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FOOT AND MOUTH DISEASE CONTROL STRATEGIES GLOBALFRAME WORK

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INTRODUCTION

Livestock sector revolution is the global phenomenon under the critical gap of Food Nutritional security to the world. Asia and the Indian dairy farming and agriculture business has grown to Himalayan heights to put the country as number one milk producer in the world today with above 106 million tons record production._The country has a huge livestock population (500million livestock) of cattle, buffaloes, sheep and goats. A small ruminant are in desert and hilly track of Indian subcontinent e.g HP, J & K, Rajasthan, Tamilnadu are the major habitant for rural economy mainly for marginal and landless community. Piggery population in NE states, Vietnam, phillipines EU and North America have significant contribution in food security even for poor people of the particular nation as a live hood programme. The production of livestock products like milk, meat, wool, hides and other byproducts have also increased tremendously in recent years. This sector has been recognized world wide as an economic agenda globally.

Improvement in the genetic potential of the livestock by means of cross breeding have made the cross breed animals, apart from increasing stressful productivity management practices have made animals more susceptible to diseases like *Foot and Mouth disease, infectious bovine rhinotracheitis, haemorrhagic septicaemia, black quarter, brucellosis production disease like mastitis.* Most of these diseases c"an be controlled by systematic vaccination and monitoring FMD and Mastitis are major economic herdle and loss to GDP more than 10billion per year which needs clear attention. Foot and mouth disease is proven globally an economically devastating and highly contagious disease of domestic and wild cloven footed animals including cattle, sheep, goat, pigs and camel. It limits access to markets for developing food producing countries and can cause costly outbreaks even in formerly FMD FREE countries eg..Netherland, UK, 2001, 2007. 100% morbidity in population, FMD virus has high mutation rate, exists as seven distinct serotypes O,A,C,ASIA1,SAT 1,2,3 in African subcontinent, the antigenic variation creates a major problem for the control of FMD.

The export market of livestock products is ever increasing. The increase is particularly significant to other Asian countries, countries of the Middle East and Europe. The world trade organization (WTO) plays a significant role in determining trade policies, it would be of utmost importance that exports products be free of important infectious diseases especially those listed by the OIE. A stringent monitoring and control policy should be implemented to prevent spread of these diseases (FAO / OIE / WTO). Global strategy with frame work to control FMD in order to get free status is the need for trade and food security.

India has followed the OIE proposal for the eradication of Rinderpest disease. Sero surveillance and disease diagnosis was vigorously followed along with compulsory vaccination of all animals. It should be noted that the OIE would recognize India to be Rinderpest free soon. An immune belt has been created along the borders of

the endemic areas. India is free from this 2004 onward how OIE agreed about India status for free Rinderpest country 2010.

Many of the developed nations are free from most of the diseases listed in OIE particularly FMD and hence their livestock products are better accepted worldwide. The prevalence of FOOT AND MOUTH DISESE in India is a major trade barrier.

Foot and Mouth disease is an acute infection caused by a virus. The virus belonging to the picornaviridae family genus Aphthovirus is the smallest virus known so far. The disease is characterized by formation of blisters, followed by ulcers on the mucosa of the. mouth cavity and on the skin of the feet, hence the name: "Foot and Mouth Disease". The disease is characterized by fever, lameness and vesicular lesions on the mouth, tongue, feet, snout and teats in infected animals scrotum in male bulls, large ruminants shows distinct evidence where as small ruminants mild manifestation, sheep was major transmitter of this disease in UK out break in 2001. FAO/EUFMD/OIE working closely to ensure vaccine bank constitution, operation and maintenance of information on the stocks of antigen and vaccine available in the member countries and other countries keep the position of the countries under review.

Animals Susceptible for Foot and Mouth Disease

All cloven-footed animals including cattle, buffaloes, sheep, goat, pigs, elephants and other ruminants are susceptible to the virus. For obvious reasons, the disease is more important in cattle, buffaloes, bulls and bullocks in India.

Disease Spread

The infected animal is the main source of infection. Infection may spread either through direct contact or by indirect means, the infected feed and fodder, infected utensils, and infected means of transportation or, through carrier cattle attenders. Infections have also been reported to travel through air. However, at most times, the wide spread of infection results from congregation of animals in cattle fairs, cattle markets or large-scale transportation of agricultural produce in bullock carts.

The incubation period for Foot and Mouth disease is as short as 48 to 72 hours and as long as 10 to 14 days. On an average, it varies from 3 to 7 days.

Seven immunologically distinct serotypes of Foot and Mouth disease viruses have been reported worldwide. There is no cross-immunity between Sero types, immunity to one does not confer immunity against any of the other types. Four serotypes 0, A, Asia-1 and C are the reported serotypes in India.

Disease Economics

Besides the acute stage of the disease, characterized by the formation of ulcers in the mouth, feet and udder, the virus of foot and mouth disease exhibits its pathology in some of the vital hormonal glands, which control the metabolic processes of the body. Disordered functioning of heat regulating centers leading to panting is one example. The disturbance in physiological process of lactation leads to a significant reduction in milk yield. In mild animals lesions on teat and udder can lead to mastitis, which may damage the teats and thereby affect the milk yield on permanently.

The economic losses to the livestock industry attributed to Foot and Mouth disease are large. The OIE / FAO / APHCA place a massive significance in their attempts to eradicate Foot and Mouth disease worldwide. 10 billion $ globally economic loss. In India $5 billion per year.

Direct losses to livestock sector are due to

I. Abortion in 25 % pregnant animals.
2. Reduction in meat production by 25 % in endemic area.
3. A drop in milk production by 50%.
4. A reduction in wool production by 25 % in affected sheep.
5. Mortality rate of up to 5.5 % of the affected cases.

Indirect loss to livestock sector is due to

a. Loss of production functions during the acute phase of disease (daily income).
b. Loss of milk yield on a permanent basis (live

hood).

c. Loss of breeding capacity including abortions (Livestock development).
d. Loss due to reduced draught capacity in working bullocks (energy saving).
e. Interference with food production programme (Nutritional security).
f. Loss in cattle trade both national and international (WTO).
g. Loss resulting from temporary cessation of A.I. programme.
h. Loss in flesh in meat-animals.
i. Mortality in young calves due to heart failure (TRIGROID Hearts).
j. Flare up ofinter-current infections like Theileriosis and Anaplasmosis.

'Prevention is better than cure' "FMD Vaccination for living not killing the animal"

This adage is very relevant in the case of Foot and Mouth Disease than other diseases. In countries where Foot and Mouth Disease is wide spread regular programme of large-scale vaccination using Foot and Mouth Disease vaccines are being followed. Herd immunity plays vital role in control strategy and finally eradication to ensure infection focus is zero level.

Prevalence

Foot and Mouth disease is enzootic in Africa, Asia. South America and parts of Europe a global frame work for control is the solution.

The disease has been reported from various parts of the world except Japan, New Zealand, Australia, Canada and the United State of America. Foot and Mouth disease is a reportable disease in most countries and attempts are made by the FAO/OIE to collect data on the prevalence of the disease in various countries. The identification of the various virus serotypes is based upon complement fixation test, liquid phase blocking ELISA and recently by nucleic acid recognition method acid recognition method (Polymerase chain reaction). Overall it has been found that the serotype '0' and the serotype 'A' occur more frequently than the other serotypes. The disease is endemic in India.

Strategy to control

Sero-type predominantly occurring in the country are mainly type '0' (70%) followed by Asia-l and type A . There has been no report or minor occurrence of type 'C' outbreak(s). In Punjab, Uttar pradesh and Maharastra in 1998 were predominantly by Type '0'. The various serotypes of foot and mouth disease virus are antigenically distinct and do not cross react. Depending upon the prevalence of the type of the virus causing disease, the vaccine used in the area is determined. If a single type of virus is seen prevalent, a monovalent vaccine (with only one type of the virus antigen) is used. If two types seen, bi-valent vaccine with two types of antigens are used. In India, a tetravalent vaccine with antigen from type A, O, and Asia-1 are used. To prevent antigenic drift, vaccines usually with more than one strain are used in manufacture "C" virus is not reported more than 10 years.

Vaccine producers maintain a reference collection of the vaccine strains of FMDV. By comparing outbreak strains with the vaccine strains could be identified. A repertoire, an appropriate vaccine strains could be identified. A repertoire of antibodies is also developed to determine shared neutralizing epitopes, thereby giving an indication of the potential value of vaccine strains in helping control the outbreak. Linkages are being established within India, with IVRI, the United Kingdom Institute for Animal Health, at Pirbright and other institutes globally via the Internet, networking resources with SAARC, Asia and Europe globally.

In 1951-52 over 900,000 outbreaks were reported in Europe, the European countries finally eradicated the disease and from 1992 the member countries of European Economic Community (EEC) are adopting, a non-vaccination and stamping-out policy. This had largely come about by the maintenance of solid vaccination coverage because the European FMD commission in 1957 accepted the systematic vaccination would be necessary for number of years to reduce the incidence of the disease so as to make other measures like slaughter policy an economically feasible. Reoccurrence in 2000 and efforts are on globally to control FMD.

FMD Vaccines

International standards for FMD vaccines can be found in the British Veterinary Pharmacopoeia, British Veterinary pharmacopoeia Codex, European Pharmacopoeia (Veterinary), I.P.Vet., OIE being followed (1993) and the OIE Manual of Standards for Diagnostic Tests and Vaccines. National Veterinary Authorities usually exercise control of the use of Foot and Mouth Disease vaccines. Indian Veterinary Pharmacopoeia is being planned to release shortly. The FAO/OIE is formulating International standards for safety, potency and antigenic mass requirements for the various vaccine strains. The dosage of the vaccine mass depending upon the epidemiological situation of the area is also being worked out purified concentrated vaccine 2ml dose is being propagated.

History of FMD vaccine development

The three critical elements of FMD vaccine production are antigen production, virus inactivation and the addition of suitable adjuvants. Historically, the original source of FMDV for vaccine production was clinically derived material, such as infected cattle tongues in 1926.

In 1951, Franked described a new technique for the production of FMDV on an industrial scale in tongue explants. It was the FMD vaccine made in this system that was used in the Netherlands in the first of the highly successful mass annual prophylactic vaccination campaigns to be carried out in Europe.

The advantages of this production system were its simplicity, low / lack of cellular protein contamination of the virus harvest and the fact that adaptations of the virus to the culture system was not required.

A significant development in FMDV antigen production was the transition to tissue culture methods of virus growth. Initially, small-scale production in roller bottles using primary calf kidney cells was instigated in Italy in 1963.

However, following the introduction in 1964 of a continuous cell line derived from baby hamster kidney fibroblasts (BHK 21) that supported the growth of FMDV, this system gained wide acceptance in FMDV vaccine production. Large scale fermentors 100 liters to 5000 liters capacity as continuous culture are in place.

Later a variety of monolayer systems were devised to increase culture vessel surface area, and thus productivity. Vector vaccine, subunit, recombinant protein is all in way for development.

However, the greatest scale-up capacity for FMDV production was the advent of technology, which exploited the ability of BHK-21 cells to grow in deep suspensions culture in fermentors (bioreactors) that are used widely now days. 5000 to 10000 liters as continuous culture.

Antigen production Commercially available FMD vaccines are still based upon inactivated whole virus particles, mostly grown in BHK-21 in a battery of fermentors 100 1200 liters capacity located in strict containment area under controlled air conditions, could be scaled up 5000 to 10000 liters

Virus growth in cell culture system is followed by a series of treatments to clarify, inactivate, purify and concentrate the viral harvest to ensure the yield and antigenic mass.

During FMD antigen production, temperature and pH have to be closely controlled because of thermal instability and the low tolerance of the virus to pH conditions outside the ranges 7.0 - 8.0. Whole virus particle (146S) content is critical to the potency of the final product and measurement of 146S is used for vaccine formulation calculations.

Inactivation

Inactivation is one of the most critical steps in the production of FMD vaccine. Initially formaldehyde was used to inactivate alum adsorbed virus. In process evaluation of this system proved to be difficult and it has mostly been superseded by the use of first order kinetics inactivants of the aziridine group of chemicals, most recently binary-ethylene-imine (BEl). Ideally this procedure is performed twice in separate inactivation vessels.

Good Manufacturing Practices (GMP)

In recent years, there has been a move away from end product testing towards the philosophy of in-

process control. This policy has been encouraged at HR Vet. Through the promotion of Good Manufacturing Practice (GMP). In process inactivation controls are performed upon the virus harvest by tissue culture titration in sensitive cells, spectrophotometeric analysis or serological assays. BSL3 and BSL4 Biocontainment and Biosafety level as per CDC- NIH guidelines is needed for plant.

Further most inactivation concentration and purification by ultrafiltration or precipitation with polyethylene glycol (PEG) could yield a final virus product with a concentration factor of up to 1000 fold. Post inactivation purification reduces the non viral protein component of the antigen harvest, which is important in the reduction of potential hypersensitivity reaction in vaccinated animals.

Adjuvants

Inactivated whole virus vaccines against FMD are formulated as mono or polyvalent products with suitable stabilizers, buffers and adjuvant to enhance their potency. In aqueous formulations, the inactivated viral antigen is adsorbed to aluminium hydroxide [AI (OH) 3] and further adjuvant with saponin.Such vaccines are used successfully world wide for the immunization of ruminants.

However, commercial aqueous vaccines have not been successful in immunizing pigs (reactions at the site of injection were observed), and concentrated, inactivated antigens formulated as oil adjuvant vaccines have been used widely in this species.

Oil adjuvant FMD vaccines are also used in cattle, particularly in South America. Improved formulations have reduced the local reactions initially seen in this species. Now India and globally oil adjuvant vaccine is choice across the species.

Advantages are claimed for the use of oil-adjuvant FMD vaccines in cattle in the areas of duration of immunity and the ability to immunize calves. Simple water-in-oil preparations can be made by the emulsification of the antigen in aqueous solution with light mineral oil and an emulsifying agent. Silverson and Ystral on-line pumps are used for the emulsification process and to ensure stability of the emulsion under field conditions.

Alternatively, a more easily injectable formulation can be made by further emulsification in a second aqueous phase to produce a stable water emulsion [double oil emulsified (DOE)]. There are several reports of the successful experiment use of these DOE FMD vaccines in cattle and pigs.

Following the completion of the blending process and addition of suitable preservatives, the vaccine bottled should be subjected to prescribed in vitro sterility test, safety *I* innocuity and potency tests in cattle, as described in the European Pharmacopoeia (Veterinary).

Safety tests are performed *in vivo* using the whole vaccine inoculated into susceptible animals and *in vitro* using eluted antigen inoculated onto sensitive cell culture.

Minimum potency assurance required is assessed by a variety of serological and *I* or animal challenge procedures.

FMD vaccines have a shelf life of one year if stored at 4^0C, two year is also in practice under I.P.Vet.

Production capacity

FMD Vaccine plant needs Biosafety and Biocontainment BSL3 and BSL4 facility under GMP/ OIE/ WHO standards. The production capacities have been increased tremendously to meet the demands of country as well as India current production capacity is 125 million doses in totality and need to increase the capacity to 250 million doses by 2015, globally 500 million doses vaccine needed which could be more under global FMD Control strategy by 2020.

Use of FMD Vaccines

In order to establish satisfactory immunity, it is usual to give a primary course of two inoculations with an interval of 2-4 weeks.

Re-vaccination may be given at 4-12 month intervals depending upon local epidemiological conditions and the quality of the vaccine. Therefore, the primary vaccination course may be delayed until four months of age in the offspring of regularly vaccinated mothers, although there is

some evidence that calves can respond at one month old or younger.

The Role of Vaccination in FMD Control Strategy

Prophylactic

The successful control of FMD in countries with endemic or epizootic disease has often been based upon the regular use of inactivated whole virus vaccines as part of a regional FMD control policy.

The short lived nature of protective immunity in cattle following vaccination compared to FMD infection has led to the need to vaccinate annually or bi-annually, and even thrice a year in areas with a high risk of exposure to the virus.

Antigenic variation within a serotype has made it common practice to include more than one strain of a particular serotype in FMD vaccines.

Mass prophylactic vaccination against FMD, usually practiced only in the cattle population, is the first step towards controlling FMD in endemic areas.

The aim of this policy is that, over a period of years the load of FMDV in the environment will be reduced as the number of outbreaks, and therefore animals, with clinical disease will fall.

Obviously, good veterinary services are essential to maintain the vaccination campaign and monitor disease status in the country.

If the level of immunity to FMD in the target population in excess of 75% is achieved, the disease should be adequately under control so that extra measures, such as importation control, quarantine and stamping out foci of infection, can be effective.

An example of the successful implementation of these policies was the reduction in outbreaks of FMD in Europe from 30000/year in 1965 to less than lOOO/year by 1975, how under vaccination, it is evident for reduction of out break even in India.

It is extremely important that an antigenically appropriate vaccine should be used. It is essential that the antigenic relationship between field isolates and the vaccine strains in use should be ascertained regularly. The next stage in FMD control is to stop mass prophylactic vaccination and, by means of stringent surveillance, rapid diagnosis and importation control, a state of freedom from infection could be achieved. This is the current situation for Uruguay and the European Union countries. Efficacy of vaccination is affected by the lack of cross protection between serotypes, as well as incomplete protection between some serotypes. In the case of FMD outbreaks, the immediate need is to detect the serotype of the circulating virus, eg Antigen TYPING ELISA or by genetic typing…vpgene1invitro vaccine matching assay are done to select most suitable vaccine strain to be used in the region country ..R value is being used presently. The antigenic similarities between vaccine strains and field isolates are estimated from their comparative reactivity test CFT, now ELISA, SNT/ VNTs

Emergency

General vaccination is recommended for countries where the disease is enzootic, or where the threat of an outbreak is very great. If an outbreak occurs, a booster vaccination with the relevant serotype will increase the resistance of the population.

The process of stamping out of infection is difficult under Indian conditions because of social reasons. Emergency vaccine bank to be maintained as National, Regional an Global level.

Mass vaccination coverage to 80% of the animal population will reduce the incidence of foot and mouth disease in endemic areas. A generation of a vaccine should be advocated to contain the disease.

Committed people, a proven vaccine, a good delivery system and effective vaccination coverage along with active support from the farmers, Governmental decision makers, government research institutes, nongovernmental agencies, and manufacturers of vaccine would effectively control foot and mouth disease in India. Regional understanding global FMD control strategy with clear frame work will certainly yield the desired result and economic benefit not only to trade, but the livelihood programme. Needs to be planned, budgeted and implementation with networking, coordination and cooperation with in

the nation and globally cooperation strategy by world organizations.

FMD vaccine application and effectiveness as future control strategy in FMD free and FMD endemic countries is global economic agenda, for efficient control of FMD, vaccination and restriction and movement of infected animals and animal products are crucial step forward. In endemic situations prophylactic vaccination every 6months or depending on local situation in FMD free countries, emergency vaccination- "**VACCINATE TO LIVE",** along with culling of infected animals may be suitable option to control FMD.

The vaccination of FMD has been proven to be an efficient disease control however the current FMD vaccines can protect from animals clinical infection, there remains the possibility of viral replication in the Oro-pharanyx of sub clinically infected animals, leaving to the carrier status in ruminants. FMD vaccine will work as a marker vaccine when purified from NSPs. Therefore development and improved of marker vaccine along with the robust company and test may help to control FMD. More fundamental immunological research is needed on host pathogen interaction in order to understand the mechanism of protection. There is also a need for research on novel adjuvant for FMD vaccines that could provide high enough TH-1 AND TH-2 responses, there by making the vaccine more efficacious. Stabilization of FMDV capsid proteins in vaccine antigen, may help us to induce stronger and longer immune response which may further help to control FMD. As an antigenic diversity of FMDV is a major concern for FMD control. Vaccine matching and selection are an important area that needs to assess to be evaluating for rapid selection of vaccine strains.

Finally a sufficient dose to cover above 90% population in the endemic countries is a major problem in endemic countries for FMD control vaccination. To over come this planning and support from the national and international agencies under global frame work are highly essential. The future scope stabilization of capsid antigen by genetic engineering may avoid the requirement of cold chains and also dissociation of capsid proteins in vivo, producing a faster stronger immune response. Using reverse genetics and substituting a stabilized/ thermo stable versions of capsid proteins a new generation of efficacy of vaccines may be developed.

The following key issues are need globally

- Although conventional vaccines can prevent clinical infection, they do not induce sterile immunity. The protection lasts approximately 06 months, often requiring frequent revaccination in the prophylactic control program and does not induce rapid protection against challenge. Furthermore, it may allow viral replication in the epithelial surface, giving rise to the carrier state in vaccinated animals following live virus challenge.
- Although vaccination could not provide complete clinical or virological protection in animals immunized for a shorter period, it reduces the severity of the disease, virus excetion and virus replication. Inoculation route and dose of challenge virus may play a major role in vaccine-induced protection.
- Enhanced stability of the capsid antigen may give a stronger immune response and durable immunity.
- High antigenic variation in foot and mouth disease virus (FMDV) causes a major problem in vaccine strain selection to control the disease.
- Comparison of sequence data generated from FMDV capsid may help in the rapid selection of a vaccine strain for better protection.
- In vitro assays may replace in vivo potency tests, which may reduce the cost of animal experiments, maintenance of expensive biocontainment facilities and possibility of virus escape.
- New-generation viral vector-based marker vaccines expressing stabilized capsid and robust tests to differentiate infection in vaccinated animals may increase the efficiency of vaccination in foot and mouth disease (FMD) control policy.
- In endemic countries, sufficient doses of foor and FMD vaccine should be made available to vaccinate susceptible animals. Water buffaloes

in South-East Asia, susceptible wildlife in Africa, particularly in national zoos, and susceptible zoo animals in FMD- free countries may need regular vaccination to control the disease.

REFERENCES

1. Anon (1996) Report from the OlE World Reference Laboratory for Foot and Mouth Disease Bulletin office International des Epizooties, 108:804.
2. Anon (1996) Report from the OlE World Reference Laboratory for Foot and Mouth Disease Bulletin office International des Epizooties, 108:867
3. Carpenter. WC, Rai D.V., Samuel A.R. & Hofner M.C, (1996) Comparison of a radioactive and non radioactive method for sequencing foot and mouth disease virus isolates Revue Scientifique et Technique, office International des Epizooties, 15: 875-882.
4. Donaldson, A.!. & Kihm, U, (1996) Research and technological developments required for more rapid control and eradication of foot and mouth disease Revue Scientific Technique, Office International des Epizooties, 15:863-873.
5. Donaldson A.I., Kitchinc R.P. .& Barnett, P.V. (1996) Foot and mouth disease. In. Manual of Standards for Diagnostic Tests and Vaccines, Lists A and B Diseases of Mammals, Birds and Bees, PP 47-56, paris, Office international des Epizooties.
6. Donaldson, A.I. (1997) Foot and mouth disease in Taiwan Veterinary Recorded, 140:407.
7. Donaldson, A.I. (1997) Risks of spreading foot and mouth disease through milk and dairy products Revue Scientifiique et Technique, Office International des Epizooties, 16: 117 -124.
8. Donaldson, A.I. (1997) Global eradication of foot-and mouth disease considerations and proposals. In: Empress Livestock Programme Rome, FAO. .
9. Donaldson, A.I. & Have,P. (1997) Criteria to consider and operational requirements for implementing emergency (ring) vaccination Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot and mouth diseases, Kibbuttz Ma'ale Hachanisha, Appendix 29: 201-207.
10. Ferris, N.P. (1997) Development and use of the ELISA in the control of foot and mouth disease. Proceedings, International Symposium on Diagnosis and Control of Livestock Diseases Using Nuclear and Related Techniques Towards Disease Control in the 21st Century, Vienna. Salf JS & Iloot MC (1997) Virus titres in the development of foot and mouth virus persistence in cattle. Report of the Session of Research Group of the Standing Technical Committee of the European Commission for the Control of Foot and Mouth Disease, Kibbutz Ma'ale Hachamisha, Appendix 4:45-51.
11. Mahy BWJ. Foot and Mouth Disease Virus. Mahy BWJ (Ed) Springer, Germany 2005.
12. Mahy BW. Overview of foot-and-mouth disease and its impact as a re-emergent viral infection. In : Foot and Mouth Disease. Current Perspectives. Sorbino F, Domingo E (Eds). Horizon Bioscience, Norfolk, UK 437-446 (2004).
13. Brooksby JB Portraits of viruses- foot-and-mouth-disease virus. Intervirology 18 (1-2), 1-23 (1982).
14. Council Directive 2003/85/EC on community measures for the control of foot-and-mouth disease repealing. Directive 85/511/EEC and Decisions 85/531/EEC and 96/665/EEC and amending Directive 92/46/EEC. Official .J.Eur.Union I 306 200346, 22nd November 2003.