titrated for each of the three valences, by serum-neutralization.

Potency test carried out by the official controller:

Generally trivalent vaccines are submitted to testing. Only those monovalent vaccines are tested which are destined to be marketed as such.

First test: Establishment of the C index in guinea pigs. (The logarithm of the index between the virus titre in vaccinated guinea pigs and the virus titre in unvaccinated guinea pigs). Four different levels of virulent virus are used and 24 animals. The vaccine is acceptable if $C \geq 2$. In such cases the cattle test may be omitted for batch control.

Second test: Establishment of the S index in calf kidney cells (serum-neutralization test). The serum from the vaccinated heifer is tested in o.1 ml amounts against 50 ID_{50} of virus produced in cell cultures. If the S value obtained is more than, or equal to, 1.5 for a batch control, the K determinations may apparently be omitted.

Third test: Establishment of the K index in cattle (The logarithm of the index between the virus titre in vaccinated heifers over the titre in unvaccinated heifers.) The unvaccinated heifers are read on day 1 after inoculation and the vaccinated on day 2 after inoculation. The K-value, when employing a high titre of virus should be $\geq$ 1.2 for

a class I vaccine, if 1.2 > K > o.6 the vaccine is class II, and if K ≤ o.6 the vaccine is rejected. Only 5% of a vaccine production may fall in class II.

Germany

Healthy cattle free from previous contact with FMD are used (three test animals and one control animal).
The vaccine has to be administered to test cattle in the form and quantity as foreseen in the directions of the producer; the control animal is not to be vaccinated.
The test and control animals are to be observed for at least 14 days. During this period they have to be kept in such a way as to exclude any possible FMD infection.
On the 15th day at the earliest or on the 21st day at the latest post-vaccination each test group has to be stabled separately and challenged with FMD virus Types O, A or C. The selection of strains is the task of the testing institute. The challenge is carried out by intensive rubbing of the tongue, the muzzle and the nostrils of the vaccinated animal with a cotton cloth. The cloth (about 30 cm by 30 cm) is soaked in a suspension of the corresponding virus types with a minimum infection titre of $10^4 LD_{50}$/ml for unweaned mice; at least 75 ml of the virus suspension are to be used for soaking the cloth. The soaking has to be repeated for each animal and the control animal is treated in the same way. The animals have to be observed for at least 10 days. Every day the body temperature has to be taken. On the tenth day post-infection, the animals must be slaughtered. An exact anatomo-pathological examination has to be made of the test and control cattle; the examination has to refer in particular to specific abnormalities on the tongue and other parts of the oral cavity, the muzzle and the hoofs.

The control cattle must contract generalized disease, (appearance of vesicles in animals under test).Virus recovered from the vesicles must accord in type with the innoculated virus as established by complement fixation.

If the reaction appears to be caused by a virus type other than the one used for challenge or if generalization did not come forth both in the test animals and the controls, challenge is to be repeated with the same virus type.

In the case of accordance between vaccine and challenge virus type:
a) the test has to be repeated if 1 test animal has generalized or two and more test animals have shown primary vesicles in the oral cavity,
b) the vaccine is to be rejected if two or more test animals have shown generalization.

In the case that according to the letter (a) testing is repeated, the number of the animals in one test group must be doubled. After repetition of the test, the vaccine will be rejected if:
one or more test cattle came down with generalized disease or if:
three or more test cattle showed primary vesicles in the oral cavity.

Instead of testing cattle, the determination of the potency for one valence (type A or C) of bivalent or trivalent vaccines can be carried out in guinea pigs.

To this effect 56 guinea pigs, weighing about 450-550 g, should be used. 28 of these animals will be given subcutaneously 1/20th of the usual cattle dose. 28 animals remain untreated as controls. Challenge is carried out 18-21 days post vaccination. Virus strains, designated by the testing institute for cattle tests to serve as infectious virus, shall have been adapted through 5-7 passages in guinea pigs in such a way that at least one secondary vesicle (generalization) appears after intraplantar injection with o.5 ml of a virus suspension of a dilution of 10^{-2} in at least 90 percent of the animals.

Potency testing of the vaccine is carried out by titration of the test virus in both the controls and the vaccinated ani-

mals. Each group of 7 controls animals is infected by intraplantar injection with 0.5 ml of virus dilutions from 10^{-3} to 10^{-6}. Each group of 7 vaccinated animals receive the test virus in the same way in dilutions ranging from 10^{-2} to 10^{-5}. The results are read 72 hours later and an animal is considered positive as soon as a secondary vesicle is demonstrated. The titre calculation for both groups is done according to the method of Reed and Münch. The protection index C is the quotient of virus titre in the controls divided by virus titre in the vaccinated animals. The protection index must amount to at least 2.0, in which case the titre in the controls must reach at least 10^5 ID_{50}/ml. If the titre lies below this figure the test can be repeated in guinea pigs. If, however, the protection index is less than 2.0, the potency test of this valence is to be repeated in cattle.

Holland

The 50% protective dose of each batch is measured in guinea pigs. A mixture of each four of five successive batches of one type is prepared and its PD_{50} is measured in cattle. Three four fold dilutions of the vaccine in the vaccine base are each injected into five steers. These are then challenged by i.d.l. injections of 100.000 cattle ID_{50} two weeks after vaccination. The test is read after 8 days when the animals are autopsied. The development of feet lesions is considered proof of sufficient protection.

It is desired to have 10-12 cattle PD_{50} pro type into each dose of vaccine, the minimum being 6 PD_{50}.

Italy

Guinea pig tests and seroneutralization tests in cell cultures are employed for in plant routine controls and also by Istituto Superiore, but the official tests are done in cattle:

The trivalent vaccine is injected in a full dose, and after dilution in buffer solution also as a 1/4 and 1/16 dose. Three groups of 15 animals (15-24 months old) are injected and in addition three animals serve as controls in each group.

Twenty days after vaccination, each group of 18 animals is challenged intradermolingually with 10,000 ID_{50} of cattle virus distributed over two different points of the tongue. The titration of the challenge virus is done in mice and only the challenge dose proper is inoculated in the cattle.

The animals are observed for 5 days: one single lesion in one foot means generalization; the controls must generalize within 48 to 72 hours. Potency is determined quantitatively by the Probits method (according to Litchfield). Eight Pb (potenza bovina) are required against the homologous virus. Antibody determination is carried out by serum neutralization (testing of pre-sera etc.) according to a standardized procedure.

U. K.

Potency is measured by the antigen extinction limit. For this purpose the final product is diluted out in a vaccine base containing the normal concentrations of adjuvants (aluminium hydroxide and saponin). The vaccine is diluted out in 5-fold steps to give the series 1:2, 1:10 and 1:50. Groups of 8 cattle similar to those used for innocuity tests are inoculated by subcutaneous injection behind the shoulder. The dose volumes are 3.0 ml for monovalent and 5.0 ml for trivalent vaccines. Results are calculated from either:

(a) Response to challenge
(b) Assessment of each animal's neutralizing antibody titre.

Challenge. Cattle are challenged at 21 days after vaccination by the intradermal inoculation of the tongue at 10 sites with $10^{4.0}$ cattle ID_{50} of the homologous strain maintained by serial passage in cattle. The challenge strain is titrated by the intradermal tongue inoculation method in 2 cattle shortly before the vaccinated cattle are challenged. These cattle also serve as controls to

the virulence of the strain and must show the rapid development of severe secondary lesions. Cattle are classed as protected if no secondary lesions develop on the feet. The test animals are observed for up to 10 days after challenge.

Antibody essays. All test cattle, whether for challenge or not, are bled for serum before vaccination and at 21 days after vaccination. Neutralizing antibody titres of each serum are measured, using the Cell Metabolic Inhibition test (CMI) with BHK 21 cells in microtrays. For the O_1-BFS.1860 and C-Noville vaccine strains, correlations of antibody levels and protection in challenge tests have been made. With the type O vaccine, cattle with serum titres of $\log_{10}$ 1.65 or higher are regarded as "protected" and, with the type C vaccine, the appropriate figure is $\log_{10}$ 1.20. At present, vaccines containing the type A component (A-Pando) are assessed by challenge only and data are being accumulated on the correlation between antibody levels and response to challenge.

Calculation of results. The response of test cattle to challenge is assessed on a quantal response basis, i.e. individual animals are classified as protected or not protected and no attempt is made at present to further quantify each animal's response by a lesion scoring method.

PD_{50} values are calculated by either the method of Spearman-Kärber or by full probit analyses. The minimum required standard for each vaccine or component of a trivalent vaccine is 6 PD_{50}.

3. 5. 4. Expiration

Belgium. No information obtained.

Denmark. Retested every 6 months if stored.

France. 12 months after the official taking of samples. May be retested.

Germany. 18 months after the official potency test.

Holland. 18 months after the potency test. Retesting is possible.

Italy. 12 months after the official potency test. May be retested.

3. 6. V a c c i n a t i o n

3. 6. 1. Rules for Vaccination

Belgium

Vaccination is compulsory for all cattle older than 3 months. The vaccination takes place in Dec.-March, and not more than 13 months may pass between vaccinations (unless the vet. inspector has decided otherwise).

Denmark

Danish cattle is not ordinarily vaccinated, but the Vet. Authorities may order vaccinations, if considered necessary in connection with acute spread of disease or may permit vaccinations, if certain areas or groups apply for it. The vaccination may only be carried out according to rules set by the Ministry of Agriculture and using a vaccine furnished by the Vet. Authorities, who decide which types of vaccine should be employed.

France

Vaccination once a year is compulsory for cattle more than 6 months old. The vaccination must be carried out using a vaccine tested and released by the control lab. authorized by the state.

Germany

Vaccination is compulsory once a year for all cattle and may be extended to sheep and goats. (3. Verordnung zum Schutz gegen die MKS § 1). The vaccination must be carried out using a vaccine made and tested and released according to the "Anlage über die Impfstoffe" attached to the regulation.

Holland

Each animal must be vaccinated once a year with a vaccine licensed by government. The vaccine is delivered in 3 bottles á 5 ml. The vet. officer is mixing the contents prior to use.

Ireland

In principle vaccination is forbidden. As the country has been free from the disease, no license for import of vaccine or its use has been issued by the Department of Health.

Italy

Annual vaccinations are compulsory for cattle, buffalos, sheep and goats from the age of 4 months (except for Valle d'Aosta) according to the specified rules. The vaccine is prepared according to the suggestions from The Ministry of Publ. Health and bought solely by the State. A 3-valent vaccine with 5 ml total vaccine dose is employed.

U. K.

The use of FM vaccine is forbidden. At the Animal Virus Res. Inst., Pirbright, a stock of vaccine is held. The only licensed producer is situated at this institute, and no one else is allowed to handle the virus. The vaccine has never been used.

The situation in the different Member States is summarized in Table 1.

3. 6. 2. Economics

The rules for payments for vaccines and vaccinations differ from one Member State to the other. It varies also according to the type of vaccination, i.e. if it is compulsory, encouraged or permitted.

The information obtained is as follows:

Belgium

No information obtained.

Denmark

Both the vaccine and the vaccination are payed for by the State when vaccination is required.

Table 2. Foot- and Mouth Disease Vaccine in Cattle

Economic Aspects

	Vaccination paid by	Indemnities in case of vaccine-associated accidents paid by
Belgium		The situation has not occurred, and no rules are fixed
Denmark	The State	The State in cases of death in immediate connection with the vaccine
France	The farmer. In some parts of France paid by local authorities	In principle the State
Germany	In part by the State in part by an epidemic diseases fund to which every farmer contribute	The epidemic diseases fund paid by the farmers
Holland	The farmer	The producer (the State)
Ireland	-	-
Italy	The State	Officially the State, but in fact the producing institute
Luxemburg		
U. K.	-	-

France

The farmers pay in general for the vaccine and vaccination, but in some provinces it is paid for through the local authorities.

Germany

The vaccination is in part paid for by the State and in part by a vaccination-fund, to which the farmers contribute according to the number of animals.

Holland

The farmer must pay.

Ireland

No vaccination.

Italy

The vaccination is paid for by the State.

U.K.

No vaccination.

The material of 3. 6. 2. is collected in Table 2.

3. 6. 3. Indemnities

In case of vaccine-associated accidents the economic responsability is in some Member States defined clearly and in other cases apparently not at all.

The information obtained is as follows:

Belgium

The situation has not occurred, and no rules are fixed.

Denmark

The State pays indemnities, if death occurs in immediate connection with the vaccination.

France

In principle indemnities are paid by the State.

Germany

Indemnities are paid by the vaccination-fund, to which the farmers contribute according to the number of animals.

Holland

The State (the producer) pays indemnities.

Ireland

Vaccine has never been used in Ireland.

Italy

Officially it is the State authorities, but in fact it is the producing institute. The Brescia Institute has a special voluntary insurance, that will cover immediate deaths in connection with vaccinations. The insurance has seldom been used.

U. K.

The vaccine has never been used in U. K.

The rules are summarized in Table 2.

3. 7. Proposals for Community Regulations

3. 7. 1. A Summing up of the present Situation

The Council Directive of 26 June 1964 (64/432/EEC) and the amendments following (66/600, 70/360, 71/285 and 72/97) state, that measures must be taken to eliminate differences between the health requirements of Member States.

There are still possibilities for nationally maintaining severe restrictions on imports and exports or transits in order to safeguard the health of man and animals in acute defined situations, but such possible rights do not suppress the duty of providing conditions that may result in the realisation of a mutual Community policy.

It is also clearly stated, that it is the duty of the individual Member State to guarantee, that animals for breeding or slaughter do not constitute a risk of spreading infection through intra-community activity.

The present day situation of F.M.D. vaccinations within the Community is quite complicated (see e.g. table 1 and 2). Vaccinations are compulsory in 6 Member States and not employed in the three new Member States, but within the 6 states there are important differences in the rules for production and control. European Pharmacopoeia Commission rules for vaccine production and standards are apparently not finalized. According to the European Treaty Series No. 50, the rules agreed upon by the Pharmacopoeia Commission should be the official standards for the member countries, so it is of obvious importance not to make any suggestions within the Community, which are in disagreement with the decisions of the Pharmacopoeia Commission.

3. 7. 2. Proposal for a Community Regulation

In view of the general duty according to the Rome treaty the following proposal is suggested:

1) As a common rule for the Community, vaccination of all cattle should be compulsory once a year employing an inactivated vaccine of agreed European standard.

2) If a region has previously been maintained unvaccinated, and no cases of FMD are diagnosed in the area, such region may be maintained without vaccination. Consequently only live cattle exported to vaccination-regions must be vaccinated. It may be decided by Permanent Veterinary Committee ruling to extend the non-vaccination areas beyond the existing ones. The right to maintain an area without vaccination does not give the right to prevent import to such areas of vaccinated live animals or of meat from vaccinated animals.

In connection with this proposal it might be reconsidered, whether vaccination for transfer to vaccinated areas is in fact reasonable. Such vaccination was originally introduced to protect the importing country, but if the level of protection is sufficiently high through vaccination of a high percentage of animals the introduction of a limited number of unvaccinated animals may not change the epidemiological situation very much, if at all, and newly vaccinated animals may, in rare instances, introduce a risk of spreading the infection.

3. 7. 3. Licenses for Vaccine Production

3. 7. 3. 1. Present Situation

There are two kinds of licenses: 1) Establishment Licenses and 2) Product Licenses.

Both for the establishment licenses and the product licenses the rules differ significantly between the Member States. In Belgium, Denmark and Holland a specific State Institute produces, and the same institute controls the vaccine. In Italy four State Institutes produce, whereas separate institutions control the product. In France, Germany and U.K. licensed private firms produce, and a speci-

fic State Institute controls . In Germany the right to issue establishment license lies within the regional (Länder) authorities, but in France it lies with the State.

3. 7. 3. 2. Proposal for a Community License

The goal of obtaining harmonization also in vaccine production might be reached through Community Licenses valid for all Member States. In order to obtain this the Community as well as the Nat. Auth. would have to establish regulations, which deal with other areas than just the vaccine standards which are treated by the European Pharmacopoiea Commission.

Proposal:

1) Establishment licenses shall be issued only after inspection resulting in a determination that the establishment complies with prescribed standards.

2) A product license shall be issued only upon examination of the vaccine and provided that the vaccine complies with the standards prescribed and that the establishment is accepted.
The details regarding License Forms etc could be worked out in such a way that the result could be a common Community License valid for all Member States. The norms are to be worked out by Permanent Veterinary Community procedures.

3. 7. 4. Standardization of Vaccine

1) Reference Lab. If a Community License is attempted and even if National License must still be obtained directly in the individual Member State, it is essential, that a reference laboratory be established. Such a laboratory could work together with the National Lab. in a trial period (probably not more than 3 years) with provisional rules. It may be sufficient just to formalize the existing collaboration, but the Reference Laboratory must be totally independent of production. After this period and after adjustment of the rules a free trade for vaccines could be established.

Proposal:

The reference lab. should provide information and recommendations, e.g. on methods and on new virus strains appearing in the Member States or in countries exporting to the Member States. It should provide seed lot virus to be used as challenge in the potency tests or make decisions on the use of special strains in special situations.

The Nat. Labs. must collaborate with this Ref. Lab. and mutual decisions must be followed by the Nat. Labs.

2) Control of production and issuing of licenses.

The establishment licenses and the production licenses are issued by the Nat. State Lab. or Nat. Authority authorized to do so. The work should be carried out according to Community Regulation. By the Community Regulations it should be stated also, if production controls could be carried out by repressive control and the types of tests carried out.

The Community regulation should be in an agreement with the Pharmacopoeia rules for storage: The vaccine should be kept at 2-6^{o}C and should not be allowed to freeze. The expiration date should be 12 months calculated from the day on which the official test for potency is begun.

Norms for labeling etc should follow the Pharmacopoiea rules and be worked out by Permanent Veterinary Committee procedures. In addition to the Pharmacopoiea rules the label should state the method of cultivation of the virus preparation.

3. 7. 5. Minimal requirements for FMD vaccines for cattle

3. 7. 5. 1. Background papers

As it seems that the German comments of 1 Aug. 1973 to PA/PH/Exp. 15V/T (70)12, 4th rev) have influenced the document of 20 March, 1975 (French) marked PA/PH/Exp. 15V/T (75)2, this later document is supposedly at the moment the nearest approach to a final decision of the Pharm. Com.. It is consequently the base of the following proposal. It has been compared also with the re-

port of the European Commission for the Control of FMD from Lelystat 22-24 October 1974. In the Recommendations of the XIII Conf. of the Permanent Commission of the OIE (1972), which is completed in the document from the XIV Conf. of 1975, it is stated, Point 5 about potency testing, that it should be carried out in cattle, and that the potency for each virus type should be expressed in minimum protective doses in completely receptive cattle.

One dose of vaccine should contain as a minimum a dose sufficient for the immediate protection of 70 per cent of the vaccinated animals.

Other methods may be applied as supplementary methods in the potency testing, if a correlation has been established between the method in question and the evaluation of protective doses in cattle, PD_{50}. "The likely performance in the field, which is influenced by many factors, can be left to the user, who is aware of any special local condition" (p 11 of the report from the European Comm. for the Contr. FMD, 1974).

3. 7. 5. 2. Proposal for minimal requirements

The proposal for the minimal requirements of an inactivated FMD vaccine for cattle (in accordance with the available documents and with the principle guide-line of following the European Pharmacopoeia Comm. decisions, when available) would be as follows:

Inactivated FMD vaccine is a liquid preparation containing one or more types or sub-types of foot-and-mouth disease virus which have been inactivated in such a manner that their antigenic activity is retained.

Preparation

The vaccine is prepared by propagating the virus either in susceptible animals which have not been vaccinated, or in suspensions of bovine tongue epithelium taken immediately after slaughter from animals free from foot-and-mouth

disease, or on cell cultures. The virus is removed from the cellular material and inactivated under appropriate conditions by a suitable agent. The vaccine may be concentrated and purified. One or more adjuvants may be added.

Identification.

The serum of an animal injected with the vaccine must neutralize the types or sub-types of the virus used for preparing the vaccine in a proper test.

Sterility.

The vaccine complies with the test for sterility of the Pharm. Comm.

Innocuity tests.

The tests for innocuity are carried out in two ways and for a vaccine to be acceptable both tests must give satisfactory results.

1. Tests in cell cultures after elution-concentration. A sample of 3 l of vaccine is centrifuged. From the sediment virus is eluted and concentrated to 1/60 of the original volume. The concentrate is inoculated in cell cultures sensitive to FMD virus, preferably of the same type which have been employed for the preparation of the vaccine. The cultures are observed during a 3 day period. Two passages into new cultures are carried out in the same way. No cytopathic effect caused by FMD virus may be observed.

2. Test in cattle. Carry out the inoculation by the intradermolingual route using 0.1 ml of the vaccine at each site. Observe the animal for at least ten days. The animals should then be killed and no lesions of foot-and-mouth disease should be observed at autopsy.

Potency tests.

The potency of a vaccine should be expressed as the percentage of protection of primary vaccinated cattle. A vaccine satisfies the minimal requirements, if it protects at least 70 per cent (confidence limit P = 0,95)

of the animals challenged with 10.000 bovine ID_{50} of the same sub.types as employed in the vaccine preparation.

For the tests 18 to 30 months old animals obtained from areas free from FMD, which have not previously been vaccinated against FMD and are free from neutralizing antibodies against FMD. The animals are vaccinated with the dose indicated on the label,and the challenge is carried out 3 weeks after the vaccination. The challenge is carried out by intradermal injection into the upper surface of the tongue of 0.1 ml in each of two sites of 10,000 bovine ID_{50}. The test is carried out separately for each type of virus. Control animals will have lesions on at least three feet. Protected animals may display lingual lesions, but only unprotected animals show lesions at sites other than the tongue. The animals are observed for 10 days and slaughtered.

Several different methods may be employed for the quantitative evaluation in cattle of the potency provided [1] that the method is widely known and [2] that it is shown, that there is a satisfactory statistical correlation between the results obtained and the prescribed degree of protection. Thus e.g. an estimation of PD_{50} could be applied. Here it seems advisable to calculate on the base of dilutions made without the addition of adjuvants, because of the better precision in the titrations. If adjuvants are employed a correlation factor should be known for the type of vaccine in question.

Another way of estimating the potency would be through establishing the K-value of the vaccine, i.e. the index between the titre of virus obtained in unvaccinated cattle and in vaccinated cattle.

3. 7. 6. Vaccination Programmes

Although identical vaccination programmes should in principle be applied in all the Member States the actual local conditions may make deviations permissible.

The vaccinations should be carried out once a year. Young animals should be revaccinated. In cases where the vaccination programmes are not sufficiently syncronized an additional vaccination could be required for intra-community trade.

The animals should be vaccinated according to what is prescribed by the producer, but must be carried out by ubcutaneous inoculation in the dewlap.

3. 8. F M D v a c c i n a t i o n o f p i g s

The European FMD situation in number of countries until around 1960 was an enzootic one with infections in cattle amounting to 15-20 per cent at the peaks of epizootics. In a single unvaccinated herd the percentage of infected animals might reach 90-100. Usually the mortality remained low. After compulsory vaccinations combined with eradication programmes the situation changed drastically and FMD disappeared almost completely in cattle.

In sheep, goats and pigs the morbidity was apparently very variable in the period before compulsory vaccinations, and it was considered to be inferior to the morbidity among cattle. As the clinical diagnosis in other animals e.g. pigs is more difficult than in cattle, it seems now possible, that the real difference is not as important as hitherto accepted. Whatever the explanation it is now apparent that severe epizootics among pigs may start in areas with high densities of pigs. This has happened in Holland in 1965-66 and in France in 1974. In this connection it has been pointed out, that even with a successful, compulsory prophylatic vaccination of cattle the young calves, the sheep, the goats and especially the pigs may easily be in such proportion, that in fact less than 50 per cent of the animals sensible to FMD are actually vaccinated. Thus an epizootic among unvaccinated animals may easily occur, especially if the vaccinated animals, due to high densities of pigs, are even less than 25 per cent of all the sensible animals.

Although prophylactic vaccination of pigs is not suggested, it has been shown, that vaccination may limit epizootics in pigs. It is suggested, that the vaccines employed should contain 8 times the dose employed for cattle.

According to French results revaccinations with a stock-virus already employed may be useful and provide partial

protection and stop an epizootic for sufficiently long at least to give time to prepare a homologous vaccine. In animals not vaccinated before, it requires the homologous vaccine to give protection.

4. SWINE FEVER VACCINE

4. 1. I n t r o d u c t i o n

A common policy of controlling classical swine fever does not exist, not even within the Member States, and a number of questions concerning the virus and the disease are still unsolved. Thus questions about factors important for the occurrence of latent infections in pigs and the relationship between bovine virus-diarrhoea mucosal disease virus are still partly unknown or open for discussions. In addition to problems concerning the interrelationship between mucosal disease virus and classical swine fever virus the possible relationship between the virus causing Border disease in sheep and the virus of cattle and pigs is also of potential interest in a vaccination programme designed for the control of classical swine fever.

4. 1. 1. Classical Swine Fever Virus

Although not yet officially classified as a togavirus the classical Swine fever virus (SFV) is at least accepted as related to the alfavirus group of togaviruses. The alfavirus group contains the "old" arboviruses of the A. type. In addition to the swine fever virus, it seems possible to include in the same group not only the closely related mucosal disease virus (virus diarrhoea virus) of cattle, but also the human rubella virus and the equine arteritis virus. The virus consists of a RNA Strand enclosed in a protein shell of icosahedron symmetry. The outer layer is an envelope, and the whole particle has a diameter of around 40 nm. The virus multiplies in pig cell cultures, but does not usually give cytopathic changes of the cells. The laboratory diagnosis has been quite difficult, but special methods have been developed. Minor antigenic differences between SFV strains exist.

The virus seems quite resistent towards drying at room temperature, and it takes extreme values of pH to cause

rapid inactivation (pH < 1.4 or pH > 13). The virus may persist a long time in pork and garbage. The virus may passively be transfered by contaminated persons.

Although the disease African swine fever (ASF) in an individual case may be clinically very difficult to distinguish from classical swine fever, the ethiological agent of ASF is a quite different virus. The ASF virus is a DNA virus of the iridovirus group, which consists of large (around 200 nm diam.) naked icosahedrons. The ASF virus is also immunologically quite distinct from the virus of classical swine fever. The ASF virus may survive for years when dried at room temperature.

The Comm. of The European Communities has published a study (October 1971) on "Properties of the virus of classical Swine fever and differential diagnosis of classical and African swine fever" containing a wealth of information.

4. 1. 2. Classical Swine Fever

Synonyms: Hog cholera, European swine fever.

The infection is usually exceedingly contagious and may be fatal. Some strains of low virulence are not very contagious and it can take many months to infect a whole herd with such strains. Only pigs are naturally infected. The infected animals may have fever, apathy, vomiting, eye-discharge, diarrhoea, haemorrhages. Some strains are mild, but others may be unusually neurotropic with frequent symptoms of encephalomyelitis. Even with attenuated virus strains transplacental infection may give still-births or diseased newborns. In the case of virulent virus strains transplacental infection becomes unimportant because of the high mortality of the infection in the sows.

The infection may spread by direct contact and by feeding contaminated garbage. Only pigs are naturally infected.

4. 1. 3. Control

As hyperimmune sera may give temporary protection simultaneous inoculation of virulent virus and antisera has been employed, but this method may lead to persistent infections and has been abandonned.

Virus inactivated by crystal violet has been employed,

but only temporary immunity is obtained in this way. Live attenuated vaccines are now being employed. They are attenuated by passages in rabbits or in cell cultures.

The problems encountered with these vaccines are, that the vaccine virus sometimes may cause abortions or malformations amongst litters when pregnant sows are vaccinated. Some workers suggest that some vaccine virus strains may sensitize pigs, so that they react with more severe symptoms than otherwise in case of a challenge through natural infection later on.

Control of African swine fever by vaccination is especially difficult because many different serotypes of the virus exist. Attempts have been made to use attenuated strains as vaccines, but vaccinated pigs may continue to carry the virus.

Both in classical swine fever and in African swine fever the vaccinated animals may become carriers, perhaps even persistent carriers of virus. Thus it is once more stressed, that the goal must remain an eradication programme, as the only real control in the long run. In all cases, where such a programme is not feasible, the vaccination programmes must be full scale vaccination.

4. 2. Legislation and regulation for vaccines against classical swine fever in the different Member States. A review of relevant documents.

Belgium

Arrêté royal du 18 juin 1968 portant des mesures de police sanitaire relatives à la peste porcine (in: Bull. Sanitaire No. 13 (1968) p. 132-168), which contains detailed descriptions of the practical control, slaughter, indemnities quarantaine etc.).

Arrêté ministériel du 1. oct. 1969 portant des mesures de police sanitaire relatives à la peste porcine (in Bull. Sanitaire No. 19 (1969) p. 276-278), which state that only vaccine of the Chinese strain or equivalents controled by the Nat. Institute and that the use of antiserum is prohibited etc.

Arreté royal du 28 octobre 1969 modifiant l'arrêté du 18 juin 1969 (must be a printing error for 1968) (in Bull. Sanitaire No. 21 (1969) p. 308-310), about indemnities.

Arrêté ministeriel du 25 mai 1970 portant des mesures de police sanitaire relatives à la peste porcine (in Bull. Sanitaire No. 12 (1970) p. 200-204, containing among other things the rules for the compulsory vaccination of certain categories of pigs.

Arrêté royal du 18 juillet 1972, modifiant l'arrêté royal du 18 juin 1968, portant des mesures de police sanitaire relatives à la peste porcine (in Bull. Sanitaire No. 17 (1972) p. 176-182) containing certain changes in rules for indemnities, protective zones etc.

Arrêté ministériel du 7 décembre 1973, modifiant l'arrêté ministériel du 25 mai 1970 portant les mesures de police sanitaire relatives à la peste porcine (in Bull. Sanitaire No. 3 (1974) p. 24-26), in which minor changes in the previous administrative rules are introduced.

Denmark

Apotekerloven af Juli 1962 (Drug act).

Landbrugsministeriets bekendtgørelse om ondartede smitsomme sygdomme hos svin (Juni 1973). (Order by the Ministry of Agriculture on notifiable communicable diseases of swine.)

France

Legislation Le code de la Santé Publ., Articles L 611 à L 617.

In addition the ministry has issued provisional regulations for the special vaccines until agreements have been reached by the European Pharmacopoeia Commission. The Pharmacopoeia rules will then be employed.

Germany

Verordnung über Sera und Impfstoffe nach § 17c des Viehseuchengesetzes (Febr. 1973).

Ausführungshinweise zur Verordnung über Sera und Impfstoffe nach § 17c des Viehseuchengesetzes (März 1973).

No special regulations for swine fever exist.

Holland

No special legislation.

Ireland

No special legislation.

Italy

La legge 7 luglio 1967, n 514 according to which the ministry may order compulsory vaccination.

Ordinanza Ministeriale 11 Agosto 1967: Vaccinazione obligatoria dei suini contro la peste suina classica (G.U. 25-8-1967), according to which vaccinations are compulsory

employing a vaccine of the Chinese strain. The order contains further details about the vaccination.

Circolare del Ministero della Sanitá. Direzione Generale dei Servizi Veterinari nr. 600.5/24486/AG.2494 in which the testing of vaccine is described.

U.K.

The Medicines Act. 1968 and 1971.

Regulations can be laid down for individual vaccines, but have not been for swine fever vaccine.

4. 3. P r o d u c t i o n

4. 3. 1. Identification of the Producer

Belgium

Different private firms if licenses for release are obtained from the Institut Nat. Recherches Vét.

Denmark

No Danish production, Imports only by permission from the Ministry of Agriculture.

France

Vaccines are produced by firms who have obtained authorization after the required tests.

According to Direction des Services Vét., Ministère de l'Agriculture, all biologicals for veterinary use produced or imported must be authorized by the ministry. Then a license may be obtained for 5 years. Prior to authorization or renewal of this the product may be tested by a State control laboratory.

Germany

Vaccines may be produced by firms authorized to do so by Bundesforschungsanstalt der Viruskrankheiten der Tiere, Tübingen.

Holland

In Holland a private firm (Philips) has a special authorization to produce, but imports are permitted also.

Ireland

No production.

Italy

Vaccines produced by institutes licensed to do so by the State. Production is made according to direction by the Ministry. (There are now the Ist. Zooprofylat. in Termo, Perugia and Brescia).

U.K.

No U.K. production is permitted. The vaccines are imported if wanted for exports of pigs.

In table 3 the material of 4. 3. 1. is compiled.

4. 3. 2. Licenses for Establishment

Little information on special requirements for establishment licenses has been found for the different Member States, where production is permitted at all.

4. 3. 3. Licenses for Release

Belgium

Only vaccines prepared employing the Chinese strain and controled and released by Institut Nat. de Recherches Vét., or other vaccines accepted by the institute as equally good, may be used.

The license for vaccines depends on fulfillment of the following criteria:

General standards for attenuated live vaccines:

1) A proper degree of attenuation.
The vaccine must be harmless for use without simultaneous use of antisera (the use of antisera is forbidden anyway) and without any special precautions or mode of application.

2) The vaccine must pass the required tests for innocuity and potency in immunosuppressed (Prednisolone) and normal piglets by vaccination and challenge according to specified rules.

3) Innocuity for embryos and fetuses is tested.

4) Resistance against contact infection must be proven

for vaccinated pigs (test method specified).

5) Stability of the attenuation must be proven (6 porcine passages should be made, and the last passage should not cause symptoms of infection, but should give a vaccination, which must be challenged).

6) An immunity of at least 3 months' duration must be obtained by the vaccination.

7) Immunofluorescense tests should be negative.

Only the institut Nat. Recherches Vét. may release vaccines. The distribution is through Vet. Authorities.

Denmark

Imports only by permission from the Ministry of Agriculture.

France

The release is given by the State laboratory. The authorized vaccines must be prepared according to specification regarding identification of the virus strain, the method of preparation, passage, quality etc, required by the Ministry of Agriculture.

Germany

Bundesforschungsanstalt der Viruskrankheiten der Tiere would be the controling and releasing institute, but the vaccine is not employed in Germany, and no specific rules exist for Germany.

Holland

The Vet. Authorities follow the system of preventive control. When a batch is produced, the producer applies to VD, who collects samples, which are sent to CDI. The VD can then release the batch before test results. Imported vaccines are sampled "at the border". Responsibility is always with the producer in Holland.

Ireland

The vaccine is in principle forbidden. Import would be

for emergencies by permission of the Department of Health (after consultation with the Department of Agriculture), but a license has never been issued.

Italy

All vaccine batches are controled by Istituto Superiore di Sanitá and released by the Ministry.

U.K.

Vet. Authorities may permit import of vaccines for use only for exported pigs.

4. 4. Controling Authorities

In all countries where the vaccines are produced at all the State in principle controls the vaccines. In some cases the control is actually carried out and in other countries like Holland the samples are taken for control. In France the samples for control are not taken systematically, but the producer must fulfill the specified requirements and at the time of establishment license the seed lots are examined for toxic effects, teratogenicity, innocuity, potency, spread of virus etc.

4. 5. The Controls and Standards required

4. 5. 1. General Rules

Belgium

Immunofluorescense tests must be negative. For Belgian products the potency test may be restricted to every fourth lot, but serum neutralization for identification must be done on each lot, and innocuity test must be carried out for all vaccines and for each lot employing piglets. The stability of the vaccine is controled by exposure to 37°C for 7 days followed by control inoculations in rabbits.

Denmark

The controls and standards that might be required in case of imports are not specified.

France

Although there is no legislation the authorities have specified requirements pending the Pharmacopoeia Commission decisions.

It is stated that the live vaccine is a product containing a strain of classical swine fever virus, which has lost its pathogenicity for pigs through adaption in rabbits. It is identified by serum neutralization tests in rabbits, where the fever provoked by intravenous injection of the virus is employed as the clinical sign of infection.

There are detailed rules for the origin of the seed virus. The vaccine may only be within 5 passages of the seed virus. After sterility tests and tests for toxicity and teratogenity, the possible spread of virus must be examined. Five days after vaccination the animals must be protected (the definition of protection being that the animals survive without a temperature rise to 41.5°C for more than 24 hours).

On the vaccine label should be stated, that the reconstituted vaccine must be employed immediately, and that it

is not recommended for pregnant sows.

Germany

No specific rules exist, only the general legislation for vaccines.

Holland

Requirements for vaccine standards and control methods are not finally settled. The Pharmacopoeia discussions are closely followed. Both crystal-violet inactivated vaccines and cell culture produced, attenuated live vaccines are employed.

The inactivated vaccines are tested in the following way:

Conventional tests for purity and innocuity. Tests in pigs only randomly. The potency not tested regularly - if it is tested U.S. requirements or British codex will be followed. In general the VD will inspect the factory and use "common sense".

The live attenuated vaccine is tested in pigs very much the way FMD vaccines are tested in cattle, but only a few times and by seed lot. There are a number of absolute requirements:

1) No clinical symptoms, not even in stressed pigs (cortison medication for 6 days), is permitted.

2) No fertility disturbance and no pregnancy disturbance are permitted. In addition it is desired, that no temperature rise is demonstrable, and that no virus is demonstrable in tonsils (in this way it may be possible to distinguish between field strains and vaccine strains). The same degree of immunity should develop in stressed and not stressed animals. If these tests are passed, then an identification test in rabbit is carried out.

Ireland

No production and no specifications for imports, which have never been necessary or permitted.

Italy

The production is carried out according to the directions of the Ministry. All batches are controled by Istituto Superiore di Sanita in Rome. There is a declared intention to follow Pharmacopoeia rules.

U.K.

No production and no special rules.

4. 5. 2. Innocuity Tests

Belgium

A test for innocuity, potency and stability is carried out in 8 piglets divided in groups, so that 2 are vaccinated employing normal procedure, 4 vaccinated with 1/10 dose after an exposure of the lyophilized vaccine to $37^{o}C$ for 7 days, and 2 serve as unvaccinated controls. The vaccinations are tested by challenge ($10^{5}ID_{50}$) or seroneutralization tests (titre $\geq$ 32 taken as positive indication) (details given).

France

Three piglets known to be without previous exposure and sensitive to classical swine fever are given inocula of 10 vaccine doses. The animals should remain in good health and not respond with elevated temperature. See also 4.5.1.

Holland

See under 4.5.1.

Italy

The rules are described in the order of the Ministero della Sanità - Direzione Generale dei Servizi Veterinari n. 600.5/24486/Ag. 2494.

4. 5. 3. Potency Tests

Belgium

Potency and identity are tested in rabbits by registering

temperature rise after vaccination (may not exceed $1^{o}C$) and afterwards by checking lack of temperature change in the vaccinated animals (test specified).

France

4 piglets are given o.1 dose and 4 piglets are given o.ol dose of vaccine. Two animals are kept as unvaccinated controls. After 14 days all the animals are challenged with a dose of virulent virus sufficient to kill unvaccinated animals. Vaccinated animal may not during 14 days respond to the challenge with temperatures exceeding $41.5^{o}C$ for more than 24 hours. The vaccine dose for pigs must contain at least 30 PD_{50}.

Holland

Potency tests are carried out in pigs. The vaccine should contain 50-100 PD_{50} for pigs.

Italy

In Brescia the test is carried out in pigs weighing 30-40 kg. The vaccine is tested in dilutions 1/1, 1/100, 1/200 and 1/400 (4 pigs per dilution and 4 controls). After 20-30 days the pigs are challenged by inoculation of $10^{6}ID_{50}$ for pigs of virulent virus. The pigs are observed for 2 weeks. The vaccine is accepted if 1/200 gives full protection.

4. 5. 4. Former U.S.A. Requirements

Although U.S.A. has discontinued the production of classical swine fever vaccine in 1971, the rules employed until then may be noted:

1) Standard requirements for inactivated hog cholera vaccines. A blood origin product from blood of pigs meeting the general requirements. Virus is inactivated in a"suitable way."

Safety and potency tests: Pigs are vaccinated twice subcutaneously. Controls are used as contact controls. Challenge virus must give grave symptoms and post mortem positive diagnosis. At least 50 per cent of vaccinated animals must remain well throughout the 14 day post-challenge period and at least 80% must be alive and well at the end.

2) Standard requirements for Hog Cholera vaccine, modified live virus: The product is prepared with living modified virus obtained from infected cell cultures.

Potency: Satisfactory vaccine must contain enough virus to give a titre of at least $10^{3.0}$ $FAD_{50} \pm 0.5$ log per pig dose.

Safety: Pooled samples of each subserial are pooled. The pigs inoculated with 2 ml of the sample must not show any signs of adverse reaction during 14 days.

4. 5. 5. Expiration

Apparently only the French rules contain anything about expiring dates. There it is stated that the lyophilized vaccine should be valid for 12 months and should be kept at $2\text{-}10^{\circ}C$.

4. 6. V a c c i n a t i o n

4. 6. 1. Rules for swine fever vaccination

Belgium

Vaccination is compulsory for commercial marketing. For the areas of East- and West Flanders and Turnout vaccination is compulsory for all pigs. A vaccination certificate is issued, and the animals are tagged according to specific rules.

Denmark

As swine fever has not been diagnosed in Denmark since 1933, vaccinations have not been employed or even planned. Vaccination may only be carried out with permission from the Ministry of Agriculture.

France

Vaccination is not compulsory, but in certain threatened areas (the northern area at the Belgian border) vaccination has been encouraged and subsidized. If the need arises, vaccination will become compulsory.

Germany

Vaccinations are not carried out.

Holland

Vaccination will be compulsory when an emergency is declared by the authorities.

Ireland

Vaccination is in principle forbidden. As the country has been free from the disease, no license for import of vaccine or its use have been issued by the Department of Health.

Italy

Young piglets must be vaccinated at the age of 60-70 days. Breeding animals must be revaccinated every year. The ani-

mals are tatooed with the authorization number of the vet. after the vaccination. It is not permitted to employ hyperimmune sera togehter with the vaccine.

U. K.

No vaccination permitted except for export animals.

4. 6. 2. Economics

Belgium

Vaccinations are payed for by the farmer. Vaccination is encouraged by a much better indemnity for vaccinated animals if slaughtering has been enforced in connection with an epizootic.

Denmark

See 4. 6. 1.

France

For certain areas vaccination is encourages and subsidized by the authorities.

Germany

In emergency cases the vaccination would be paid for by the State.

Holland

Just as for FMD vaccination in pigs the swine fever vaccines have been used only for emergencies and are then paid for by the State.

Ireland

See 4. 6. 1.

Italy

As long as vaccination remains compulsory, vaccination is without cost for the farmer. The vaccine is bought and distributed by the Ministry of Public Health.

U.K.

See 4. 6. 1.

4. 6. 3. Indemnities

Belgium

No special provisions are made.

Denmark

No special provisions are made.

France

Responsibility remains with the producer.

Germany

For the emergency vaccination the State would pay endemnities for damage in immediate connection with the vaccination.

Holland

In all cases the producer or the seller remains responsible for the product.

Ireland

See 4. 6. 1.

Italy

The producer remains responsible for the vaccine.

U.K.

No special provisions made.

In table 4 the general economic aspects of swine fever vaccination in the Member States are summarized.

Table 4

Classical Swine Fever Vaccine

Economic aspects

	Vaccination paid by:	Indemnities in case of vaccine-associated accidents paid by:
Belgium	The farmer	No provisions made
Denmark	-	-
France	In certain areas subsidized by the State, otherwise the farmer	The producer
Germany	The State	The State
Holland	The State	The producer
Ireland	-	-
Italy	The State	The producer
Luxemburg		
U.K.	-	-

4. 7. Proposals for Community Regulations

4. 7. 1. 1. Summary of the present situation

As mentioned in connection with proposals for Community Regulations (3. 7. 1.) the council directive of 26 June 1964 (64/432/EEC) and the amendments following this directive state, that measures must be taken to eliminate differences between the health requirements of Member States. It is still possible to maintain severe restrictions on imports and exports or transits in order to safeguard the health of man and animals in acute, defined situations, but such possible right does not suppress the duty of providing conditions, that may result in the realisation of a mutual Community policy. It is the duty of the individual Member State to guarantee, that animals for breeding or slaughter do not constitute a risk for spread of infection through intra-community activity.

The actual situation, both epidemiologically and from a regulation point of view, varies considerably from country, but in none of the Member States does the legislation as such mention a general, compulsory vaccination. The Belgian rules require the vaccination of pigs before marketing as live pigs and for general vaccination in certain regions of Belgium. The Italian order of 1967 contains the requirements for compulsory vaccination, but in principle this is not against what would happen in other Member States in acute situations, and the regulation from 1967 only gives the authorities the right to do what was actually done. The other Member States are either not vaccinating at all or only in certain regions or in acute situations. It thus seems possible to formulate general regulations within the framework of what is actually the case to-day.

4. 7. 1. 2. Standardization of laboratory diagnosis

Effective control of swine fever can only be obtained, if the sources of infection and the spread of infection are

properly traced. The specific clinical diagnosis of swine fever may be so difficult in the individual case, because so many other infections may give similar symptoms, that it is quite important for the control that proper laboratory diagnostic methods be set up and standardized. The Member States should standardize the diagnostic procedure through collaboration with a reference laboratory. The direct immunofluorescence test on frozen tissue sections of the tonsils would seem to be the method of choice. Consequently standard conjugates for this test should be made by a reference laboratory, and techniques for virus isolation and virus neutralization tests should also be standardized.

4. 7. 2. Proposal for Vaccination Procedure

In principle, the situation should be maintained as it is to-day: The proper autjorities in each country should decide, if the situation calls for compulsory vaccinations in certain regions or for a whole country. The decision should be reached in collaboration with a Community Ref. Lab. and the Nat. Lab. according to rules worked out by Permanent Veterinary Committee procedures.

The European Pharmacopoeia Commission rules for vaccine production and standards are under discussion and are not finalized. It is of obvious importance not to make any suggestions within the Community, which are in disagreement with the decisions of the Pharmacopoeia Commission, so cooperation should be as close as possible with this commission.

4. 7. 3. Licenses for Vaccine Production

4. 7. 3. 1. Present Situation

There are two kinds of licenses: 1) Establishment licenses and 2) Product licenses. The general rules are in fact not very different in the different Member States,who produce vaccines,but in Denmark, Ireland and U.K. no vaccines

are produced and in cases of emergency or for other reasons needed, the vaccines would be imported. Vaccines are produced in Belgium, France, Germany, Holland and Italy, and they are all controled by the Nat. State Institute authorized nationally to do so, i.e. in all cases producer and controler are different establishments, and both domestic and foreign vaccines are accepted in some countries. Thus it should not be very difficult to obtain harmony according to the Rome treaty for Classical Swine Fever vaccines. In principle it would not be very important, if Germany maintains, that the right to issue establishment licenses remains with the regional (Länder) authorities. Apparently only the French provisional rules contain anything specific about the conditions for obtaining an establishment license (see 4. 3. 1.)

In order to obtain harmony within the Community both the Community and the National Authorities would have to establish regulations, which deal with other areas than just the vaccine requirements treated by the European Pharmacopoeia Commission.

4. 7. 3. 2. Proposal for a Community License

1) Establishment licenses shall be issued only after inspection resulting in a determination that the establishment complies with prescribed standards.

2) A product license shall be issued only upon examination of the vaccine and provided that the vaccine complies with the standards prescribed and that the establishment is accepted.

The details also regarding License Forms etc. could be worked out in such a way that the result could be a common Community License valid for all Member States. The norms are to be worked out by Permanent Veterinary Committee procedure.

4. 7. 4. Standardization of Vaccine

In close agreement with what has been proposed for FMD vaccines (3. 7. 4.) it is suggested, that reference laboratories be established, preferably as an extension of existing institutions.

If a Community License is attempted it is essential that such a reference lab. exists, but even if National Licenses must still be obtained directly, it is still essential to have a reference lab. Such a laboratory could work together with the National Lab. in a trial period (probably not more than 3 years) with provisional rules. After this period and after adjustment of the rules a free trade for vaccines could be established.

Proposal: The reference lab. should provide information and recommendation. It should provide seed lot virus to be used as challenge in the potency tests.

The Nat. Labs. must collaborate with this Ref. Lab., and mutual decisions must be followed by the Nat. Labs.

Control of production and issuing of licenses. The establishment licenses and the production licenses are issued by the Nat. State Lab. or Authority authorized to do so. The work should be carried out according to rules made in collaboration with the Ref. Lab. Rules should be set up for this collaboration.

Community Regulations should also state what part of the production controls may be carried out by repressive control.

Just as for the licenses,release papers should be valid for all Member States.

4. 7. 5. Minimal Requirements for Classical Swine Fever Vaccines

4. 7. 5. 1. Background Papers

Of the papers available for study French, Italian, Belgian and Dutch information are especially important in the field of production control and minimal requirements for vaccines. As the European Pharmacopoeia Commission work on Swine Fever vaccine is not finalized, the proposed reference laboraotry should use the material obtained so far as guidelines in the trial period, unless final Pharm. Com. decisions are reached, in which case they should be followed. This would be in close agreement with what is the declared intentions of the countries, in which the vaccine is produced to-day and also with the European Treaty Series No. 50, where it is stated that the rules agreed upon by the Pharm. Com. should be the official standards for the member countries.

The following papers in particular are employed in the proposals of 4. 7. 5. 2.:

The PA/PH/Exp. 15V/T (73)31 (Comments of the Netherlands Delegation).

PA/PH/Exp. 15V/T (74) 10 (proposed by Florent concerning control by titration of neutralizing antibodies);

The paper by Precausta and Perrenot: Titrage des anticorps neutralisants anti peste porcine classique par la méthode d'immunofluorescence (Rec.Méd. Vét. (1973) 149 p.1567-1576),

The conclusions and recommendations on diagnostic methods for swine fever by the group of the Seminar on Diagnosis and Epizootiology of Classical Swine Fever, Amsterdam, 1975.

PA/PH/Exp. 15V/T (74) 4, first rev. (on freeze-dried swine-fever (lapinised) live vaccine),

The draft prepared for the Pharm.Com. meeting of April 25, 1975 in Louviers marked H bis of March 27, 1975: Vaccine vivant cryodesseché contre la peste porcine classique obtenu en culture cellulaire, and

The Iffa Merieux paper of April 1974 on Pestiffa: Notice technique concernant la preparation et le controle d'un vaccin vivant contre la peste porcine classique à l'aide de la

souche chinoise "CL" adaptée à la culture cellulaire.

4. 7. 5. 2. Proposals for Minimal Requirements

Freeze-dried swine-fever live vaccine is a preparation obtained from a strain of classical swine fever virus, which has lost its pathogenicity for animals of the porcine species by adaption to other animal species than porcines or to cell cultures.

The vaccine virus strains

The vaccine virus strain must not possess any observable residual pathogenicity for pigs. The following properties must be found for the seed lots of virus:

1) Absence of pathological changes in pigs, in particular the absence of leucopenia.

2) The strain of virus must be innocuous for the foetus. Thus neither infertility nor still-birth nor abortion may be caused by the virus, and no other signs of disturbance of pregnancy of sows may be observed.

3) The virus should not be transmissible to unvaccinated pigs. (Apparently spread of vaccine virus does occur occasionally so this is a difficult property to ensure).

4) The strain must retain its apathogenicity even after six serial passages in pigs.

5) The virus strain must be free from contaminating microorganisms, including viruses.

A strain should be chosen that may be differentiated in the laboratory from wild strains. Such virus strain markers could be growth in certain ways in special cultured cell systems, adaption to a particular animal, a certain temperature optimum for multiplication, specific reactions to immunofluorescence tests or other ways. The producer should indicate such marker for the particular virus strain.

In order to fulfill the demands of 1-3 the following tests must be carried out:

1. Tests on pregnant sows

At least 10 non-immunized pregnant sows are inoculated intramuscularly during the first month of gestation with a dose

of the seed lot corresponding to 2 vaccinating doses (as indicated by the producer). A group of at least 10 unvaccinated sows of the same origin are used as controls. The seed lot must not cause any disturbances in the gestation or have any detrimental effect on the piglet.

2. Tests on pigs weighing about 30 kg carried out for:

safety, potency, non-diffusibility of the vaccinal virus, and time taken to acquire immunity.

The tests are carried out on pigs from healthy herds weighing about 30 kg and free from specific antibodies. In the course of these tests the animals' temperature is taken twice a day.

At least 50 pigs are distributed as follows:

Group No. 1. At least 10 pigs are inoculated with 10 doses of the vaccine (safety test);
Group No. 2. At least 10 pigs are inoculated with 1 dose of the vaccine (test for immunity on 21st day);
Group No. 3. At least 10 pigs are inoculated with 1 dose of the vaccine (time taken to acquire immunity);
Group No. 4. At least 10 pigs are used as contact controls;
Group No. 5. At least 10 pigs are used as controls of the test virus.

The whole series of tests extends over 8 weeks and is divided into 4 periods.

First period (adaptation of the animals to the laboratory). This lasts one week and comprises the randomisation of the animals to make up the seed lots mentioned previously, the weighing and the elimination of parasites, the determination of the absence of antibodies and the checking of the stability of the thermal curve.

Second period (immunization). This lasts 3 weeks. Groups No. 1, 2 and 3 are inoculated as indicated above. These animals are inoculated with the volume recommanded by the manufacturer. In the case of a dose or its multiples the commercial diluent is used to make up the volume.

The animals are then reweighed. During this period the thermal curve is checked. This must remain normal in all the animals. The period of immunization for the 10 pigs of Group No. 3 used to determine that the time taken to acquire immunity is only 7 days. At the end of this period the animals are inoculated with the quantity of virus specified below.

Third period (challenge). This lasts two weeks. The immunity of the pigs (Group No. 2) is tested on the 21st day by inoculating a quantity of virus representing at least 10^5 lethal doses for unvaccinated pigs, whereby death ensues within 7-14 days. The unvaccinated pigs used as contact controls (Group No. 4) are separated from the vaccinated animals and challenged in the same way 15 days later. The pigs of Group No. 5 are also inoculated with the same quantity of virus.

During this third period, an autopsy is carried out on the dead animals to confirm the causes of death.

Fourth period (post-challenge). At the end of the third period, the surviving animals are killed, examined for possible lesions and the presence or absence of test virus is checked.

The seed lot is judged on the basis of the following results:

1. The form of the weight curve before and after vaccination.
2. The reaction of the contact controls and the unvaccinated controls.
3. The potency.
4. The time taken to acquire immunity.

1. The weight curve must remain unchanged and a comparison between the mean daily gains must not display any significant difference unfavourable to the second period.

2. The contact controls kept during the test in the same place as the vaccinated animals must react to the lethal

challenge in the same way as non-vaccinated controls kept away from the vaccinated animals and take the same time to die.

3. For each type of treatment (Groups 1, 2 and 3) those animals shall be regarded as protected who have survived the test without any notable change in their thermal graph and without displaying any acute lesions under necroscopic examination after being slaughtered on the fifteenth day. The presence of scarry lesions will be tolerated, but if the test virus is observed to be present in an organ the seed lot must be discarded. All the vaccinated animals must comply with this requirement.

4. The time of onset of immunity in pigs after vaccination with 1 vaccinating dose will be 7 days. In this test, those surviving animals shall be regarded as protected whose temperature does not exceed 41.5° for more than 24 hours. All the vaccinated animals must comply with this requirement.

Requirements for each batch of finished vaccine

Each batch shall be prepared from a seed lot of virus, which complies with the above specifications for the vaccine virus strains.

The vaccine batch must not be more than 5 passages past the seed lot tested according to these requirements.

Each batch of vaccine must comply with the Pharm. Com. sterility tests.

Innocuity tests are carried out by intramuscular injections in 3 susceptible piglets weighing about 30 kg and littered from sensitive sows. Each piglet receives 10 doses of the vaccine reconstituted as indicated on the label. The animals are observed for 21 days. The thermal graph must remain normal,and the animals must remain in apparent good health and display normal growth.

Potency tests are carried out in two ways:

1) It must be shown that the vaccine contains a minimum of

10 protective doses for pigs. The test is carried out by intramuscular injections of 1/50 of a vaccine dose into 4 piglets weighing about 30 kg.

2) The thermal stability of the vaccine must be tested by intramuscular injections into 4 piglets weighing about 30 kg of 1 dose of vaccine, which has been kept for one week at 37°C.

3) 2 piglets of the same age, weight and origin are used as controls. After 14 days all the animals are challenged and examined in the same way as described for potency testing of the seed lot. The vaccinated animals must be protected and may not show lesions or rise in temperature. The two control pigs die.

By Permanent Vet. Comm. procedure and in collaboration with the reference laboratory it may be decided, that some controls are carried out by repressive controls, and that alternative methods not employing pigs may be employed in certain cases.

Storage

The lyophilized vaccine is stored in darkness at 2-6°C. The vaccine must be used immediately after reconstitution.

Expiration date

The vaccine may be expected to retain its potency for 12 months from the date of the last test for potency if stored under the prescribed conditions.

4. 7. 6. Vaccination programmes

The vaccines employed to-day reduce the disease incidence, but there is no assurance that SF virus can be eliminated, while programmes of vaccination continue. In some countries vaccination must be used to control swine fever effectively at present. The available information indicates, that the vaccine virus strains employed are genetically stable and do not, or only occasionally, spread from vaccinated pigs.

Information about the possible persistence of virus in vaccinated pigs is rather incomplete, but it has been reported, that the virus may be present in pigs six months after vaccination.

A number of questions regarding the vaccination of piglets especially from immunized mothers are not sufficiently elucidated. The above mentioned problems should be kept in mind, when vaccination programmes are decided.

For vaccination programmes the following is proposed:

The animals should be vaccinated according to what is prescribed by the producer.

Identical programmes should be employed in all the Member States in accordance with the decisions reached through Permanent Vet. Com. procedures. The questions should be studied with the goal in mind of harmonization, so that identical situations are handled in the same manner in all Member States.

5. NEWCASTLE DISEASE VACCINE

5. 1. I n t r o d u c t i o n

Control and prevention of Newcastle Disease in domestic poultry flocks within the EEC depends on a number of factors besides the nature of the virus, e.g. the pattern of production and the movements of wild birds, domestic poultry, eggs and poultry meat. To this must be added the quality of the vaccines and the character of the vaccination programmes.

In the tropic areas natural reservoirs of Newcastle Disease virus (NDV) exist. Through the import of wild birds foreign virus types are introduced into domestic poultry populations. This introduction of virus sometimes of high virulence may overcome the effects of traditional vaccination programmes.

In the integrated production system of EEC prevention against exotic infections should take the form of control of importation of exotic birds, live poultry, eggs and poultry meat from Third Countries, but it is at least as important to reduce the spread of field virus within the Community. Theoretically an efficient solution would be to prohibit Intra-Community trade. In practical terms, however, this is only partly a solution, as the regional problems still exist, and some material will always pass frontiers. In addition this solution will be neither desirable nor legal. Consequently other means of safe-guarding the flocks must be introduced to reduce the risk of spreading N.D. infections. Hanson has (R.P. Hanson (1964); Newcastle disease virus: An evolving pathogen, Madison, Univ. and Wisconsin Press) reviewed the problem.

5. 1. 1. Newcastle Disease Virus

NDV belongs to the paramyxoviruses. It contains RNA in a nucleoprotein helix surrounded by an envelope containing lipids. On the surface are projections containing haemag-

glutinin and the enzyme neuraminidase. The size is around 100-200 nm in diam., but filamentous particles occur. Only one serotype is found, but it has been reported, that immunological differences amongst strains exist, anyway.

Virus infectivity is sensitive to lipid solvents and is heat labile and unstable at high and low pH. It has long been considered, that NDV, contrary to other paramyxoviruses, may withstand drying. Consequently the virus may remain active in the dust of infected farms, but laboratory studies do not support this. It has therefore been suggested, that previous findings, based on epidemiological evidence, that chickens may become infected in deserted, cleaned buildings long after ND cases, are really misinterpretations of evidence. The explanation may be, that subclinically infected chickens were introduced in the cleaned farm rather than recurrency has resulted from insufficient cleaning and disinfection, so that the virus survives in the building and surroundings.

5. 1. 2. Newcastle Disease

Synonyms: Avian pneumo-encephalitis, fowl-pest (which then would include also fowl plaque, a disease caused by avian influenza virus).

A fatal disease may be caused in birds by NDV. Respiratory or nervous symptoms may be seen and sometimes both nasal discharge and watery diarrhoea may be seen also. Milder strains (the lentogenic ones) occur as well as more virulent strains (the velogenic ones). The milder strains cause low mortality, but may affect egg production. The infection may be spread through drinking-water or may be airborne. The respiratory tract is considered the most important portal of entry rather than the intestinal tract. Virus has been isolated from eggs, from vaccinated hens. Healthy carriers are frequently found.

5. 1. 3. Control

Whole-sale slaughter or vaccination are the two alternatives for control. The vaccinations are now usually carried out with live attenuated virus vaccines, but some inactivated vaccines are used in special situations.

Various procedures of prevention and control are applied in different countries to reduce the losses due to Newcastle Disease. The methods employed depend on the production pattern and the geographical situation. Vaccination is usually preferred, because it establishes protection quickly and easily without expensive improvements in the standards of hygiene. If pathways are opened for infections between flocks and countries however a permanent risk of spread of new exotic virus types from the outside will result. In addition a spread of classical virus infections might cause a break through a low barrier of immunological protection and result in disease.

The combined influence of the spread of virus from infected flocks followed by more or less systematic employment of vaccines of varying quality leads to irregular fluctuations in the health situation in many poultry populations. The result is an unbalanced animal production causing economic loss or bad economy due to overproduction.

The ideal solution is the establishment and maintainance of poultry without NDV infections. This principle is followed in certain countries by the combined activity of producers, who build up closed flocks and participate in a common health scheme, in which breeding farms, development and production establishments operate within a vertically integrated and closed system. Efficient control and elimination by slaughter of flocks in which ND is diagnosed is an essential part of the scheme. This system functions in some areas and should be considered the long-term solution for a number of European areas, where the only realistic solution of the problems of protecting many Community flocks is vaccination.

This stresses the importance of getting a better quality of vaccine and better, more uniform vaccination procedures, so that a permanent high immunity may be obtained for egg-layers and a good protection of broilers before slaughter. A special problem is the possibility, that field virus may be disguised in vaccinated flocks, but at least the concentration of infective virus may be reduced in the carcass. If the carcasses are processed without head, legs and viscera, this danger may be further diminished.

5. 2. Legislation and regulations for vaccines against Newca stle Disease in the different Member States. A review of relevant documents

Belgium

Arrêté royal du mars 1974, portant des mesures de police sanitaire relative à la peste aviaire et à la pseudo-peste aviaire. This regulation which contains rules and regulations regarding control measure(in Bull. Sanitaire No. 7, April 1974, p 84-99) against the disease including vaccinations, but nothing about the vaccines.

The control of NDV vaccines is covered by the general legislation requiring the control of all vaccines by the Vet. State Lab.

Denmark

Apotekerloven af Juli 1962 (Drug Act). Bekendtgørelse om foranstaltninger til bekæmpelse af ondartede smitsomme sygdomme hos fjerkræ (August 1963) (Order on measures for the control of notifiable communicable poultry diseases). This order is in general terms rather close to the Belgium regulation, except that the vaccinations, which are potentially possible, are not encouraged. They have in fact never been permitted. In the sporadic cases that have happened, the disease spread has been stopped by slaughter.

France

No regulations exist. While waiting for Pharmacopoeia decisions provisional rules have been issued by the Ministry of Agriculture. These are concerned with standards and control tests for inactivated and live attenuated vaccines.

The general rules are in the Code de la Santé Publique, articles L 611-L 617.

Germany

Verordnung über Sera und Impfstoffe nach § 17c des Vieh-

seuchengesetzes (Februar 1973).

Ausführungshinweise zur Verordnung über Sera und Impfstoffe (März 1973).

Verordnung zum Schutz gegen die Geflügelpest und die Newcastle Krankheit (Geflügelpest-Verordnung) von December 1972.

Richtlinien für die Herstellung und Prüfung von Lebendvakzine zur Immuniesierung gegen atypische Geflügelpest (Newcastle Disease) (undated material received from Poul-Ehrlich-Institut, September 1974).

Staatliche Prüfung von Impfstoffen gegen Geflügelpest (Febr. 1969, from Staats-Anzeiger für das Land Hessen p. 374).

In principle Germany is also waiting for Pharmacopoeia rules.

Holland

Verordening N.C.D.-bestrijding, Marts 1974 (Regulation on Newcastle Disease control), which contains rules for compulsory vaccinations and serum testing of poultry.

Uitvoeringsbesluit I and II of Verordening N.C.D.- bestrijding 1974 containing information about how the regulation is used in practice. Stichting Gesundheidzorg voor Dieren of Jan. 1975: Instruktie dierenartsen ten aanzien van de Uitvoering N.C.D.-entingen, which contains the instructions for the veterinarians regarding the NDV vaccinations.

The Vet-Service decides what strains and what kind of products may be used. The accepted firms are mentioned by name (including a large Belgian firm), and they have accepted inspection by Vet. Department-inspectors, who sample for repressive control. The repressive control functions in such a way, that if 3 lots out of 5 lots were not found satisfactory, a preventive control is enforced, until the product is up to standard. If this does not occur, the producer may loose his license.

Ireland

Animal Remedies Act of 1956. Diseases of Animals (Disinfection) Order of 1931.

Imports of vaccine would be by permission from the Department of Health (after consultation with the Department of Agriculture), but a license has never been issued.

Italy

L. Ravaioli, Z. Orfei, M. Granieri: Controllo dei sieri, dei vaccini e dei prodotti diagnostici per uso veterinaria, Istituto Superiore di Sanita, Roma 1964. The vaccine must contain at least $10^6 DI_{50}$/dose.

F. Cessi and L. Nardelli: Requirements for testing oil emulsion inactivated Newcastle Disease vaccine (1974), about the controls for inactivated vaccines.

The general rules require that only the first batch of the producer be controlled by the Istituto Superiore di Sanita. After this stage official control is done only irregularly. The systematic control is done by the producer.

Luxemburg

Follow Benelux-rules.

U.K.

The Medicines Act (1968) which requires both manufacturing establishments and each product to be licensed. Manufacturers are subject to inspection and have to have adequate premises, methods and records. Each product has to be shown to be safe, effective and of satisfactory quality.

British Vet. Codex 1965, Supplement 1970.

5. 3. P r o d u c t i o n

5. 3. 1. Identification of the Producer

Belgium

Authorized private firms.

Denmark

No production. Vaccinations have never been permitted, but vaccine would be imported if considered necessary for emergencies by the Vet. Auth.

France

Authorized private firms. The authorization is obtained after the testing of the trial products and after inspection of the premises.

Germany

Authorized private firms.

Holland

Authorized private firms.

Ireland

No production. If considered necessary by Vet. Auth. import would be permitted. This has never happened.

Italy

Both the Publ. Health Institutes and private firms are authorized to produce.

Luxemburg

Is part of a Benelux-agreement.

U.K.

Private firms who have obtained a license to do so. According to the Codex live vaccines are not permitted, but Centr. Vet. Lab., Weybridge informed (in Sept. 1974) that only lentogenic strains are allowed. This means that the strains Hitchner B 1 and La Sota have been permitted and now are the only permissible.

5. 3. 2. Licenses for establishment

It seems that in all the producing countries (Belgium, France, Germany, Holland, Italy and U.K.) the authorization is given by the State after control of the product and inspection of the premises. Only in Germany the establishment license is given by the local authorities (Bundesland-authorities), but this remains a theoretical difference, as the State Lab. has the production control even for the primary product.

5. 3. 3. Licenses for Release

Belgium

The State Vet. Lab. releases vaccines after tests.

Denmark

Vaccinations may only be performed with vaccines, which have been imported by permission from the Ministry of Agriculture, and they are only obtainable from the State Vet. Serum Laboratory. Such permits have not been given.

France

Each vaccine batch whether French or imported is sampled, but tests are carried out at random. The State Lab. releases.

Germany

Poul-Ehrlich-Institut (Bundesamt für Sera und Impfstoffe), i.e. the State Lab.

Holland

The State Lab. (Centraal Diergeneeskundig Instituut).

Ireland

The State would release by giving permission for imports. Such permission has never been given.

Italy

The State Lab. (Istituto Superiore di Sanita, Roma).

U. K.

The State Lab. (The Central Vet. Lab., Weybridge).

5. 4. Controling Authorities

In table 5 the information on the producers and the controling authorities is collected. Whereas the establishment licenses in all the producing countries require that the samples from the primary production be tested, production control is often carried out either by repressive control or by testing the collected samples at random or even by sampling less frequently.

5. 5. The Controls and Standards required

5. 5. 1. General Rules

Belgium

All vaccines are controlled on quality, innocuity and quantity of immunizing particles, but the specific requirements are not stated. There is a special Benelux-agreement to have the same quality and controls and a declared intention of following Pharmacopoeia rules. Apparently only the lentogenic, live vaccines are employed (the La Sota or the Hitchner B 1 strains). It has not been stated whether other strains are permissible.

Denmark

No specifications. Imports have never been permitted.

France

Inactivated vaccines.

The vaccine may contain an approved anti-microbiological compound and must pass conventional sterility tests.

Tests performed on live, attenuated, lyophilized vaccines.

The vaccine is prepared from a lentogenic strain in eggs or in cell cultures. In both cases the absence of specific avian pathogens must be demonstrated. The virus strain must have an index of neurovirulence o.25 (the index specified). This test is only obligatory for seed lots.

The purity of the vaccine virus is tested through a neutralization test (described in detail) in embryonated eggs, which shall not show any abnormalities or possess hemagglutinins. The vaccine must also be tested for encephalomyelitis virus, but for leucosis (COFAL test) and Marek disease (serum neutralization tests on chicken 42 days after vaccination with a 10 fold vaccine dose). It is sufficient to test the seed lot. Tests for mycoplasma and bacteria are also carried out.

Germany

Inactivated vaccines.

The vaccine must primarily be tested by the producer, but under the supervision of a State representative. The vaccine must contain a bacteriostatically active substance which must be declared.

Sampling is described in detail in the legislation. The control tests by the State are concerned with sterility, innocuity of the antibacterial substances (tested in 2 mice), residual virus activity and potency. The production records and test results obtained by the producer must be submitted to the State control institute.

Tests on attenuated live vaccine.

For the vaccine the strain Hitchner B1 or another correspondingly lentogenic virus strain must be employed. The virus may be produced in eggs or in cell cultures, in both cases the host system must be free from avian pathogens (specified). The SPF state must be controled by tests each month in the chicken flocks used. The test records must be kept for inspection. The production must take place in rooms and with equipment, which are not employed for work with other viruses, bacteria or fungi.

The seed virus to be employed as vaccine must be controled for purity, innocuity and potency. The identity of the virus is controled by establishing a neutralization index employing a NDV-antiserum and inoculations in eggs. The presence of contaminating viruses is controled by passing neutralized material in eggs (details are given).

The avirulence of the NDV strain is checked through a determination of a neurovirulence index (in the same way as the French test and as Hanson (1956) Am. J. Vet. Res. 17, 16). Only lyophilized vaccine with a minimum of additions may be marketed. The finished vaccine is tested for contamination with bacteria, fungi and mycoplasma.

Holland

No special information received, apart from the fact that a Benelux agreement exists, and the Pharm. Comm. is closely followed.

Ireland

No requirements specified. No imports have been permitted.

Italy

Few official regulations for controls or tests exist. The Brescia institute follows the British Codex and the material published by Cessi and Nardelli on the inactivated vaccine. These would give a more strict control than the official tests. The internal control by the Brescia institute is carried out in the following way:

Inactivated vaccines with adjuvants are prepared in 11 day old embryonated eggs. The allantoic harvests are clarified by centrifugation and inactivated using beta-propiolactone. The inactivated virus is emulsified with mineral oil to which emulsifier is added (Freundt's incomplete adjuvant). The vaccine is tested for sterility, emulsion stability and viscosity. Live attenuated vaccines are tested for purity as follows: Ten chickens are vaccinated and may not show any signs of disease during a 15 day period. In eggs 3 different routes are employed (each for 5 eggs) for inoculations of micture of virus and antiserum. Except during the first 24 hours, the embryos must not die or react in any way to the inoculation.
The controls which are carried out by the Istituto Superiore di Sanità for the registration of a live attenuated virus vaccine are always carried out in SPF eggs.

U.K.

Tests for inactivated vaccine.
The vaccine may be prepared in eggs or cell cultures and inactivated with a suitable agent (e.g. beta-propiolactone or formalin). Conventional sterility tests are carried out.
Test for live attenuated vaccines.
The chicken flocks must be SPF for all the important avian

pathogens (tests specified).

Seed virus must be tested for extraneous agents like fungi, bacteria, mycoplasma and viruses. Controls for avian encephalomyelitis virus and Marek disease virus and other agents are carried out by inoculations in 5-6 days old and 14 days old chickens employing 10 times the intended vaccine dose. A leukosis test is made in chick embryo fibroblast cultures and, in addition, inoculations in embryonated eggs using 3 different routes are prescribed followed by a further embryo passage. No deaths or abnormalities may occur.

For vaccines using the Hitchner B1 strain the vaccine shall be shown to have an ICPI of not greater than o.3. For the La Sota strain the tests are essentially the same except for details of the innocuity tests (see 5. 5. 2.).If a parenteral administration is intended,the tests are essentially similar.

5. 5. 2. Innocuity Tests

France

Inactivated vaccine.

Innocuity is tested by intramuscular inoculation in at least 10 chickens (3-6 weeks old) employing 2 vaccine doses per animal. The animals are observed for 3 weeks. All animals must survive and remain healthy.

Live vaccines.

A group of 20 chickens is vaccinated and as many are kept as controls. All animals must survive and remain healthy.

Germany

Inactivated vaccine.

The vaccine is tested by letting 5 chickens receive each 10 ml of vaccine. They should remain well, with no signs of NDC. The same applies for inoculations in embryonated eggs. Details in the tests are given.

Live, lentogenic vaccine.

In addition to the vaccination experiment mentioned under

potency-testing the following test is carried out: Intramuscular inoculations are performed of o.1 ml of a 1/5o dilution into 20 days old chickens, which must remain well and alive after 14 days.

Italy

Inactivated vaccine with adjuvants. Innocuity is tested by inoculation in 25 10-day embryonated eggs to control the possible residual virus activity both in inactivated virus suspension and finished vaccines (both living and dead embryos are tested as pools). One passage of pooled material should give a product without HA-activity.

A test in 20 chickens is carried out employing intramuscular injections with 1 ml vaccine. No abnormal reactions may develop during the observation period (14 days).

Live, attenuated vaccines are tested for innocuity by vaccinating 25 5-6 day old chickens from SPF eggs according to the prescribed method. They are observed for 21 days, and if more than 8 per cent die with respiratory or nervous symptoms of disease, the vaccine is not accepted. Characterization of the vaccine virus is obtained by determination of the index of intracerebral pathogenicity (IPI) and the index of intravenous pathogenicity (IPIV).

U. K.

Inactivated vaccine. Innocuity tests are carried out in eggs and chickens. The materials from eggs are collected in two pools: one from the eggs with live embryos and one from the eggs with dead embryos. Both materials are passed into new eggs, which must be free from HA reaction after harvest. The chickens must remain healthy.

Live, attenuated vaccines. For the Hitchner strain intranasal instillation at the field strength is applied to 25 day old SPF chickens. No more than two deaths and no respiratory or nervous symptoms after 21 days may occur.

The test for the La Sota strain is essentially the same, but 25 SPF chickens of age 10-21 days are employed, and the surviving birds shall be challenged.

5. 5. 3. Potency Tests

France

Inactivated vaccine.

Potency is tested by vaccinating 20 animals in the prescribed way. After 14-21 days the vaccinated animals as well as a corresponding control group are given $10^5 LD_{50}$ of a virulent NDV. The virus should kill 100 per cent of the controls, and 90 per cent of the vaccinated chicken must survive without any signs of disease.

Live, lentogenic vaccine.

A group of 20 chickens are vaccinated and as many are kept as controls. They are challenged with $10^5 LD_{50}$ of virulent NDV. The dose should kill all the controls, and 90 per cent of the vaccinated chicken must survive without any symptoms of disease.

The titre of infectiosity may not be lower than $10^6 ID_{50}$ per vaccine dose. This test is carried out in embryonated eggs through HA demonstration.

Germany

Inactivated vaccine.

Potency is tested using 160 2-week-old chickens. Of these chickens, 100 chickens are employed for testing a standard vaccine (employing 2 doses with a factor 10 in between) for comparison. Of the remaining animals 50 are employed for the vaccine to be tested, and 10 animals serve as unvaccinated controls. After 14 days a challenge dose of $5 \times 10^6 LD_{50}$ of virus is given, and after a further 10 day period the control animals must all have died. The vaccine tested must give a protection between the one obtained with the two standard vaccine at the doses employed (details given).

Live, lentogenic vaccine.

The potency of the virus harvest is tested in eggs. The titre must be $10^{8.5}$/0.1 ml before lyophilization. The finished vaccine is tested for potency as follows:

A dose sufficient for 100 animals must contain 4 x 10^8 ID_{50} measured in eggs. A vaccination experiment is made in 20 3-7 day old chickens employing the vaccination method prescribed by the producer. The animals must remain well. After 14-21 days the vaccinated animals and a corresponding group of unvaccinated animals are challenged using 10^4 egg ID_{50} of a virulent strain of NDV. Out of the 20 controls 18 at least must show typical ND symptoms and at least 18 of the 20 vaccinated must be protected during a 14 day observation period.

Italy

Inactivated vaccine with adjuvants.

Potency is tested by a titration of the vaccine employing 1/25, 1/50 and 1/100 of the recommended dose. A challenge of at least 2 x 10^5 egg LD_{50} of Herts 33 strain is given to the 75 vaccinated and at least 10 unvaccinated controls, which must all die during a 10 day period after challenge. By a probit analysis a strength of at least 75 PD_{50} must be found for the vaccine. The lower 95% confidence limit must be at least 50 PD_{50}. (Cessi and Nardelli 1974).

Live, attenuated vaccine.

The vaccine should contain 10^6 ID_{50}/ml and protect to at least 80 per cent, when 20 vaccinated chickens and as many controls are challenged with a dose of virulent virus, which gives a mortality of at least 80 per cent after 15 days in the controls.

U. K.

Inactivated vaccines.

1/25, 1/50 and 1/100 of the vaccine is inoculated in groups of 25 chickens and challenge using 2 x 10^5 ELD_{50} of virulent virus is performed after 14-18 days. Unvaccinated controls (10 chickens) are included. If any controls survive after 10 days, the test must be repeated. The potency is evaluated statistically. By storage at 5°C the potency should be preserved for at least a year. Freezing must be avoided. Dosage is 0.5 ml for chickens.

Live, attenuated vaccines.

The vaccine dose shall contain not less than $10^6 EID_{50}$. A challenge test using at least $10^6 EID_{50}$ in 25 vaccinated chickens and 10 unvaccinated chickens must show protection with no signs of disease in 23 vaccinated chickens, and all controls must die.

If a parenteral administration is intended, the tests are essentially similar.

Table 5. Schematic presentation of Newcastle Disease vaccines

	Producer	Controling authority	Rules for vaccination
Belgium	Authorized private firms	The State	Encouraged any time (by indemnities) compulsory when decided by Vet. Auth.
Denmark	Imported if considered necessary by Vet. Auth.		Vaccinations have never been permitted
France	Authorized private firms	The State	Encouraged, but ordinarily not compulsory
Germany	Authorized private firms	The State (Paul Ehrlich-Institut	Compulsory
Holland	Private authorized firms	The State (sampling by VD testing by CDI)	Compulsory for the commercial farming
Ireland	No production No import		If needed the ministry would have to give special permissions
Italy	Private or semi-private firms	The State	Permitted, but not compulsory
Luxemburg			
U. K.	Private firms with license	The State The Central Vet. Lab., Weybridge	Encouraged, but not compulsory

5. 6. V a c c i n a t i o n

5. 6. 1. Rules for vaccination

Belgium

If the poultry has been vaccinated at least 10 days, but less than 4 months before an epizootic,clinical healthy animals may be excepted from slaughter and kept under observation. If the Vet. Off. decides for slaughter in spite of the proper vaccination, the owner may, within the budget available, receive indemnities. Vaccination may, when needed, be made compulsory locally or in general. Within the budget available, the vaccination is paid for by the public.

Denmark

Vaccinations have never been employed.

France

Apparently no specific information about the rules has been ceived. It was, however, stated: "A too early discontinuation of vaccination programmes should be avoided, because the virus persists,even if the vaccinations may eliminate the manifestations for some time". From this statement it may probably be concluded, that vaccination in general is encouraged, but that it is not compulsory.

Germany

The farmer must let a veterinarian vaccinate all chickens and repeat the vaccinations, so that a sufficient immunity is obtained. The vaccination may be carried out with inactivated vaccine or live vaccine containing the Hitchner B or the La Sota strain of virus.

Holland

Vaccinations are compulsory for all commercial marketing. Every flock of broilers for slaughter must be vaccinated, and also laying hens and breeders. This is intensively controlled by HI-tests on sera from the poultry. The sera of breeders must be tested (o.5 per cent of the animals with

a minimum of 24 animals), and it is recommended to test the laying hens. There is a special vaccination plan for the different categories of poultry with 2-4 vaccinations.

Ireland

No vaccinations have been carried out.

Italy

Vaccinations are permitted, but not compulsory.

U. K.

Vaccinations are encouraged, but are not compulsory.

5. 6. 2. Economics

Belgium

Normally the farmer pays for the vaccination, but may then have his poultry exempted from slaughter during an epizootic. The compulsory, emergency vaccination is in principle paid for by the State.

Denmark

Apparently no information received.

France

Apparently no information received.

Germany

The farmer pays the vaccination.

Holland

The farmer pays the vaccination.

Italy

The farmer pays the vaccination.

U.K.

The farmer pays the vaccination.

5. 6. 3. Indemnities

Belgium

Apparently no special provisions made.

Denmark

No special provisions made.

France

Apparently no information received.

Germany

The producer remains responsible for the vaccine.

Holland

The producer remains responsible for the vaccine.

Italy

The producer remains responsible for the vaccine.

U. K.

The producer remains responsible for the vaccine.

In table 6 the economic aspects of NDV vaccines are compiled.

Table 6. Newcastle Disease Vaccine

Economic Aspects

Member State	Vaccination paid by:	Indemnities in cse of vaccine-associated accidents paid by:
Belgium	Within the budget available the vaccination is paid for by the public	Has not been paid
Denmark		
France		
Germany	The farmer	The producer
Holland	The farmer	The producer
Ireland	-	-
Italy	The farmer	The producer
Luxemburg		
U. K.	The farmer	The producer

5. 7. Proposals for Community Regulations

5. 7. 1. Summing up of the Present Situation

The present day situation within the Community is quite complicated not only regarding rules and regulations (see table 5), but also with respect to the presence or absence of NDV in different regions and countries.

A very serious spread might occur once the rapprochement envisaged between the regulations of the Member States become a reality, because field virus may be disguised in vaccinated flocks, and vaccinations are compulsory in some of the countries and forbidden in other countries at present. In addition virus may be excreted weeks after recovery and apparently survive at least months even under quite unfavourable conditions.

In the Memorandum for the Meeting of the Working Subgroup "Newcastle Disease", May 1974 (Comm. Europ. Comm., VI/H/2 File No. 7.9, 1603/VI/74 E) it is stated that control of Newcastle disease cannot be established without taking the pandemic nature of the disease into consideration. Rules for controling the spread of virus from the "outside world" (through imports and movements of wild birds, domestic poultry, eggs, and poultry meat) should be very strict and common for Newcastle disease and a number of other diseases that might be spread in similar ways. Examination of imported poultry carcasses for presence of virus should be performed on a routine basis.

The rules 64/432/CEE of the Council of June 1964, which are intended for cattle and pigs, might be employed in principle for poultry as well. It seems possible at least, that similar rules will be applied. Thus it should be possible nationally to maintain severe restrictions on imports and exports or transits for reasons connected with the safeguarding of health of man and animals. These possible rights do not, however, suppress the duty of providing such conditions, that may

result in rapprochement and realization of a mutual Community policy. Included in such a policy is the duty of the individual Member State to guarantee that animals for breeding or slaughter, which are exported by intra-community activity, do not constitute a risk for the spread of infection. It does not seem quite clear, if the Rome treaty in fact permits national regulations concerning control of imports from other Member States by taking samples for virus detection or if the product (or the bird) is covered by certificates from the authorities of the country of origin. This problem should be brought to the attention of the Permanent Vet. Commission for clarification.

The above mentioned sub-group on ND worked out the following papers, which have been employed as background material:

Annex A: The implications of vaccination against Newcastle Disease with special regard to production of vaccine (standardization, control) and the methods of administrating vaccine.

Annex B: Safeguards to reduce the risk of spread of Newcastle Disease infection by trade of fresh poultry meat.

Addendum I. G. Eissner: Proposals for requirements for the production and control of "Newcastle-Disease vaccine, freeze-dried, live-virus".

Addendum II. Standardization of vaccines.

Addendum III: Document de travail. CCE sur la vaccination contre la maladie de Newcastle (Station exp. d'aviculture. Ploufragan).

Addendum IV: W.H. Allan: Newcastle Disease. The guarantee of safe conditions for countries or regions not at present affected.

The subgroup has summarized the situation in its introductory note. If the contents of the note is sharpened somewhat the situation might be summarized as follows:

Ideally poultry flocks should be established and kept-without NDV. This system is functional in some Member States and should be the long term goal, because by vaccinations the infections may not be eliminated, and because the less strict systems allow spread of other diseases as well, against which there may not exist efficient vaccines. On a short term basis and accepting the actual situation (organisation, economics, presence of infection etc.) the only realistic method of protecting many Community flocks is by vaccination.

In addition to the subgroup I papers the British Codex and supplementary British information has been employed in the following proposals, as well as the Pharmacopoeia Comm. papers PA/PH/Exp.3/T(73)8 third rev. and PA/PH/Exp.3T/(73)21 2 rev.

As also declared by the different Nat. Auth. that the Pharm. decisions will be followed as soon as they are finalized. In the meantime and in a trial period the following proposals could be followed.

5. 7. 2. Proposal for Community vaccination procedure

As a common rule for the Community it seems, that vaccination of all poultry meant for slaughter and marketing should be compulsory and carried out at a suitable early period. Breeding farms should be permitted and even encouraged by legislation (and perhaps some kind of an insurance) to be kept NDV free and without vaccination in a "one-way-traffic system" in otherwise closed units. At their own risk and after certifying that the chickens come from NDV free unvaccinated breeding farms and provided that NDV is not diagnosed in the area, commercial breeders may be permitted to omit vaccination even for broilers.

5. 7. 3. Licenses for Vaccine Production

5. 7. 3. 1. Present situation

The present situation concerning NDV vaccine production

corresponds in many ways to the Swine fever situation. In all producing countries private firms produce the vaccines, and the State lab. that is doing the required official controls. There are many differences in the actual requirements, but nothing in principle would prevent Community Licenses as soon as the requirements were agreed upon. Both the Community and the Nat. Authorities would have to establish regulations, which deal with other areas than just the vaccine requirements treated by the European Pharmacopoiea Comm.

The vaccine is produced in Belgium, France, Germany, Holland, Italy and U. K.

5. 7. 3. 2. Proposal for a Community License:

1) Establishment licenses shall be issued only after inspection resulting in a determination that the establishment complies with prescribed standards.

2) A product license shall be issued only upon examination of the vaccine and provided, that the vaccine complies with the standards prescribed, and that the establishment is accepted.

The details regarding License Forms etc. could also be worked out; the result could be a common Community License valid for all Member States. The norms are to be worked out by Permanent Veterinary Committee procedures.

5. 7. 4. Standardization of Vaccine

In close agreement with what has been suggested for FMD vaccines (3. 7. 4.) and Swine fever vaccines (4. 7. 4.), it is suggested, that reference laboratories be established, preferably as an extension of existing institutions. The reference laboratory should provide information and recommendations, e.g. if new vaccine virus strains or new methods for production and application are considered. The reference laboratory should provide seed lot virus to be used as a challenge in the official potency tests.

The Nat. Laboratories must collaborate with this Ref. Lab., and mutual decisions must be followed by the Nat. Labs, but the issuance of establishment licenses and the control of vaccine seed lots and production are carried out nationally.

By Permanent Vet. Comm. procedure it should be decided how many tests may be done, apart from the tests and inspections required in connection with the establishment licenses and with seed lot tests which should always be carried out.

The reference laboratory system should have a trial period of at most three years. In this period the licenses should still be approved in each country, but after this time the principle of "free trade" should be employed.

5. 7. 5. Minimal Requirements for NDV Vaccines

5. 7. 5. 1. Inactivated Vaccines

In general it is suggested that the rules in British Vet. Codex 1965, Supplement 1970, with the addition found in Cessi and Nardelli Requirements for testing oil emulsion inactivated Newcastle Disease vaccine. (Int. Symp. on Requirements for Poultry Virus Vaccines (1973)) should be followed.

It seems, that there is still a need for an inactivated vaccine for revaccination, and that such vaccines may even give a long lasting immunity.

Definition: An inactivated NDV vaccine is a suspension of Newcastle Virus inactivated without destroying its immunogenicity.

Preparation: The vaccine is obtained from cell cultures or embryonated eggs from healthy animals. The harvested virus suspenison is inactivated by physical or chemical means. The vaccine should contain an adjuvant and may contain a suitable anti-microbiol. substance, but not penicillin or streptomycin.

In chickens the vaccine must stimulate the formation of hemagglutination inhibiting and neutralizing anti-

bodies.

Sterility tests should be carried out in the usual Pharm. Europ. manner.

The innocuity is tested by inoculation in at least 10 3-6 days old chickens. The vaccine is given intramuscularly in double vaccine dose. The animals are observed for 21 days. No death or clinical signs of disease may appear.

Potency tests. 20 SPF-chickens are vaccinated employing the dose and the method prescribed on the label by the producer. The chickens should be of the minimum age indicated on the label. After 14 or 21 days the vaccinated animals together with as many unvaccinated of the same flock serving as controls. The challenge dose should be $10^5 LD_{50}$ of the reference challenge virus for chickens less than 10 days old. After 10 days 90 per cent of the vaccinated animals must survive without any signs of disease, and all control animals must have died.

Expiration date. The vaccine should be kept at 2-6°C, and the expiration date is 12 months after the date of a satisfactory potency test.

5. 7. 5. 2. Live, attenuated Vaccines

Production of live virus vaccine is based on a seed lot system. The final NDV vaccine must not be more than five sub-cultures from the seed lot, on which were made all the tests required for accepting the strain, as lentogenic and otherwise suitable.

The vaccine is obtained by the culture of the virus in the allantoic cavity of fertile hen eggs from specific pathogen-free flocks or in cell cultures. If the cell cultures are of avian origin they must be obtained from specific pathogen-free sources. The viral suspension is collected, titrated, and diluted in a suitable stabilising solution and freeze-dried. The virus titre must be at least $10^{6.5} ID_{50}$ per dose.

Tests on seed lots. The lentogenic character of the strain is verified by determining the index of neuropathogenicity. The seed lot is diluted with sterile liquid adjusted to pH 7.0 containing antibiotic so that $10^{5.7}$ EID_{50} is contained in 0.05 ml.

0.05 ml of the dilution is injected intracerebrally into at least ten one-day old chicks. The chicks are observed for 8 days and, each day, the number in good health, the number showing signs of disease, and the number that die, are noted. The three totals are calculated. The total of healthy chickens is multiplied by 0, the total of those showing disease is multiplied by 1, and the total of deaths is multiplied by 2. The sum of the three products is divided by the sum of the total number of healthy, diseased and killed animals. The index of neuropathogenicity, so calculated, must not be greater than 0.25.

The vaccine virus is identified in an HI test employing a monospecific antiserum. The vaccine is tested for extraneous virus, especially avian encephalomyelitis virus, avian leucosis, Marek disease, mycoplasma and for bacterial contamination in a manner decided through agreement with the Reference Laboratory.

Innocuity tests are carried out by the intranasal route to each of at least ten SPF chickens of the minimum age recommended for vaccination. The chickens are observed for 21 days after vaccination. If more than two chickens die from non-specific causes during the prescribed period, the test must be repeated. The vaccine passes the test, if none of the chickens develop serious respiratory or nervous symptoms or dies.

Potency tests are carried out as for the inactivated vaccines (5. 7. 5. 1.).

Virus titre. Reconstitute the vaccine as indicated on the label and make titrations in eggs or in cell cultures. The titre in the vaccine must be not less than 10^{6} ID_{50} per dose.

Batch tests. For each batch innocuity and virus titre should be tested as for seed lots. The exact requirements corresponding to the description of the test should be worked out by Permanent Vet. Comm. procedure, unless the Pharm. Comm. monographs are finalized during the trial period.

5. 7. 6. Vaccination Programmes

Methods for vaccination. It is suggested, that application by spraying, in spite of a number of problems, is the best method, provided that the procedure and apparatus are suitable, and that the vaccination is carried out by a qualified person. The inactivated vaccines are employed using intramuscular and subcutaneous injections, and the same routes could be used also for the live vaccines. A certificate by an auth. vet. must always be issued.

Vaccination schedules. Except for chickens from previously NDV Stricken flocks, vaccination may be carried out on day old chickens. With an inactivated vaccine it seems possible to obtain protection for 6 months, whereas the reported duration of protection when using lentogenic live vaccines seems to be 10-12 weeks. In accordance with this the following schedules are suggested:

For broilers vaccination on day 4 and on day 21-25 and for breeders vaccinations at 6-7 weeks, 10 weeks and 18 weeks. There should be 20 days between vaccination and slaughter. With such a schedule a good level of immunization should be maintained, but changes in vaccine quality and in the epidemiological situation may make other vaccination schedules desirable. Consequently they may be subject to changes according to recommendations through the Ref. Lab.

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