AGA: EUFMD/RG/91

REPORT

of the

Session of the Research Group of the Standing Technical Committee

of the

European Commission for the Control of Food-and-Mouth Disease

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INTRODUCTION

A Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease was held at the SAP Enstitusu (FMD Institute), Ankara, from 1 to 5 October 1991.

Dr. M. Aksin, Director of the Institute, welcomed the participants. He recalled that the Research Group had previously held a Session at the SAP Institute in 1970, and stated that it was a pleasure for him and his staff to assist the Group in the conduct of this Session. He wished them a successful meeting.

On behalf of the Research Group of the European Commission for the Control of Foot-and-Mouth Disease, Dr. M. Eskildsen, Chairman of the Group, expressed appreciation for the facilities made available for the hosting of this Session of the Research Group in Turkey. He briefly traced the history and the importance of the close cooperation with Turkey. He extended a special welcome to the new members of the Group and to the observers from EC and 0IE.

Mr. J. Doorenbos, FA0 Representative in Turkey, stressed the economic role of animal husbandry in Turkey with some 80 percent of the farming population deriving their income fully or partly from this sector. He underlined the importance of research which is given high priority by the Turkish authorities and is being continuously strengthened. A meeting of this highly qualified Group at the SAP Institute was both timely and welcome, he stated.

Professor E. Istanbulluoglu, Deputy Under-secretary, Ministry of Agriculture, addressed the Group on behalf of the Minister. The Ministry of Agriculture acknowledged the very important role of the European Commission for the Control of FMD in providing guidelines and assistance in the control of FMD and in stimulating relevant research activities. He expressed concern for the deteriorating economic situation in some countries of this area due to the difficulty to enforce and maintain adequate animal disease control policies following the recent political disturbances. It was essential, he stated, that concerted action be taken to implement disease control policies for FMD and Rinderpest.

The Session was chaired by Dr. M. Eskildsen, Chairman of the Research Group. Members of the Group present were: Dr. R. Ahl, Germany, Dr. G.F. Panina, Italy, Dr. C. Terpstra, Netherlands, Dr. E. Domingo, Spain, Dr. S. Uluturk, Turkey, and Dr. A. Donaldson, (ex-officio) United Kingdom. (Appendix 8)

Observers attended from EC, 0IE, Italy, and Turkey.

Dr. P. Stouraitis and Ms J. Raftery of the European Commission for the Control of FMD provided the secretariat.

The following Agenda was presented and adopted:

1. Adoption of Agenda

2a. Emergency action to be taken in non-vaccinating countries following an outbreak of FMD

2b. Review of security requirements for laboratories working with FMD virus

3. Further information about the stability of FMD vaccines prepared from stored antigen

4. Guidelines for importation into Europe of live animals, meat and offal (replacing earlier recommendations)

5. Epidemiology of recent FMD outbreaks in Turkey and characteristics of field isolates with reference to both cattle and small ruminants

6. Results of the Ankara/Pirbright FMD vaccine trial in cattle

7. Follow-up discussion on the carrier state, including a review of the recommendation from 1977, with a view to finalizing this item at the next open meeting

8. Visit of the Ankara FMD laboratory

9. Election of Chairman

10. Adoption of the draft report

11. Any other business
    - FMD outbreak in Bulgaria
    - Examination of bovine semen samples for recovery of FMD virus
    - Date and place of next Session

Item 2a - Emergency action to be taken in non-vaccinating countries following an outbreak of FMD

At the Twenty-ninth Session of the Commission in April 1991, the present and future policies for the control of FMD in Europe were discussed. The Commission, in recognising the cessation of prophylactic vaccination in Europe identified a need for non-vaccinating countries to have available contingency plans and established policies for emergency action in the event of outbreaks of FMD. The Commission requested the Research Group to prepare a working paper for discussion.

The paper introduced by Dr. Donaldson (Appendix 1) was prepared in response to this request and reviewed by the Group. The importance of a comprehensive approach to the problem and the need for a link to be made between the emergency actions to be taken and the financial and logistic resources required was stressed.

Dr. Marchant (EC) stressed that these guidelines were also relevant to vaccinating countries. Dr. Eskildsen stated that this document should now form the basis for further discussion at the Fifty-fourth Session of the Executive Committee and thereafter at the Thirtieth Session of the Commission. The title of the document was modified as follows: "Recommendations for FMD contingency plans including emergency actions in and around FMD infected premises in non-vaccinating countries".

A number of amendments were introduced for the purpose of clarifying the wording and avoiding misinterpretation of the concepts. It was agreed that Section 5 be included as an annex to this document under the title "Emergency actions in non-vaccinating countries". The Group also discussed and identified the criteria which need to be considered before strategic ("ring") vaccination is employed as an adjunct to stamping out. (Appendix 1, Annex 1).

Item 2b - Review of security requirements for laboratories working with FMD virus

The Chairman introduced the paper (Appendix 2). Following an in-depth study, a Sub-group of the Scientific Veterinary Committee of the EC has updated the "Minimum Standards for laboratories working with FMD virus in vitro and in vivo" as adopted by the Commission at its Twenty-sixth Session in April 1985.

The Research Group discussed the revised document and proposed some modifications and amendments. The Group agreed that in its present form it represented an update of the previous requirements in the light of the most recent scientific developments in this field and taking the non-vaccination strategy into consideration. The Group recommended that it be submitted to the Commission for adoption as FA0 minimum requirements (Amended).

Item 3 - Further information about the stability of FMD vaccines prepared from stored antigen

Dr. Eskildsen opened the discussion, stressing the importance of the item with regard to the present non-vaccination policy of Europe. As no paper specifically relevant to the subject had been submitted, it was considered that it should be placed on the Agenda for the next Open Session of the Group.

A paper dealing with the antigenic variability of type C FMD viruses isolated over six decades was presented by Dr. E. Domingo (Appendix 3). Evidence was presented of two major patterns of antigenic variation:

i) a gradual increase of divergence due to accumulation of amino acid replacements at positions 138-150 and 186-209 of VP1, and

ii) an abrupt antigenic change caused by one replacement at either position 145 or 146 of VP1. Such a change might have contributed to the emergence of new C subtypes.

The presence of distinct evolutionary lines of type C FMDV strains in the field was recognised.

On the basis of these results, the Group recommended a careful selection of the C type strains to be included in the vaccine bank.

It was recognised that new field variants could overcome the immunity induced by vaccines, as in the case of the C5 sub-type in South America. Further research should aim at defining additional critical B and T cell epitopes involved in cross-protection among C type FMDV strains. It was also recommended that similar analyses should be extended to FMDV strains of serotypes A and 0.

Item 4 - Guidelines for importation into Europe of live animals, meat and offal (replacing earlier recommendations)

The Chairman introduced a paper which was prepared in response to the Commission's request that recommendations discussed at the Twenty-ninth Session in April 1991 be further reviewed and should take account of the non-vaccination policy adopted in Europe.

The Group discussed the paper and agreed further amendments (Appendix 4).

Item 5 - Epidemiology of recent FMD outbreaks in Turkey and characteristics of field isolates with reference to both cattle and small ruminants

Two papers were presented under this heading. In the first presentation, Dr. Uluturk gave results obtained at the SAP Institute, Ankara, of the serotyping of isolates of virus obtained from field outbreaks in Turkey during 1990 and 1991 (up to June) (Appendix 5). Of 943 viruses identified, 911 (96.6%) were type 01 and 32 (3.4%) were type A22. A higher incidence of disease was recorded during the first half of 1991. Carrier rates of 5.4% for cattle and 3% for sheep were found in animals sampled by "probang" in the first half of 1991 (see Item 7). Serological surveys using VN and ELISA tests on 882 cattle sera from vaccinated animals collected in 1991 indicated that certain levels of antibodies against type 01 and A22 were present in 56% and 68% respectively, of the samples tested. In another survey of 464 samples from special farms, the values were 85% (type A) and 75.5% (type 0).

The second presentation was given by Dr. Donaldson and reported work at the WRL, Pirbright, on genetic and antigenic analyses of FMD virus isolates collected in Turkey in 1991 (Appendix 6). An unusual finding was the presence of serotypes O and A in two samples of epithelial tissue collected from individual animals.

The Group noted the agreement between the serological results obtained by the SAP Institute and the WRL and reiterated the need for submission and investigation of field isolates. It also noted that in the strategic vaccination zone an estimated 70% of the animal population is vaccinated and in the rest of the country the coverage is 30%.

The Group recommended that a better vaccination coverage be obtained and that a greater number of field samples be sent for examination both to the laboratory in Ankara and to the WRL.

Considering the number of outbreaks in the strategic vaccination area, stricter control of animal movements was recommended.

In addition, the Group recommended that a further serological survey should be carried out in the Thrace area at some suitable time in the future as soon as possible, taking into account the presence of recently vaccinated animals in a zone near the Bulgarian border.

Item 6 - Results of the Ankara/Pirbright FMD vaccine trial in cattle

Dr. Donaldson presented a detailed report on the potency test of FMD vaccine undertaken at the SAP Institute by personnel from the SAP Institute and the WRL. The test was carried out according to protocols agreed with the Turkish authorities. The vaccine failed to protect any vaccinated cattle against the homologous challenge with 01 Manisa and because of this the proposed follow-up heterologous challenge test was cancelled. The Group noted that the conditions under which the challenge test was carried out might have influenced the results; in particular, the ventilation system was deficient and consequently the environment was hot and humid, thus cattle were subjected to abnormal stress.

It was noted that a few vaccinated animals had pre-challenge serological titres of 1:100 but were not protected. The analysis of the 146S antigen content in the vaccine after completion of the test indicated that the concentration may have been below that required to give protection. The identity of the vaccine and challenge strains had been confirmed in separate experiments.

Dr. Ulütürk from the SAP Institute confirmed the poor environmental conditions of cattle during the potency test. She expressed surprise about the results in that the same vaccine batch had proved to be fully protective for three cattle in a potency test against the same homologous O1 Manisa challenge virus carried out independently by the SAP Institute.

Dr. Stouraitis expressed concern for the possible consequences on the vaccination policy in the strategic vaccination zone, while Drs Panina and Domingo highlighted the importance of stricter in-process controls of antigen quality, before the final formulation of vaccine.

The Group recognised that the vaccine batch produced by the SAP Institute had failed the test though this had not been performed under acceptable environmental conditions for cattle. It was agreed that one or more batches of antigen produced at the SAP Institute for use in the 1992 spring vaccination campaign will be selected for repeat testing. Before the trial proceeds, the infectivity titres and the 146s content of the 01 Manisa virus used in the vaccine will be controlled and shown to be adequate. For this purpose, the SAP Institute will deliver to the WRL, Pirbright, tissue culture supernatants taken just before and immediately after inactivation with BEI. The virus will be produced under the standard conditions of SAP Institute and checked at the WRL for infectivity acceptable 146s quantity and quality.

The Group recommended that important kinetic parameters of antigen production be carefully monitored and optimised to improve vaccine quality at the SAP Institute. The Group also recommended that technical assistance be provided to the SAP Institute, Ankara, on a full-time basis.

Item 7 - Follow-up discussion on the carrier state, including a review of the recommendation from 1977, with a view to finalizing this item at the next open Session

The Group reviewed the recommendations of 1977 and agreed that they were no longer valid and that new ones should be proposed on the basis of the recommendations agreed at the 1990 Session of the Group. Limited information is available for the carrier state in small ruminants, however, further results may become available from studies currently taking place in Turkey (see Item 5). The Group agreed that further discussions were required at the next Session before final recommendations can be made.

Item 8 - Visit of the Ankara FMD Laboratory

The Group visited the SAP Institute during the morning of 3 October and saw the vaccine production facilities, the animal accommodation, and the diagnostic laboratory. They discussed technical aspects of the work of the Institute with the staff.

Conclusions and recommendations

i) The SAP Institute is well equipped for vaccine production and the equipment is appropriate for up-to-date vaccine production.

ii) There is a need for repair and maintenance of buildings, air conditioning and air filtration systems.

iii) There is a need for technical assistance to increase virus yields during vaccine production as the infectivity levels are less than those normally expected. The members of the Group provided advice on various ways for improving procedures.

iv) The final formulation (bivalent) vaccine should be potency tested by cattle challenge. Dr. Ahl proposed that a careful control of all batches of tissue culture components should be implemented. Dr. Domingo suggested that a detailed characterisation of the different strains of BHK-21 cells for the antigen yield under the conditions at the SAP Institute should be undertaken. Dr. Terpstra expressed his concern about the limited time span over which the yearly production of vaccine takes place (9 months). Dr. Amadori suggested, for practical purposes, an increase in the concentration of Al(0H)3 in the formulated vaccine, to allow a more uniform blending of the antigen.

It was also recommended that

v) Greater use should be made of the ELISA for routine diagnosis.

vi) The quantity and quality determination of 146s should be routinely employed to monitor antigen during vaccine production.

vii) More effort should be made to collect diagnostic material from suspected outbreaks of FMD in sheep and goats so that their importance in the epidemiology of the disease can be determined.

viii) The air conditioning and air filtration systems should be repaired and maintained.

ix) The National Authorities should give their full support to the laboratory and its staff to meet the national needs for FMD control.

A detailed discussion followed as to how to implement technical assistance to the SAP Institute. Dr. Marchant, EC, informed the Group that a technical agreement with the Turkish authorities would be signed in the near future, involving a three-stage programme of long-term assistance in the fields of FMD vaccine production, control and disease diagnosis. Dr. Panina warned against short-term technical assistance in which varying advice may be given by different experts.

Item 9 - Election of Chairman

The Group unanimously elected Dr. Donaldson (representative of the WRL, Pirbright, UK) as Chairman. Dr. Donaldson thanked the outgoing Chairman, Dr. M. Eskildsen for his valued contribution during his six year term of office.

Dr. M. Eskildsen was elected Vice-Chairman.

Item 10 - Adoption of draft report

Following discussion of the draft report, the Chairman called for its adoption, subject to incorporation of the amendments agreed, and to any necessary editorial changes.

Item 11 - Any other business

- Examination of bovine semen samples

Dr. Ahl presented a paper on the recovery of FMD virus from bovine semen samples to which a defined amount of virus had been added (Appendix 7). Samples were from batches of semen destined for export. The cell suspension plaque assay in BHK-21-CT cells was used for the detection of virus. With type 01 virus recovery was in the range of 50 to 80 %, depending on the individual bull, whereas with type A5 virus recovery was

considerably lower. The cell suspension plaque assay was shown to be a sensitive test for detecting low levels of contamination with FMD virus. In addition it has been demonstrated that extended bovine semen had an inactivating effect on virus.

- **Recent FMD outbreaks**

(a) <u>Europe</u>

Dr. Donaldson reviewed the field data available for the recent outbreak in **Bulgaria** and provided laboratory results obtained at Pirbright of nucleotide sequence analysis of the Bulgarian isolate and a range of type 01 reference strains. The main finding was that the Bulgarian isolate was very closely related to a number of other Middle East type 0-1 strains, in particular strains SAU 35/90, TUR 13/91 and ISR 1/91 from Saudi Arabia, Turkey and Israel, respectively.

Dr. Stouraitis highlighted the present epidemiological situation at the border between Turkey and Bulgaria and expressed his concern that a bivalent vaccine containing A22 antigen had been used along the Turkish border during the emergency. The Secretary also stressed the need for representatives of the countries concerned to meet regularly to review and discuss FMD control problems in the area.

The Group stressed the need for free exchange of information on current field isolates between laboratories, in particular from the WRL.

(b) <u>Surrounding areas</u>

Dr. Marchant reported the findings of recent visits by an EC mission and an expert to **Morocco**. Early this year an epizootic of type 01 occurred with 13 outbreaks reported up to July 1991. The possibility of a national vaccination programme in Morocco was referred to as well as the need for diagnostic facilities and training.

The Group recognised the serious threat posed to Europe by the existence of FMD in North Africa.

- **Date and place of next Session**

The Chairman informed the Group that an informal invitation had been extended by the Director of the Institut Vaccinal Federal, Bâle, to hold the next Session in Switzerland during the second half of September 1992.

Items suggested for inclusion in the Provisional Agenda were:

1. Persistence of FMD virus in ruminants including game animals.
2. Potency and stability of FMD vaccines prepared from stored antigens.
3. Results of FAO Collaborative Study (Phase XIII)
4. Improved and new techniques for the diagnosis of FMD (PCR, monoclonal antibodies etc).

5. Differentiation of antibodies induced by vaccination and by infection.
6. Items referred by the Commission to the Group.

The importance of preparing papers on the relevant agenda items in time for circulation prior to the meeting was once again stressed. In addition, it was recommended that authors provide a summary (not more than four pages) of their contributions which would then be included as an appendix to the Report of the Session.

Closing remarks

In closing the Session, Dr. Donaldson, on behalf of the Research Group, extended thanks to Professor Istanbulluoglu, Deputy Under-secretary, Ministry of Agriculture and Rural Affairs, for arranging to have the Session held in Turkey. He thanked Dr. Aksin and all the staff of the SAP Institute for the excellent arrangements which had been made for the meeting and for their active and valuable contribution to the discussions. The Group was particularly appreciative of the frank exchange they had had with Dr. Semiha Uluturk and Dr. Hasan Ozturkmen during the laboratory visit. The outstanding hospitality offered by the Turkish authorities during the stay of the Group in Ankara was also acknowledged.

Thanks were also extended to the speakers, to those who had presented papers, to the drafting groups, and to the secretariat.

APPENDIX 1

RECOMMENDATIONS FOR FMD CONTINGENCY PLANS INCLUDING ACTIONS IN NON-VACCINATING COUNTRIES*

WORKING DOCUMENT
(subject to review by 54th Session of Executive Committee and 30th Session of Commission)

1. Legal powers
2. Financial provisions
3. National disease control centres
4. Local disease control centres
5. Expert teams
6. The sources required for disease emergencies (Personnel)
7. The sources required for disease emergencies (equipment and facilities)
8. Instructions for dealing with FMD outbreaks
9. Diagnostic laboratories
10. Contingency plans for vaccination
11. Training
12. Publicity - disease awareness

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* Based on "European Community Contingency Plans for FMD" CEC document VI/1324/91-EN, 1991 and "National Contingency plans for emergency action to be taken in case of an FMD outbreak (Aide memoire)". Report of the European Commission for the Control of FMD, 28th Session, 9-12 May 1989, Rome, FAO pp 61-70.

Revised text for

SECTION 1 - LEGAL POWERS

1.1 Each country should have legal powers to ensure that a campaign against FMD is rapidly and successfully concluded. The legal powers should be clearly understood by all involved with disease control and should be described in the instruction manual for dealing with FMD outbreaks (see Section 8).

1.2 The legal powers should encompass :

- the compulsory notification of any suspected case of FMD,
- authority to collect samples for laboratory investigation,
- the killing of infected and contact animals,
- the payment of compensation,
- zoo-sanitary and other procedures on infected premises,
- the control of movement and other restrictions (including the designation of protection and surveillance zones),
- emergency vaccination.

SECTION 2 - FINANCIAL PROVISIONS

2.1 Each country should ensure that it has the budgetary powers and financial resources to cover the cost of dealing with all aspects of an FMD epidemic.

The main areas of expenditure are :

- the cost of personnel over and above normal running costs,
- the cost of capital equipment and consumable items,
- the cost of killing, disposal of carcasses/contaminated material and zoo-sanitary measures,
- the cost of compensation payments to stock owners,
- the cost of emergency vaccination.

2.2 The farming community can be expected to co-operate only if valuation is fair and compensation for killed stock is paid promptly. National authorities should endeavour to ensure that payments are made no later than 60 days after the killing of stock.

SECTION 3 - NATIONAL DISEASE CONTROL CENTRES

3.1 Each country establish a permanent DISEASE CONTROL CENTRE at the national level. In the event of an outbreak of FMD the centre should co-ordinate all control measures within the national territory. Although the centre is established primarily for the control of FMD, the facilities may also be used for the control of other List A diseases.

3.2 The centre should be in proximity to the office of the Chief Veterinary Officer (CVO) who is ultimately responsible for the co-ordination of all control measures. The CVO may delegate day-to-day responsibilities in this area to a veterinarian designated as the head of the control centre.

3.3 The National disease control centre should be kept in a state of readiness for a disease outbreak. Its main task is to direct and monitor the operations of the local disease control centre or centres and the responsibilities include :

- the overall direction of control strategies,
- the deployment of staff and other resources to local centres,
- the provision of information to OIE and FAO and to neighbouring countries, to national agricultural and trading bodies and to the press and other media, the release of vaccine for use within the country and the determination of vaccination zones,
- liaison with the national diagnostic laboratory.

3.4 The Centre should be equipped with :

- all suitable means of communication including telephones, telex and telefax; facilities for the press are desirable,
- maps covering the area of the country (preferably at 1:50,000 scale),
- lists of national organizations which will be affected by and must be contacted in the event of disease outbreaks (e.g. AI organizations),
- lists of staff and other persons who can be called upon immediately to serve at local disease control centres or in Expert Teams in the event of a disease outbreak. These lists should record practical experience or training in the control of List A diseases and language abilities.

3.5 The staffing of a National disease control centre is dealt with in Section 6.

SECTION 4 - LOCAL DISEASE CONTROL CENTRES

4.1 Each country should establish one or more Local disease control centres which may be the existing local veterinary offices. The number and location of the centres should be such that staff operating out of a centre can easily reach any livestock holding within the area under its control and return to the centre within a day. In those countries which have a small land area the national centre may also serve as the local centre.

4.2 The National disease control centre should maintain a list of Local disease control centres and for each the name of the veterinarian in charge, the address of the centre, its telephone, telex and telefax number and a map showing the area under its control.

4.3 In the event of a disease outbreak a temporary disease control centre may be set up that is convenient to the location of the infected premises. This centre should preferably be within the surveillance zone surrounding the primary outbreak.

4.4 The local centres should be under the charge of a veterinarian who is directly responsible to the veterinarian at the National disease control centre. All staff allocated to a centre for the period of the disease emergency should be under his/her *de facto* command. He/she should have the necessary authority to:

- designate a holding as an "infected premises" (after consultation with, and the sanction of, the National disease control centre if that is considered necessary).
- deploy the necessary staff and equipment to infected premises,
- arrange valuation and slaughter of infected and contact stock, the disposal of carcasses and contaminated material and zoo-sanitary procedures,
- advise on the delineation of protection and surveillance zones and the measures to be taken within them,
- impose movement restrictions within the protection and surveillance zones; close markets and abattoirs as necessary,
- liaise with police and other authorities over the nomination of infected premises and to maintain these movements and other restrictions.

4.5 Local disease control centres, whether permanent or temporary, should be equipped with :

- adequate telephone, telex and telefax communications. At least one line should be reserved for communication with the national disease control centre,
- record systems; preferably these should be computer-based,

- maps covering the territory overseen by the centre (1:50,000 and if possible 1:10,000),

- lists of persons and organizations in the area covered by the centre who must be contacted in the event of a disease outbreak. These will include :
  * artificial insemination organizations,
  * milk cooperatives and dairies,
  * local authorities responsible for control measures,
  * police,
  * customs,
  * other official services likely to visit farms,
  * markets and auctioneers,
  * private veterinarians,
  * livestock and meat hauliers,
  * animal disposal contractors,
  * livestock valuers,
  * feed suppliers,
  * rodent control companies,
  * local veterinary associations,
  * hunting and shooting organizations; race tracks,
  * slaughterhouses and meat processing plants,
  * farmers union,
  * telephone companies,
  * local environmental health and waste disposal authorities

These lists should be kept up to date and a notification procedure established.

- facilities for informing the press and other media so that all persons are fully aware of the restrictions in force,

- equipment stores (see Section 7),

- facilities for cleaning and disinfecting personnel, clothing and vehicles.

SECTION 5 - EXPERT TEAMS

5.1 The prompt identification of the source and the possible consequences of a primary outbreak of FMD is crucial to the rapid eradication of the disease. Expertise in dealing with FMD outbreaks is already becoming scarce and all countries are recommended to create one or more Expert Teams that can provide a nucleus of expertise. The teams should be alerted when disease is first reported and deployed in the field as soon as it is confirmed. The staffing of these Expert Teams is dealt with in Sector 6.

5.2 The Expert Teams have two main responsibilities :

- to conduct an epidemiological investigation and where appropriate collect samples (epithelium, blood, milk, probangs etc) for submission to the National diagnostic laboratory to determine the extent and pattern of infection,

- through the head of the Expert Team to provide an epidemiological report for the head of the Local or National control centre,

- to advise the head of the Local disease control centre on the advisability of taking of samples (e.g. milk) from contiguous or other herds.

5.3 The epidemiological report from a primary outbreak should describe:

- the situation on the infected premises,

- the number and species of susceptible and other livestock; the method of husbandry,

- the number of clinically affected animals and the age of the oldest lesion(s),

- the size and location of the premises and its relationships with other holdings, public roads, etc.,

- the local meteorological situation unless this is available from a nearby meteorological station,

- the recent movements (livestock and personnel) on and off the holding.

5.4 On the basis of these findings the head of the team should advise the Local or National disease control centre on:

- the possible origin and the date of the introduction of infection,

- the likely period of infection on the premises/holding*,

- the holdings most at risk from airborne spread or from movements.

5.5 The expert Team should <u>not</u> be responsible for the killing and disposal of stock or for tracing movements on and off the infected premises. These tasks are the responsibility of the Local disease control centre. However, countries may wish to consider including experts in zoo-sanitary measures and carcass disposal in their teams so that they can advise disease control centres on these aspects.

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* "premises/holding" includes a village or area of transhumance activity consider including experts in zoo-sanitary measures and carcass disposal in their teams so that they can advise disease control centres on these aspects of control.

5.6 The team should be provided with sampling equipment (for 250 animals) and communication equipment. Mobile accommodation may be provided and sited just beyond the disinfection barrier outside the infected premises.

5.7 The Expert Team(s) should train staff in FMD control techniques and advise the National disease control centre on the development of new control initiatives.

SECTION 6 - THE RESOURCES REQUIRED FOR DISEASE EMERGENCIES - PERSONNEL

6.1 Experience has shown that the resource factor most critical to effective disease control is a sufficient number of trained and experienced personnel.

6.2 Each National Authority should maintain lists of staff available to deal with a disease emergency. The lists (to be held at the National disease control centre) should identify :

- the name and location of the staff members,
- qualifications e.g. veterinarian,
- practical experience of List A diseases (specifying the disease),
- language abilities - in the event of a call for assistance from another country,
- training undertaken (see Section 11).

6.3 If some personnel are not under the direct control of the Chief Veterinary Officer there should be a firm agreement preferably in writing between the Chief Veterinary Officer and the employers of such personnel for their immediate release.

6.4 National Disease Control Centres

The veterinarian in charge of a National disease control centre would have at his/her command veterinarians and other staff who have been trained in the management of diseases emergencies (see Section 11).

6.5 Local Disease Control Centre

The staff at Local disease control centres should include :

- Senior Veterinarian in charge,
- veterinarians trained
  * in the diagnosis of FMD
  * in slaughter, zoo-sanitary and other procedures at infected premises,
  * the operation of movement controls and other restrictions,

- support staff who are trained in
  * the procedures at infected premises,
  * the operation of movement controls and other procedures,

- office staff trained in the maintenance of record systems required for FMD control.

6.6 All staff who are, or may be, allocated to disease control centres should be regularly retrained in disease control procedures and the clinical diagnosis of FMD (see Section 11).

6.7 Expert Teams

Each country should create one or more Expert Teams. The responsibilities of these teams are described in Section 5. Each team should consist of :

- a Senior Veterinarian experienced (or trained) in FMD clinical diagnosis including the ageing of lesions. He/she should also be trained in the epidemiology of the disease.

- a laboratory scientist experienced in laboratory tests for FMD,

- a meteorologist knowledgeable of the weather conditions which may aid the spread of FMD.

6.8 Personnel Resources in the Member State

Each National Authority should ensure that it has immediately available sufficient trained staff to deal with, at any one time, up to 10 outbreaks and to properly maintain surveillance in the 3 Km radius protection zone associated with each. It has been estimated that a trained veterinarian can visit and examine stock at no more than 5 holdings on one day if he/she properly undertakes the required disinfection procedures at each place.
Note : These scope of activities may need to be modified in some countries according to the local livestock husbandry situation.

6.9 If a National Authority does not have the resources suggested in para 8 above, contingency arrangements should be in place to arrange for deployment from other countries.

SECTION 7 - THE RESOURCES REQUIRED FOR DISEASE EMERGENCIES, - EQUIPMENT AND FACILITIES

7.1 The effective control of FMD depends on the immediate availability of equipment and immediate access to facilities provided by public and private sector utilities.

7.2 Each country should have readily available at Local disease control centres or at some other convenient place at least the following equipment :

- protective clothing,
- disinfectants effective against FMD virus, detergents and soaps,
- pumps, shovels and scrapers,
- humane killers and ammunition, lethal drugs and other approved means of killing animals,
- autopsy and sampling equipment,
- sign post/warning notices for use in infected premises in protection/ surveillance zones,
- maps (1:50,000 and 1:10,000),
- vaccination equipment,
- exhaust air filters for bulk milk tankers.

7.3 The veterinarian in charge of the Local disease control centre should have standing arrangements for access to :

- vehicles,
- combustible materials,
- digging equipment,
- flame guns,
- knapsack sprayers and other means of disinfection.

7.4 Whilst it is preferable to dispose of carcasses by burning or burying them on the holdings, in some situations this may not be possible. If this is so, countries should develop arrangements for transporting carcasses in sealed vehicles to sites for disposal or destruction.

7.5 Each local disease control centre should have available office equipment including:

- office furniture, photocopiers, etc.,
- record systems specifically designed for FMD outbreaks; preferably these should be computer based,
- preprinted proformas such as:
  * formal notices of restriction,
  * valuation,
  * notices for public display,
  * epidemiological reports,
  * tracing requests and reports,
  * movement permits.

SECTION 8 - INSTRUCTIONS FOR DEALING WITH FMD OUTBREAKS

8.1 Each National Authority should prepare a set of instructions (a manual) for dealing with FMD outbreaks.

8.2 The instructions should cover :

The legal basis for

- the notification of suspected FMD,
- the killing of animals, valuation and compensation payments,
- zoo-sanitary and other procedures at infected premises,
- the control of movements,
- emergency vaccination.

8.3 The responsibilities of and the working arrangements for:

- the National disease control centre,
- Local disease control centres,
- the Expert Teams.

8.4 When FMD is reported the action to be taken:

- in the case of a suspected case,
- a case confirmed on clinical grounds only,
- a case fully confirmed (clinical and laboratory tests),
- the submission of material to the National FMD Laboratory,
- the notification of police, local authorities, agricultural associations etc.

8.5 The producers at an "infected premises"

- the isolation of the premises,
- valuation and compensation,
- the slaughter of livestock,
- carcass disposal; contaminated material disposal,
- zoo-sanitary measures (disinfection, decontamination, cleaning),
- restocking.

8.6 Creation of protection and surveillance zones :

- census of all livestock holdings,
- regular surveillance of all livestock holdings,
- movement controls; arrangements with police and other authorities,
- prohibition of AI services, markets, agricultural shows, etc.,
- notices and other publicity,
- handling of milk according to approved procedures.

8.7 Movement tracing

A national Authority should employ a uniform tracing system.

8.8 Equipment and facilities

Arrangements for providing the equipment and facilities detailed in Section 4.

8.9 Epidemiological information

- to be provided by the Expert Teams,
- to be collected at Local disease control centres,
- to be provided to the National disease control centre,
- to be provided to the Animal Production and Health Division, FAO.

8.10 National publicity

SECTION 9 - Diagnostic Laboratories

9.1 Each National Authority should ensure that it has available at all times the services of an FMD diagnostic laboratory either in its own country or elsewhere.

9.2 In all suspected primary outbreaks (of FMD) appropriate samples must be collected and quickly transported, according to a set protocol, to an approved FMD laboratory.

9.3 The responsibilities and duties of National FMD laboratories are:

1. Access to rapid laboratory diagnosis, especially in initial cases of FMD is required. This applies whether or not regular prophylactic vaccination is employed.

2. The National laboratory should be equipped and skilled for rapidly providing an initial diagnosis. For this purpose, an ELISA test system of high sensitivity is recommended. The laboratory should keep in stock reference strains of all serotypes of FMD virus including relevant exotic strains (inactivated), and immune sera against the viruses, as well as all other reagents necessary for rapid establishment of an initial diagnosis.

   Likewise, appropriate cell cultures should currently be available for confirming a definitive negative diagnosis.

3. In all suspected primary outbreaks, epithelial samples from vesicles must be collected and packed according to a set protocol. The samples should be quickly transported to the National Laboratory. In anticipation of a possible case of FMD, the appropriate equipment for sample collection and transportation to the National laboratory should be stored in readiness locally.

4. At the earliest possible occasion, the National Laboratory should send a sample of virus from the primary case to the World Reference Laboratory (SRL) for confirmation and further characterization (including advice on the antigenical relations of the field strain to vaccine strains).

   Ideally, an aliquot of field material should be sent; if this is not possible, animal passage material, obtained from the original host species, or low cell culture passage material is acceptable. The history of animal or cell passage material should be provided.

5. Representatives from the National Laboratory, the Meteorological Office and the State Veterinary Services, respectively, should currently be prepared to form an epidemiological team for emergency action.

6. The National Laboratory should be equipped and skilled for large scale serological examinations.

7. All laboratories manipulating FMD virus must operate under high security conditions as recommended in the FAO Guidelines on Minimum Standards.

   The high security systems should be regularly inspected.

8. Members of the field section of the State Veterinary Services should have the opportunity of seeing clinical cases of FMD in the WRL or National Laboratories as part of their training.

9.4 National FMD laboratories must be equipped and skilled for providing a rapid initial diagnosis and also for large-scale serological surveillance.

SECTION 10 - Contingency plans for vaccination

10.1 Emergency vaccination may be introduced when FMD has been confirmed and threatens to become extensive.

10.2 As part of their contingency plans countries should establish or have access to facilities that will allow the prompt provision of vaccine.

10.3 "Cold chain" facilities should be established for the distribution of the vaccine so that it is, at all times, kept under cool temperature conditions e.g. as specified in the European Pharmacopoeia. These facilities should be available:

- at a point where vaccine is delivered to a country for further distribution,
- at or near Local disease control centres for distribution to the veterinarians who will administer this vaccine.

Refrigerated storage and transport facilities should be available which will allow the distribution of the vaccine well within the shelf-life of the product.

10.4 Administration

Vaccination equipment should be held at Local disease control centres or at some other convenient place. Sufficient vaccination needles should be stocked so that each herd can be vaccinated with a fresh unused needle.

Each country should prepare a list of personnel who can be called upon to undertake an emergency vaccination programme. Ideally these persons, if called upon, should have had no recent contact with FMD contaminated material or have visited an FMD infected premises. If they have been in contact with contaminated material or infected premises they should undergo rigorous disinfection including a full change of clothing before commencing vaccination duties.

Before a herd is vaccinated it must be examined by a veterinarian to ensure that the animals are not already showing FMD lesions.

SECTION 11 - TRAINING

11.1 Each country should ensure that staff are regularly trained in procedures for diagnosing and dealing with FMD.

11.2 Training of staff involved in FMD control

National Authorities should arrange for the regular training of all veterinary and support staff who may be engaged in dealing with FMD outbreaks. The training programmes should be led by members of Expert Teams with the support of staff who have experience of FMD. The training programme should include:

- the diagnosis of FMD (video presentations, etc),
- procedures at infected premises,
- procedures at Local disease control centres,
- procedures at National disease control centres,
- tracing exercises, record keeping,
- notification and publicity procedures.

The competence of staff in this area should be maintained by regular training exercises at national and local level. These should include simulated disease control operations.

SECTION 12 - PUBLICITY/DISEASE AWARENESS

12.1 State veterinary services rely on stockowners or on veterinarians attending farm animals to report the possibility of FMD. Reporting needs to be prompt and accurate

so that all outbreaks are identified as soon as possible without raising too many false alarms.

12.3 Prompt and accurate reporting can only be achieved if the veterinary profession and stockowners are aware of danger of FMD and are conversant with the signs of the disease. To this end countries should maintain awareness of the disease both within the veterinary profession and in the agricultural community.

The veterinary profession - Material should be aimed at veterinary students and at practising veterinarians. Veterinary schools should be provided with material that is simple but comprehensive and describes :

- the clinical disease,
- the epidemiology of the disease,
- the notification procedures,
- the control measures,
- the epidemiological situation in neighbouring states and in those of trading partners.

The veterinary profession at large should be regularly provided with information that covers:

- current notification and control procedures,
- the epidemiological situation within the country and elsewhere.

Refresher courses arranged for veterinarians should include all List A diseases.

12.4 The agricultural community

Disease awareness campaigns should be targeted primarily at stockowners and non-professional personnel who regularly visit herds or flocks e.g. AI personnel and livestock hauliers.

The campaigns should emphasise:

- the importance of FMD,
- the clinical signs,
- the importance of prompt reporting,
- the availability of compensatory funds.

ANNEX 1

EMERGENCY ACTIONS IN NON-VACCINATING COUNTRIES

1. Notification

- Suspicion of disease to be reported immediately to the appropriate authority.

2. Investigation

- Immediate investigation by a veterinary officer of the appropriate authority.
- If FMD suspected - farm restrictions imposed immediately if not already in force to stop all movement of livestock, persons or inanimate objects onto or off the premises.
- Special arrangements may be needed if suspected FMD is reported at a market or slaughterhouse.
- Samples collected for immediate transportation to diagnostic laboratory by fastest possible route.
- When the disease has been confirmed in the area a total standstill on the movement of animals within three km of the infested place shall be imposed and remain in force.
- On farm investigations to continue and to include:
  * total stock count,
  * preliminary epidemiological investigation to include details of livestock movements on and off the premises, potential personnel contact with disease, or other potential sources of infection such as waste food. Also an assessment of potential for disease spread from the infected place,
  * assessment of likely arrangements needed for slaughter and disposal of livestock, and any welfare problems anticipated while under restriction.

3. Confirmation

All confirmations of the existence of FMD to be made by the National Authority.

4. Infected premises procedures

After confirmation of disease rapid killing and disinfection is essential to reduce virus emission.

(a) **Valuation**

Procedures listed below may and usually will take place simultaneously:

- immediate valuation of livestock to assess compensation payable
- valuation to include contaminating feeding stuffs and bedding that cannot be disinfected,
- formal appeal procedure needed to ensure that disputes over valuation do not delay killing of stock.

(b) **Killing and disposal of stock**

As soon as valuation is completed all susceptible stock to be killed on the premises, starting with the animals exhibiting lesions. heads and feet to be covered by plastic bags while awaiting disposal.

- disposal/destruction of carcasses to be contained on premises by burial (or cremation) subject to approval by Local Environmental Authorities,
- consideration may have to be given to disposal elsewhere, possibly via a destructor plant if it is possible under secure conditions,
- cleansing and disinfection,
- preliminary disinfection - yards, driveways, buildings surfaces to be sprayed with a disinfectant approved against FMD, to be repeated as soon as slaughter is completed,
- after removal of carcasses, thorough cleansing and disinfection of premises, equipment and materials. Any contaminated items, which cannot be satisfactorily disinfected to be buried or burned. Fumigation considered if necessary.
- special attention must be given to large quantities of slurry which may need chemical treatment and/or prolonged storage before release.

(c) Movement restrictions

- Protection Zone* - A minimum of three km radius around the Infected Premises (IP) must be established as soon as FMD is confirmed. Farmers should be warned of the need to regularly inspect their stock and notify any suspicious signs if their farms are located within the protection Zone. For 15 days after confirmation movements only permitted direct to slaughter, licensed, in emergency. Subsequently licensed slaughterhouse in same Protection Zone, provided 15 days elapsed since killing on IP, and subject to satisfactory inspection by Veterinary Officer. No markets, exhibitions or collecting centres

permitted.

- Surveillance Zone* - a minimum of 10 km radius around the Infected Premises. Must provide for adequate slaughtering facilities within clearly defined boundaries. animal movements limited and permitted only under licence within the area. Artificial insemination banned except for semen stored on the farm where it is to be used. Resumption at discretion of officer-in-charge of Disease Control Centre.

- Animals may not be removed from the Surveillance Zone during the first 15 days. Between the 15th day and the 30th day the animals may not be removed from the said zone except to be transported under official supervision directly to a slaughterhouse for the purpose of emergency slaughter.

- Controlled Area - generally in force for a short period, can be very wide area to limit animal movement and to allow veterinary staff to trace and inspect potentially infected animals that may have been distributed widely, such as through a market. Movements licensed and animal gatherings prohibited other than markets for slaughter stock.

- Although movements of animals are permitted through Surveillance and Controlled Areas, under licence, none may pass through a Protection Zone.

- Collection of milk from farms within the Protection zone Infected Area is controlled and a detailed code practice is followed. Vehicles and personnel follow rigorous cleansing and disinfection procedures, and air filters are fitted to the exhaust outlets of bulk milk tankers.

8. Removal of infected premises restrictions

Farm restrictions withdrawn six weeks after completion of slaughter or 30 days after completion of preliminary cleansing and disinfection, whichever is the earlier but subject to continued area controls where other infected premises have been identified. Withdrawal also subject to satisfactory inspection of premises and livestock on continuous premises.

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* The definition of these zones should take into account natural boundaries, supervision facilities, meteorological conditions and other relevant factors and should be reviewed if necessary.

Strategic ("ring") vaccination

The objective of strategic ("ring") vaccination used under emergency conditions is to dampen down the amount of virus circulating in the region and to reduce the risk of further spread.

The inner part of the strategic zone of vaccination should be beyond the area predicted to be already potentially contaminated on the basis of analysis using airborne prediction models and other epidemiological assessments. The area of the zone will be determined by a prediction of the area at risk, the geographical features, the number of doses and the manpower resources available.

The criteria on which the decision on whether or not to apply strategic vaccination should include the following:

- species of animals and their densities in the area;
- predicted risk of airborne viruses spread;
- geography and other features of the area and practicability of delineating a strategic vaccination zone;
- an assessment of the economic consequences for trade;
- the presence of valuable breeding stock in the area at risk;
- environmental and welfare considerations and public opinion.

APPENDIX 2

SECURITY.9

SECURITY STANDARDS FOR FMD LABORATORIES *)

Foot-and-mouth disease is one of the most contagious diseases known and manipulating the virus in the laboratory without adequate precautions is a hazard. The escape of a single infectious unit of FMD virus from a laboratory could potentially cause an outbreak.

The main sources of virus or infectious RNA (in increasing risk of hazard) are:

1. Infected tissue cultures.
2. Infected baby mice, guinea pigs, rabbits etc.
3. Physical and chemical processing of large quantities of virus outside closed vessels (e.g. concentration, purification, inactivation etc.).
4. Infected pigs, cattle, sheep, goats and other susceptible animals.

Ways by which the virus or infectious RNA may escape or be carried out from laboratories include:

Personnel
Air
Effluent and other waste
Equipment

Therefore all laboratories manipulating FMD virus must work under high containment conditions. The safety precautions must preclude every kind of escape of virus and special attention must be given to:

- the prevention of illegal entry into the restricted area
- the presence of changing and showering facilities
- the responsible behaviour of personnel within and when they leave the laboratory
- application of rules for primary containment
- the use of inactivated virus where possible
- the decontamination of exhaust air
- the decontamination of effluent
- the disposal of carcasses in a safe manner
- the decontamination of equipment and materials before removal from the restricted area

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*) *Revision of "Minimum standards for laboratories working with FMD virus in vitro and in vivo" as adopted by the European Commission for the Control of FMD, FAO, at its Twenty-sixth Session in April 1985.*

To achieve this containment a variety of technical installations and a comprehensive set of disease security regulations are required under the supervision of a Disease Security Officer.

The Disease Security Officer must regularly receive technical reports about the various installations and monitor their performance. On the basis of day-to-day records he prepares an annual report on security (incidents, improvements etc.) to the director.

FMD laboratories may be authorized to manipulate live FMD virus for:

DIAGNOSIS
and/or
LARGE SCALE VIRUS PRODUCTION,
VACCINE TESTING INCLUDING INFECTION OF LARGE ANIMALS, and
EXPERIMENTAL INFECTION OF LARGE ANIMALS.

MINIMUM REQUIREMENTS

I. Personnel

1. Control of access to the premises - Prevention of illegal entry to the restricted area*).

2. Personnel must be appropriately trained for the position held.

3. Entry into - and exit from - the restricted area must take place only through changing and showering facilities. This means a complete change from private to working clothes on entry - and another complete change as well as a shower on exit.

4. Personnel must be regularly trained in disease security. A code of disease security practice, including instructions for entry into - and exit from - restricted areas, must be available for all employees on site and for visitors. The disease security regulations must have been read and signed by each employee at the beginning of their employment.

5. All staff members must be appropriately informed and regularly trained in emergency evacuation procedures with special attention being given to security requirements in cases of fire.

6. Personnel must contractually agree not to keep any animals which are susceptible to FMD, nor reside on premises where such animals are kept, nor in the same household as other persons working with such animals and to abide by minimum standards of quarantine, i.e. no contact with animals susceptible to foot-and-mouth disease for specific periods according to exposure at work as follows:

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*) Restricted area: Area where virus is manipulated and rooms in direct and indirect contact therewith.

(a) After involvement with normal laboratory techniques: at least 3 days.

(b) Following contact with large animals infected with foot-and-mouth disease virus or entry into a vaccine production plant where large quantities of infectious FMD virus are handled: **at least 5 days.**

(c) Visitors: at least **7 days.**

7. Regular supply of appropriate laboratory clothing for use within the restricted area.

II. BUILDINGS

8. General construction of buildings and their surfaces, including ducting of the air conditioning system:
   - well maintained condition with a high standard of airtightness
   - insect, rodent and bird proof

9. Windows:
   - non-opening and able to withstand operating pressures
   - shock proof in animal rooms

10. Doors:
   - warning sign emblem at entrances:

ACCESS FOR AUTHORIZED PERSONNEL ONLY **BIOLOGICAL HAZARD**

   - access restricted by locked doors
   - airlocks provided with self-closing doors or with gastight doors
   - doors fitted with windows where appropriate

11. Walls, floors, ceilings:
   - appropriate surfaces
   - easily cleaned
   - sealed (airtight) entry of service lines

III. AIR

Ventilation systems

12. Air is removed from virus manipulating areas through a HEPA filtration system which guarantees a negative pressure to atmosphere of at least 35 pascals (3.5 mm water) for laboratory rooms and rooms for small experimental animals and 50 pascals (5 mm water) for large scale virus production rooms and for large animal rooms.
13. The exhaust air from rooms where experiments in large animals are carried out must pass through two HEPA filters in series.

14. The filter installations must allow for their testing and safe changing in situ.

15. Manometers measuring the pressure drop across filters and/or negative pressure in facilities must be installed. They must be monitored and recorded regularly and, where appropriate, incorporate alarms.
Input and extract fans should be interlocked so that the failure of an extract fan shuts off input air and prevents reflux of contaminated air to the exterior, e.g. by means of a valve in the air intake duct. In any case, every effort should be made to prevent a positive pressure within the building when either shutting off or turning on the ventilation system.

16. The laboratory power supply should be equipped with a back-up source of electricity which starts without a delay of more than a few minutes in the event of power failure, or the commercial power supplier must guarantee a supply from an alternative source within a few minutes.

17. Vertical flow safety cabinets (**Class 2**), approved and checked, with absolute filtration of exhaust air must be available for the handling of FMD virus outside closed vessels.

Monitoring of ventilation systems

18. Ventilation systems should be continuously monitored to ensure proper function.

19. Before installation all filters must have passed an overall test for efficiency (normally done by the manufacturer).

20. When HEPA filters are installed or replaced an efficiency test must be carried out to ensure proper installation and function of the filters. This testing must be done at least once every two years, or when needed, e.g. when there is a sudden change in pressure over the filters. Testing of filters should be carried out by properly trained staff.

21. Among the methods acceptable for testing efficiency of filtration are:

    A. A smoke challenge and photometric detection system using either DOP (dioctyl phthalate) or Shell Ondina (ANNEX 1).

    B. A lithium flame photometer test for highly efficient filters (ref.: Hans Flyger and H.C. Rosenbaum, in American Industrial Hygiene Association Journal, Vol. 26, 409-412, 1965 (ANNEX 2).

    C. Electronic particle counting (ANNEX 3).

Change of HEPA filters

22. Filters must be changed when the pressure difference exceeds certain limits in accordance with the instructions given by the manufacturer, or whenever needed.

    Replacement of both pre- and absolute (HEPA) filters must be safe and must take place in accordance with an authorized procedure. Strict precautions must be taken to prevent spread of virus from filters or via contaminated air. Replacement of filters from outside must take place after decontamination in situ or in "safe change" air-handling units.

    Decontamination of filters after use: Wet autoclaving or incineration.

23. Filters in safety cabinets must be tested following installation and thereafter on a regular basis using the same or equivalent methods as used for testing of the general HEPA filtration system. When changed, filters from safety cabinets must be autoclaved or otherwise decontaminated before removal from the restricted area.

IV. EFFLUENT

24. Effluent from laboratories and from facilities holding animals should be treated in a manner which ensures that the inactivation of FMD virus has been achieved. For this purpose heat or chemical treatment can be used in a system which ensures that all the material is exposed to the specific treatment. The entire effluent treatment system, including the system for transport of effluent to the treatment unit, must comply with high containment conditions. There must be sufficient storage capacity (tanks) for the storage of untreated effluent.

25. The equipment should have automatic monitoring systems to ensure proper function. It must be ensured that the required temperature/pH is reached, and that the installation will stop automatically when required limits are reached, e.g. sterilisation temperature, time and maximum temperature or pH for discharge of the effluent.

Liquids
(slurry, waste water)

26. Heat treatment:
    100^0 C for 1 hour or an equivalent heat effect.

27. Monitoring:
    Automatic and continuous temperature/time/flow rate recording at different stages of the process.

28. Chemical treatment:
    NaOH or Na_2CO_3 or other alkaline treatment at pH 12 for at least 10 hours. Thorough mixing of the materials must be ensured. After treatment the mixture must be neutralized and

the pH must be checked before the effluent is released.

29. Monitoring:
Automatic and continuous control and regulation of pH.

Solid waste
(animal carcasses, feedstuffs etc.)

30. Wet heat treatment (autoclave, 115^0 C in the center of all material for 30 min. or an equivalent heat effect, e.g. in a rendering process) on site.

31. Incineration on site. The incinerators must comply with current safety standards and be fitted with afterburners.

32. The system must exclude the possibility of re-contamination.

33. Monitoring:
The heating systems should be continuously monitored and recorded as part of a fail/safe system. In the event of failure the system must be protected as far as possible against the release of potentially infectious material.

V. EQUIPMENT AND MATERIALS

Laboratory fittings

34. Benches: - impervious surfaces

35. Centrifuges, sonicators, homogenizers etc.: - must be designed so as to contain aerosols

36. Laboratory facilities and equipment must be cleaned and appropriately disinfected at regular intervals. Cleansing and disinfection must be supervised and recorded.

Handling of FMD virus

37. Primary containment must be given proper attention. Handling of FMD virus outside closed vessels must be done in approved (Class 2) safety cabinets.

38. Processing of large quantities of virus must take place in closed systems. Proper attention should be paid to the decontamination of effluent air from vessels and pipe work and to the decontamination of the processing systems before opening for cleaning/maintenance/repair etc.

39. Inoculation of animals and the keeping of infected animals must take place within the restricted area under negative pressure. Personnel must wear appropriate protective clothing when handling virus suspensions and when inoculating or hand-

ling infected animals. On exit from animal rooms protective clothes and footwear must be left inside the room or appropriately decontaminated before being taken out.

Removal of equipment and materials

40. Before removal from restricted areas equipment must be decontaminated according to the size and use of the equipment:

41. either by: heat (wet autoclave), if possible, at 115^0 C for 30 min. or an equivalent heat effect.

42. or dry heat at 50^0 C for 48 hours, in special situations, e.g. for certain sensitive instruments.

43. or after surface disinfection, fumigation with formaldehyde (10 g/m^3 at 70 % RH) for at least 10 minutes or (3 g/m^3) for 24 hours or equivalent with other aldehydes, e.g. glutaraldehyde, or ethylene oxide (0.8 g/litre for 1.5 hrs. at 50^0 C)

44. or thorough wash in an appropriate chemical disinfectant such as:

- 4 % washing soda (Na_2CO_3)
- 0.5 % caustic soda (NaOH)
- 0.2 % citric acid
- 4 % formaldehyde or equivalent with other aldehydes. e.g. glutaraldehyde
- or other disinfectant officially approved for the purpose

<u>Note:</u> The efficiency of these chemical disinfectants is considerably improved by the addition of a non-ionic detergent (concentration 0.005 %).

45. Decontamination of clothing before removal from the restricted area for laundry: heat (wet autoclave) at 115^0 C for 30 min. or equivalent heat effect. The laundry process must involve at least a hot (80^0 C) standard detergent wash at some stage of the cycle. Laundering must be done on site.

46. Treatment of papers before removal: 50^0 C for 48 hours, or an equivalent heat effect, or, for single sheets, fumigation. Removal of books should be exceptional and under the control of the Disease Security Officer. Fax or photocopy barrier systems should preferably be used.

47. All contaminated materials (including dead laboratory animals) must be placed in sealed bags inside leak-proof containers and decontaminated (e.g. by autoclaving) or transported for inci-

neration or rendering on site. The surface of these containers should be disinfected before removal from the restricted area.

48. Before sending non-FMD biological material to another laboratory the necessary precautions must be taken to ensure that the material does not contain FMD virus. The recipient laboratory must be informed about the potential risk of material coming from a laboratory manipulating FMD virus. The recipient laboratory must further sign a statement that it is prepared to receive the material and that it will take the necessary precautions.

A study of the antigenic variation of FMDV of serotype C over six decades: potential for further variation and implications for the choice of vaccine strains.

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We have compared the amino acid sequence of capsid protein VP1 of 30 strains of FMDV type C isolated during 6 decades (from circa 1926 until 1988) in Europe, South America and The Philippines. The effect of amino acid replacements at antigenic sites A (VP1 residues 138 to 150) and C (the carboxy-terminus of VP1, positions 186 to 209) on the reactivity with neutralizing monoclonal antibodies (MAbs) has been quantitated. The results, reported in detail elsewhere (1), indicate two mechanisms of antigenic diversification of the immunodominant site A. One mechanism is a gradual increase in antigenic divergence (defined as the number of dissimilar reactivities with a panel of 11 MAbs that recognize different epitopes on site A) due to accumulation of amino acid replacements at two hypervariable segments (two amino acid triplets at positions 138 to 140 and 148 to 150) within site A. The second mechanism consists on an abrupt antigenic change caused by one replacement at a critical position. We have found such critical replacements in FMDV C_5 Argentina 69, C Philippines 1/88 and 3/87 (these three viruses included Gln at position 146 instead of His, present in related viruses), in C_1 Haute Loire Fr 69 (Val at position 145 instead of Ala), as well as in mutants selected from persistently infected cattle and in cell culture. Such critical replacements resulted in the loss of reactivity of the viruses (in immunodot) and of VP1 (in western blot assays) with all the MAbs directed to site A that were tested. We suspect that the presence of Gln in VP1 position 146 contributed to assign C_5 Argentina 69 to a different subtype (C_5 rather than the most common C_3 in South America) and to the unusual antigenic behaviour of the C Philippines isolates.

The number of different amino acids found at each position within antigenic sites A and C is much lower than the number theoretically possible.

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This suggests that FMDV type C harbors great potential for additional antigenic variation that has not been realized in six decades of natural evolution. Also, it is intriguing that those critical amino acid replacements that could have caused a major antigenic change were fixed rather infrequently during FMDV evolution. This is surprising in view of the likely advantage that such mutants would have in front of immune responses (natural or induced by vaccination) afforded by the more abundant, standard virus types. Indeed, at least with some swine and rabbit sera we have detected a significant (5-to 10-fold) decrease of neutralization by polyclonal antibodies of virus mutants harboring those critical replacements.

This screening of isolates from 6 decades has also demonstrated that even within serotype C, viruses with quite divergent antigenic specificities coexist. For example, C_1 Brescia It/89, C_1 Loupoigne Bel/53, C_1 Santa Pau Sp/70 and C_1 Vosges Fr/60 constitute an antigenically related group different from the group formed by C_2, C_3 and C_4 viruses, which are also quite distinct from C_1 Haute Loire Fr/69, C_5 Argentina/69 or the C Philippines isolates. At least concerning the immunodominant site A one virus from each of those groups should be placed in a depository of vaccines intended to control a possible emergency of FMD caused by a type C virus of unknown origin. Choice of such vaccine strains might be modified, however, when we know more about other antigenic sites and their contribution to the immune response; such sites (other than sites A and C) have been described for viruses of serotypes O and A (2, 3). Surveys of the antigenic evolution of FMDV of the other six FMDV serotypes over many decades, using MAbs and polyclonal sera, are important for the rational choice of vaccine strains. Obviously, animal protection tests are the ultimate assay for ascertaining vaccine efficacy. However, such tests are expensive and time-consuming and surveys of the type reported here for FMDV type C should help in the choice of candidate vaccine strains before undertaking animal protection tests. There is always an uncertainty regarding the antigenic characteristics of a new RNA virus strain (mainly if it comes from natural reservoirs such as wild animals or from carrier ruminants) (4), but depositories of vaccines should cover as great a range as possible of antigenic specificities. This seems particularly relevant in the face of a non-vaccination policy for Europe if depositories of vaccines are organized with the aim of coping with emergencies.

Our results have also shown that FMDV CGC Ger/26 (one of the earliest viral isolates available for molecular studies) is antigenically intermediate

between the two major groups represented by C_1 and C_3 viruses. It appears as if CGC Ger/26 belongs to an evolutionary line, now extinct, that was replaced by the modern viruses isolated subsequently. Additional studies are required to confirm the unique genetic characteristics of this isolate and to define its relation to other isolates.

REFERENCES

(1) Martínez, M.A., Hernández, J., Piccone, M.E., Palma, E.L., Domingo, E., Knowles, N. and Mateu, M. G. (1991). Two mechanisms of antigenic diversification of foot-and- mouth disease virus. Virology, in press.

(2) Thomas, A.A.M., Woortmeijer, R.J., Puijk, W. and Barteling, S.J. (1988). Antigenic sites on foot-and-mouth disease virus type A_{10}. J. Virol. 62, 2782-2789.

(3) Kitson, J.D.A., McCahon, D. and Belsham, G.J. (1990). Sequence analysis of monoconal antibody resistant mutants of type O foot-and-mouth disease virus: evidence for the involvement of the three surface exposed capsid proteins in four antigenic sites. Virology 179, 26-34.

(4) Holland, J.J. and Domingo, E. (1992) Complications of RNA heterogeneity for the engineering of virus vaccines and antiviral agents. Genetic Engineering, Principles and Methods, Vol. 14 (J.K. Setlow, ed.), submitted for publication.

Appendix 4

(Draft)

MINIMUM CONDITIONS FOR THE IMPORTATION INTO EUROPE OF LIVE ANIMALS, FRESH MEAT AND OFFAL OF THE BOVINE, OVINE, CAPRINE AND PORCINE SPECIES. *)

1991

CONSIDERATIONS

1. Following infection with FMD virus and recovery from clinical disease cattle and other ruminants may carry the virus for as long as 6 months or more.

2. The carrier state may also develop in vaccinated cattle following exposure to FMD virus, e.g. during outbreaks of the disease.

3. If, by accident, an inadequately inactivated vaccine was used in an area, infection could be inapparent at first, but in all likelihood it would eventually lead to the appearance of clinical disease.

4. Studies on the carrier state, in particular in sheep and goats, still remain to be finalized. There is no evidence for a true carrier state in pigs, that is persistence of virus beyond one month after the acute phase of disease.

5. During maturation at an ambient temperature of more than $+2^0$ C for at least 24 hours, the pH of normal cattle carcass muscles will drop to a level where FMD virus is inactivated. However, organs such as lungs, liver, kidneys, brain, bone marrow and lymph glands do not develop this degree of acidity post mortem, and FMD virus will survive in such tissues during maturation as above. The same consideration applies to masseter muscles although some virus inactivation takes place in these tissues.

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*) **Replacing earlier recommendations of the Commission on *"Movement of slaughter stock and meat from areas where exotic FMD virus has occurred or inactivated vaccines were applied, in Europe"* as amended in 1987, and**

***"Minimum conditions for the importation of beef into Europe from countries where FMD is endemic and is caused by viruses not considered exotic to Europe"* as amended in 1987.**

6. The possible influence of maturation on the survival of FMD virus in carcass meat from sheep and goats and pigs is still not fully documented. Furthermore, in general terms it is impractical to de-bone sheep, goat and pig carcasses prior to export. Therefore, the following recommendations on the use of maturation and de-boning only apply to cattle.

RECOMMENDATIONS

General requirements

1. The exporting country must have an effective State Veterinary Service as well as an effective and reliable veterinary infrastructure at the local level.

2. A laboratory capable of diagnosing FMD must be available to the Veterinary Services for rapid laboratory diagnosis.

3. Notification of FMD must be compulsory. If outbreaks occur the type and subtype of field virus and any changes thereof must be notified to the appropriate authority in the importing country. All new strains of virus must be forwarded to the World Reference Laboratory.

4. Approval of abattoirs, de-boning, cutting and processing plants should be under the responsibility of the competent National Veterinary Authority.

5. If vaccine is used, proper procedures for virus inactivation must have been applied, including the application of a first order inactivant as well as in-process control of the inactivation slope (linearity). Testing for safety must have been carried out properly and with a satisfactory result.

Special requirements

A. IMPORTATION OF LIVE ANIMALS

A.1. Importation should be allowed from a country which has been free from FMD for at least 2 years, which has not practised vaccination for at least 12 months and which does not allow on to its territory animals that have been vaccinated less than one year previously, **provided that the imported animals**

- have not been vaccinated against FMD,

- have remained in the territory of the country concerned for at least 6 months before importation or since birth,

and provided that

- a national plan for dealing with FMD outbreaks has been developed by the exporting country and made available to the importing country. The main aim of the plan should be the rapid elimination of the virus from the country concerned. To this end the plan must include a total stamping out strategy and other appropriate strict control measures.

A.2. Importation should be allowed from a country which has been free from FMD for at least 2 years, which has practised vaccination within the last 12 months and which allows vaccinated animals on to its territory, **provided that the imported animals**

- have not been vaccinated against FMD,

- have remained in the territory of the country concerned for at least 6 months before importation or since birth,

- have been isolated in a quarantine station in the country of origin under the surveillance of an official veterinarian for at least 2 weeks prior to exportation, with no contact with animals other than those forming part of the consignment. During this period the animals must have reacted negatively to a probang test (cattle, sheep and goats) and to a serological test for antibodies against FMD virus carried out not less than 7 days after entry into the quarantine. Furthermore, this should be followed by a quarantine period in the country of destination,

and provided that

- a national plan for dealing with FMD outbreaks has been developed by the exporting country and made available to the importing country. The main aim of the plan should be the rapid elimination of the virus from the country concerned.

- a properly inactivated vaccine conforming to international standards is used,

For the purpose of paragraphs A.1. and A.2., a country may continue to be considered as having been free from FMD for at least 2 years, even if a limited number of outbreaks of the disease have been recorded on a limited part of its territory, on condition that the disease was eradicated within a period of less than 3 months subject to evaluation on a case by case basis.

A.3. Importation should be allowed from a country which has been free from FMD for less than 2 years, which has practised vaccination within the last 12 months and which allows vaccinated animals on to its territory, **provided that:**

- the same conditions as under A.2. are fulfilled,

- the animals have remained on the holding for at least 6 months,

- additional requirements as may be specified by the importing country are satisfied.

B. IMPORTATION OF FRESH MEAT

B.1. Importation of fresh meat *) should be allowed from a country which has been free from FMD for at least 12 months prior to the date of slaughter and which has not practised vaccination for at least 12 months and which does not allow on to its territory animals that have been vaccinated less than 12 months previously, **provided that:**

- the meat originates from animals which have remained in the territory of the country concerned for at least 3 months before slaughter or since birth,

- a national plan for dealing with FMD outbreaks has been developed by the exporting country and made available to the importing country. The main aim of the plan should be the rapid elimination of the virus from the country concerned. To this end the plan must include a total stamping out strategy and other appropriate strict control measures.

B.2. Importation of beef *) should be allowed from a country which has been free from FMD for less than 12 months and/or which has practised vaccination within the last 12 months, **provided that**:

- the beef originates from animals that come from holdings in which there has been no outbreak of FMD in the previous 60 days, and around which within a radius of 25 km there has been no case of FMD for 30 days,

- the animals have remained in the territory of the country concerned for at least 3 months before slaughter or since birth,

- the animals have been transported directly from their holding of origin to the approved slaughterhouse concerned without passing through markets, without contact with animals which do not comply with the conditions required for export of meat to Europe and, if conveyed in a means of transport, the latter has been cleaned and disinfected before loading,

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*) **Fresh meat (including beef) means meat which has not undergone any treatment other than cold treatment to ensure preservation.**

- the animals are slaughtered as soon as possible, and not later than 72 hours, after their arrival in approved slaughterhouses which conform to international standards and where the animals will be subjected to ante-mortem inspection by Government veterinarians and post-mortem inspection under the direct responsibility of Government veterinarians, and found to be free from FMD during these inspections. The ante-mortem inspection should be carried out within 24 hours of arrival in the slaughterhouse and repeated if the animals are not slaughtered within 24 hours. The ante-mortem inspection should include examination for clinical evidence of FMD and where appropriate a detailed examination of feet and mouth.

- lairage facilities at approved slaughterhouses are adequate and capable of being cleansed and disinfected effectively,

- **the beef does not contain any bones or major lymphatic glands**. The de-boned beef must originate from carcasses which have matured at an ambient temperature of more than $+2^0$ C for at least 24 hours before the bones were removed. After maturation and before removal of the bones, the pH value of the beef must be less than 6 as measured in M. longissimus dorsi, which should be controlled in each half carcass by probe ("spear") electrode or other proven method. Measurements should be recorded.

- the beef is clearly marked in an agreed manner so that the identity of the slaughterhouse of origin can readily be recognized.

- if FMD is found at ante or post mortem inspection, further preparation of beef for export to Europe will only be authorized after slaughter of all animals present, removal of all meat, and the total cleaning and disinfection of the establishment(s) under the control of an official veterinarian. Furthermore, all precautions must be taken to ensure no possibility that contaminated meat is exported.

C. IMPORTATION OF BOVINE OFFAL

C.1. Importation of offal should be allowed from a country which has been free from FMD for at least 12 months and which has not practised vaccination for at least 12 months and which does not allow on to its territory animals that have been vaccinated less than 12 months previously, **provided that:**

- the offal originate from animals which have remained in the territory of the country concerned for at least 3 months before slaughter or since birth,

- a national plan for dealing with FMD outbreaks has been developed by the exporting country and made available to the importing country. The main aim of the plan should be the rapid elimination of the virus from the

country concerned. To this end the plan must include a total stamping out strategy and other appropriate strict control measures.

C.2. Importation of offal should be allowed from a country which has been free from FMD for less than 12 months and/or which has practised vaccination within the last 12 months, **on the following conditions**:

- the offal must come from animals originating from holdings in which there has been no outbreak of FMD in the previous 60 days, and around which within a radius of 25 km there has been no case of FMD for 30 days, and the animals must have been found to be free from FMD before and after slaughter,

- the animals have remained in the territory of the country concerned for at least 3 months before slaughter or since birth,

- diaphragmatic muscles ("skirts") from which lymphatic glands, adhering connective tissues and fat have been removed, should be allowed for importation as fresh meat for human consumption after maturation at a temperature of more than $+2^0$ C for 24 hours prior to freezing, provided they come from carcasses which fulfill the requirements under **B.2.**

- masseter muscles, from which lymphatic glands, adhering connective tissues and fat have been removed and which have matured at a temperature of more than $+2^0$ C for 24 hours prior to freezing, should be allowed only for manufacture of cooked meat based products or for the production of petfood. The processing should ensure total inactivation of any FMD virus possibly present, i.e. the internal temperature of the product must reach at least 70^0 C for at least 30 minutes when processed by retort cooking or 80^0 C must be attained in the centre of the product for non-retort processing.

- all other offal, appropriately trimmed and matured as described above, should be allowed only for specified technical purposes, e.g. production of petfood/pharmaceuticals, provided that any FMD virus possibly present in the raw material will be totally inactivated during manufacture (heat treatment or other proven method). Any packaging, wrapping or residual processing material should be disposed of to avoid any animal health risks.

- the raw materials mentioned under 3rd and 4th indent should be clearly identified and transported under strict control directly from the port of arrival in Europe to the processing plant in leak-proof and sealed containers.

- in the processing plant appropriate cleansing and disinfection procedures must be carefully applied. Special attention should be paid to the necessity

of carefully separating processed from unprocessed materials.

It is permissible for an importing country to impose stricter conditions.

Selected references

EEC Council Directive	64/432 of 26 June 1964	as amended
EEC Council Directive	64/433 of 26 June 1964	- -
EEC Council Directive	72/462 of 17 December 1972	- -
EEC Council Directive	85/511 of 18 November 1985	- -
EEC Council Directive	90/423 of 26 June 1991	
EEC Council Directive	91/266 of 21 May 1991	
EEC Commission Decision	89/18 of 22 1988	

Blackwell, J.H. et al. (1982). Effect of Thermal Processing on the Survival of Foot-and-Mouth Disease Virus in Ground Meat. J.Fd.Sci., **47,** 388-392.

Blackwell, J.H. (1984). Foreign animal disease agent survival in animal products: Recent developments. JAVMA, **184,** 674-679.

CEC, AGRICULTURE (1986). Investigation on the possible effect of electrical stimula tion on pH and survival of foot-and-mouth disease virus in meat and offals from experimentally infected animals. Report EUR 10048 EN.

Roberts, P.C.B. (1970). Foot-and-mouth disease, its relation to meat processing. J.Fd. Technol. **5,** 313-323.

Wittmann, G. (1990). Virusträger bei Maul- und Klauenseuche. Über sichtsreferat. Berl. Münch. Tierärzl.Wschr. **103,** 145-150.

APPENDIX 5

EPIDEMIOLOGY OF RECENT FMD OUTBREAKS IN TURKEY, AND CHARACTERISTICS OF FIELD ISOLATES WİTH REFERANCE TO BOTH FROM CATTLE AND SMALL RUMINANTS

INTRODUCTION:

Due to its special location between East and West, Turkey has accepted the international duty as to prevent FMD (Foot and Mouth Disease) contagıouns which can spread in Europe, and because of its great effects on the Turkish economy great attention has also been paid to fight against the FMD.

For these reasons, it has also been targetted to achieve positive results by getting into close co- operation with United Nations Food and Agricultural Organization (FAO) / Office International Des Epizootie (OIE) / European Commission for the control of FMD and European Economic Community (EEC). In the controlling of FMD, as well as the method of vaccination quarantine has been accepted and generally the social economical conditions, geographical position and climate of our country has been taken into consideration.

Applications of FMD vaccination on a large scale in our country have started in october-1962. In these applications, Thrace region has been accepted as a buffer zone. The number of provinces comprised in this buffer zone have been increased in 1970, all of the provinces in Thrace and Çanakkale, İstanbul, Balıkesir, Bursa, Kocaeli, Sakarya, in Marmara region, in the Southeast Anatolia, Syria, Iraq border villages of Hatay, Gazeantep, Şanlıurfa Mardin provinces which are on the line of 20 km inside the border has been included in this buffer zone (Table 1).

In 1983 Hakkari, Van, Ağrı, Kars provinces have also been included in the buffer zone. In 1988, the number of provinces in the buffer zone has been increased to 28 by including Afyon, Bolu, Eskişehir, Kütahya, Manisa, Uşak and Zonguldak provinces.

According to the decisions which have been taken in the FMD Assembly, held in Brussels- October 30-31 1989 by FAO/OIE/EEC; the buffer zone has been removed from Thrace to Marmara region. The applications of vaccination have been stopped in 1990 - 1991 and a new buffer zone has been constituted with 12 provinces in western Anatolia and Marmara region (Table 2).

In these buffer zones, the cattle is vaccinated twice a year and the sheep is vaccinated once a year. The potency and inocuity tests of every vaccine batch produced in our Institute is being done suitably to European Pharmacopy. The necessary legal measurements are taken regarding the sensitive animals and animal products which will enter Thrace. The stamping out policy in Thrace is applicable with compensation and the entrance of non-vaccinated animals into Marmara buffer zone are being prevented. The necessary great attention is being payed to the controlling of animal movements, the quarantine applications in diseases outbreaks, the training of farmers and technical personnel in the field.

EPIDEMIOLOGICAL STUDIES

In fighting against FMD, a project has been prepared in september 1989 aiming the application of Epidemiologic studies by actual methods in the framework of Turkish - German Technical Co-operation programme. Project studies have started primarily in 3 provinces (Samsun, Konya and Balıkesir) as a pilot region.

Within this programme a cold chain project is started and a frigorific truck is provided so that the vaccines are protected from environmental temperatures during their transportation from FMD Institute to application points (cold-hot). Research and studies directed to field scannings have been continued for the determination of vaccine potency. More samples have been sent from province to FMD Institute and from FMD Institute to World Referance Laboratory.

In this fromework the studies given below have been carried out in our Institute for the determination of the epidemiologic position of FMD in Turkey.

1-Outbreak studies

2-Determination studies of carrier ratio

3-Determination of vaccine potency

4-Diagnosis and characterization of field strains

1-OUTBREAK STUDIES

A-Samples brought to our Institute,

B-Denounciations of the epidemiologists in provinces or counties,

C-Datas which were provided to our Institute specialist's from the disease denounciations;

have been made use of in examination of FMD and in the determination of its entrance points to Turkey. These have also been made use of in identifying the spreading number of diseased, infected or if existing the dead animals, the monthly or yearly distrubution of FMD outbreaks, position and type of FMD;

According to datas obtained in the first half of 1990-1991; 911'0'and 32'A' type have been determined in 943 outbreaks (Table 3).

If we examine the tables, an increase can be seen in the number of outbreaks in the first half of 1990 (graph 1), (Table 4,5).

Animal movements, geographical position of the country and the

reason of individual stock-breeding play an important role in the wide spread of FMD.

To prevent the spreading of FMD by noncontrolled animal movements and to control these movements in a certain system, the primary control centers in Giresun, Tokat, Sivas, Malatya, Gaziantep and secondary centers in Kocaeli, Bursa, Balıkesir and Çanakkale have been established (Table 6) In 1991,the controlling of animal transportation at constant control centers, determined in certain regions, have been started.

In these controls, the important points are the controlling of the disinfention of transportation vehicles and the controlling of the FMD vaccination of the animals.

From these centers in the first half of the year a number of 322005 certificated animals and a number of 2637 non-certificated animals has passed. This shows that the succes of the applicated system is 72%.

2- DETERMINATION STUDIES OF CARRIER RATIO

It is known that the diseased and then recovered cattle and sheep are still carrying the FMD virus in epithelial cells on the oesophageal-pharengeal areas in certain duration and although they don't show any clinical disease symptom, they infect the other animals around them. The determination of carrier ratio in a region or a country plays an important role in the succes of the epidemiological studies and in the controlling of the disease.

To determine the FMD carrier in cattle and sheep in Anatolia, a project has been started in. 1990 which will be completed at the and of this year, and according to the datas obtained from the project, the carrier ratio has been found as 5,4% for cattle and 3% for sheep.

In this study the recovered animals were chosen as sample and samples have been taken by the Cluster sampling method.

In 1989, a similar study carried out in Thrace region as well as in Ankara FMD Institute and IAH Pirbright, stated that the animals in Thrace have no FMD virus.

3- DETERMINATION OF VACCINE POTENCY

The controlling of the FMD requires safe and high quality controlling of the vaccine.

Although, there must be a paralellism between the vaccine potency value and it's field value, sometimes this relation doesnit take place for some reasons. In the encouragement of the herd immunity, it is necessary to vaccinate all of the animals and know the number of vaccination in order to increase the formation of antibody in 90% of animals.

It is certain that if an animal is challenged, a virus will get into that animal however the animal will provide enough immunity to be protected. This situtation is different in the herd. The possibility of an animal getting a disease depends on its contact with an animal carrying a virus or on a carrier like air current or etc.

In the analysis of herd immunity, it is necessary to find out the ratio of the immuned animals in contrast to the absolute protection value required in the vaccination/challenge tests which measure the usefulnes of the vaccine in an individual level.

For this reason; results of the antibody scanning of the SNT and ELISA tests done on 852 cattle blood serum which has been sent from the provinces where cattle was vaccinated show that, 56% of immunity against O type and 68% of immunity A type in the first half of 1991 (graphic 2).

On the other hand 75,5% of immunity against O type and 85% of immunity against A type has been determined from 464 cattle blood serum which was sent by special farms for the same purpose (graphic 3).

It is obvious that the desired immunity obtained by means of vaccine is affected by a series of factors.

According to our control programme against FMD, there has been an application of vaccine to at least 70-80% (twice a year for cattles and once a year for sheeps) of the animals in the buffer zones, this ratio has decreased to 30% in other provinces. It is known that even if we provide the animal with the necessary conditions before during and after vactination the formation of antibody titration which is at most 80 % in the herd decreases to 20% in 6 months.

A good herd immunity can be obtained after 2 or 3-4-5 vaccination and the required antibody titration can be achieved in 90% of the animals.

4- DIAGNOSIS AND CHARACTERIZATION OF THE FIELD STRAINS

Selection of a vaccine strain seems to be very important because of the FMD virus' properties of mutation and its quick changing of antigenic character.

If the contagioun is still seen in the field after the vaccinations, it is known that, this can happen due to changes in the antigenic character of field virus or may depend on the problems regarding the vaccine production or the application.

The antigenic comparison of strains which provide the contagioun in the field and vaccine strains used in the vaccine and the determination of the relation between the strains help us in choosing vaccine strain used for the routine controlling of the FMD.

ELISA TEST is being used as routine in our Institute to find antigenic relation between the disease strains in the field and the vaccine strain. After the type determination of the samples which come from the field, suitable strain is passaged in the BHK-21 cell and subjected to the ELISA test for the determination of the antigenic relation.

Therefore, in the first half of 1991 there has been a study on the number of 14 samples which come from the various provinces.

Results of the studies show that;

There is very close relation between the strains isolated from the field and our vaccine strain of 01 Manisa (Table 7).

In the same way when the results of the World Reference Laboratory is examined it is seen that the field strain and the 01 Manisa is very close to each other in antigenic way (Table 8).

Consequently, there is no objection to the use of 01 Manisa as a vaccine strain because there is a antigenic relation between the 01 Manisa and the strain isolated from the field. The using of 01 Manisa as a vaccine strain has also been proposed by the World Reference Laboratory.

According to the monoclonal antibody technique which will be put into application in the near future, the values obtained by the nucleotid sequencing methods and the results of the epidemiologic studies, we may understand the reason of the occurence of field virus whether it is occured by the change of the antigenic charecter by a virus which escaped from the laboratory or by an inactivated vaccine application. These will help us make reasonable decisions in choosing the vaccine strain.

NOTE: Every strain isolated from the field are cattle originated. The samples obtained from the sheep during the year were not enough to be studied.

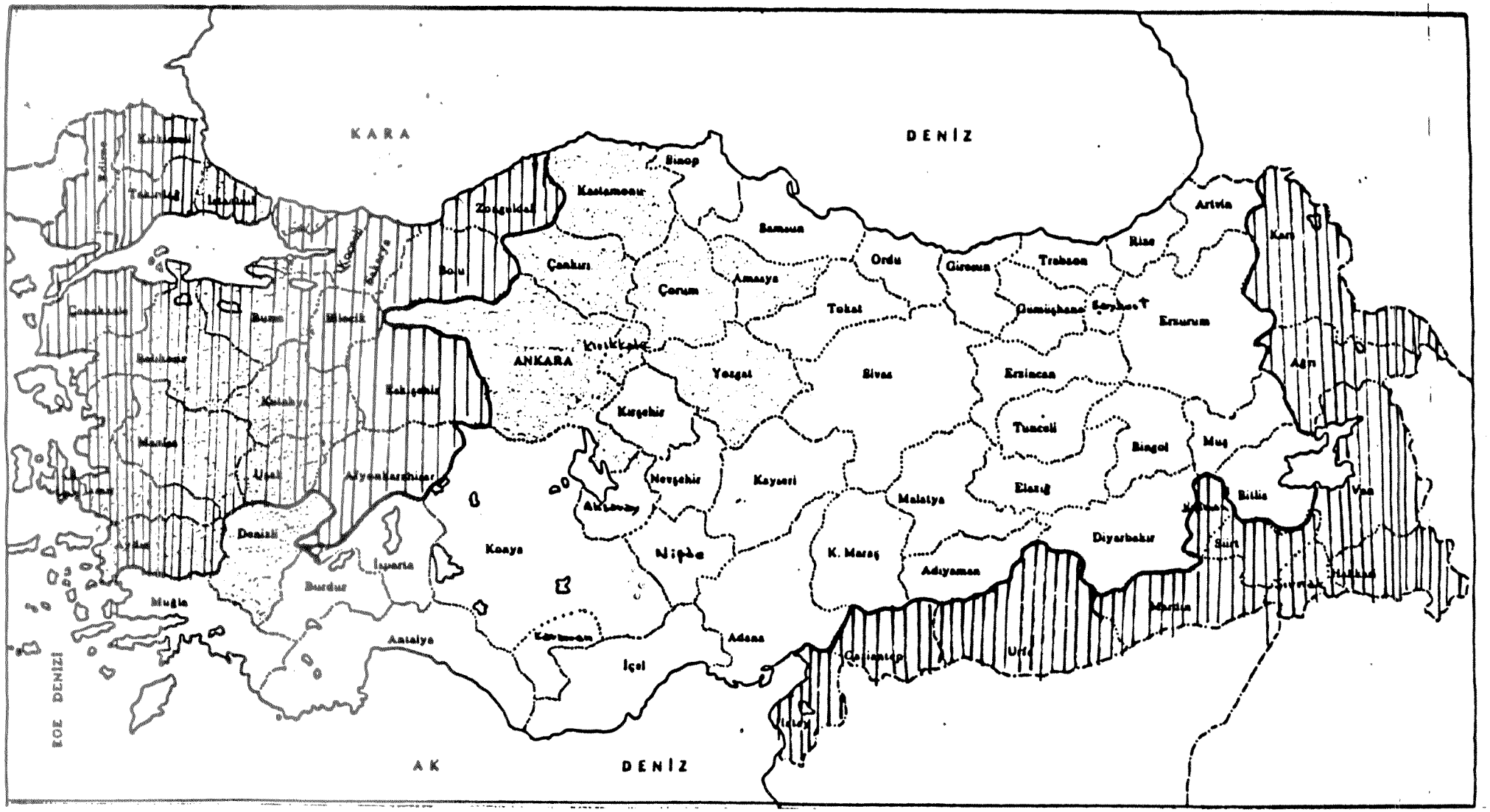
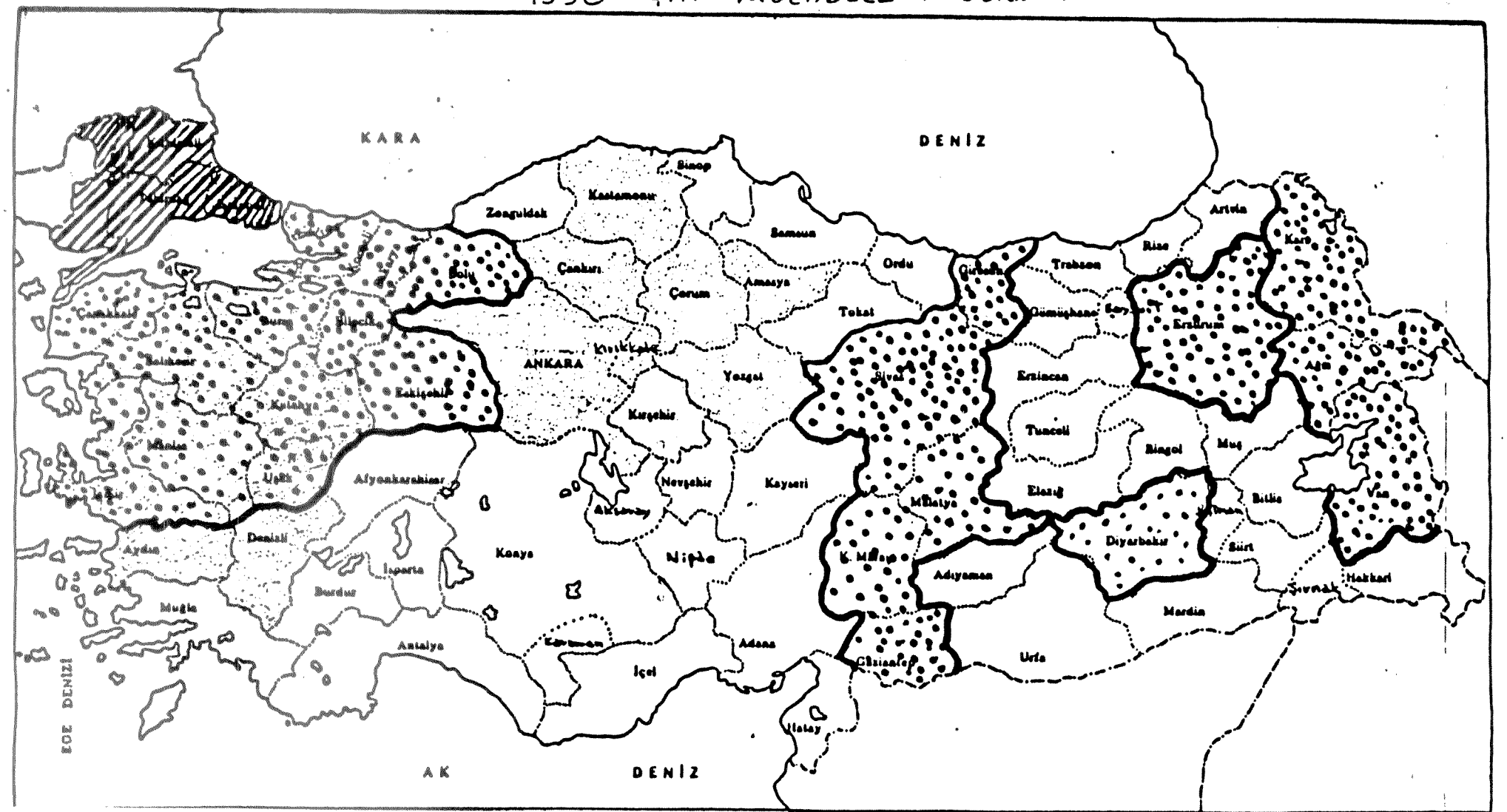


TABLE-1 BUFFER ZONE (1962 - 1989)

1990 ŞAP MÜCADELE PROGRAMI



Free Zone

Buffer Zone

(1990-1991)

TABLE-2

FOOT AND MOUTH DİSEASE OUTBREAKS
IN TURKEY (1990-1991)

YEARS :	MONTHS :	O 1 TYPE :	A 22 TYPE :	TOTAL:
1990	JAN.	5	0	5
	FEB.	2	3	5
	MAR.	3	6	9
	APR.	7	2	9
	MAY.	26	2	28
	JUN.	58	2	60
	JUL.	74	1	75
	AUG.	86	0	86
	SEP.	64	0	64
	OCT.	79	0	79
	NOV.	34	0	34
	DEC.	36	0	36
1991	JAN.	70	0	70
	FEB.	55	0	55
	MAR.	45	0	45
	APR.	75	2	77
	MAY.	91	6	97
	JUN.	101	8	109
TOTAL :		911	32	943

TABLE-3

FOOT AND MOUTH DISEASE OUTBREAKS IN TURKEY (1990-1991)

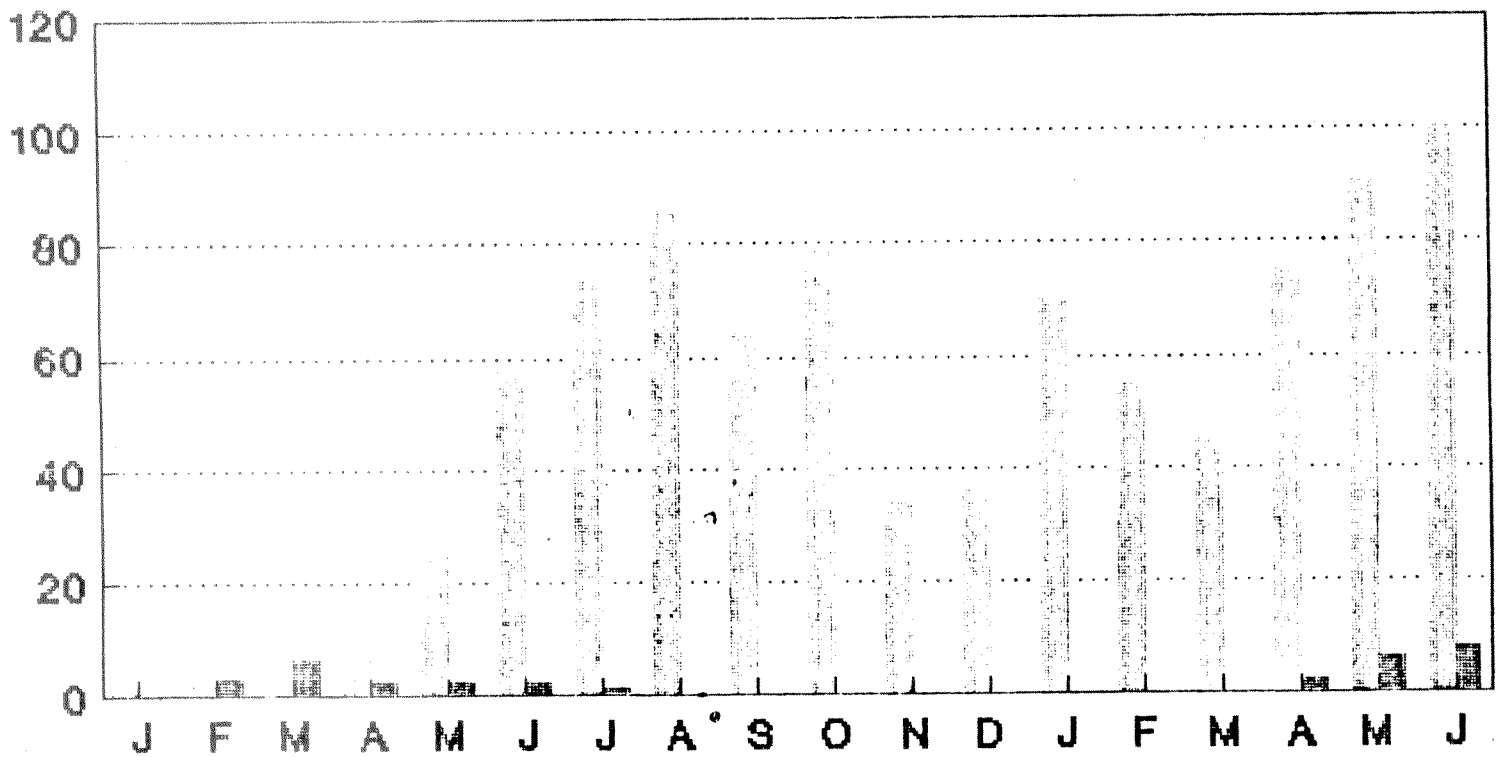


TABLE 1

O TYPE A TYPE

Graphic-1

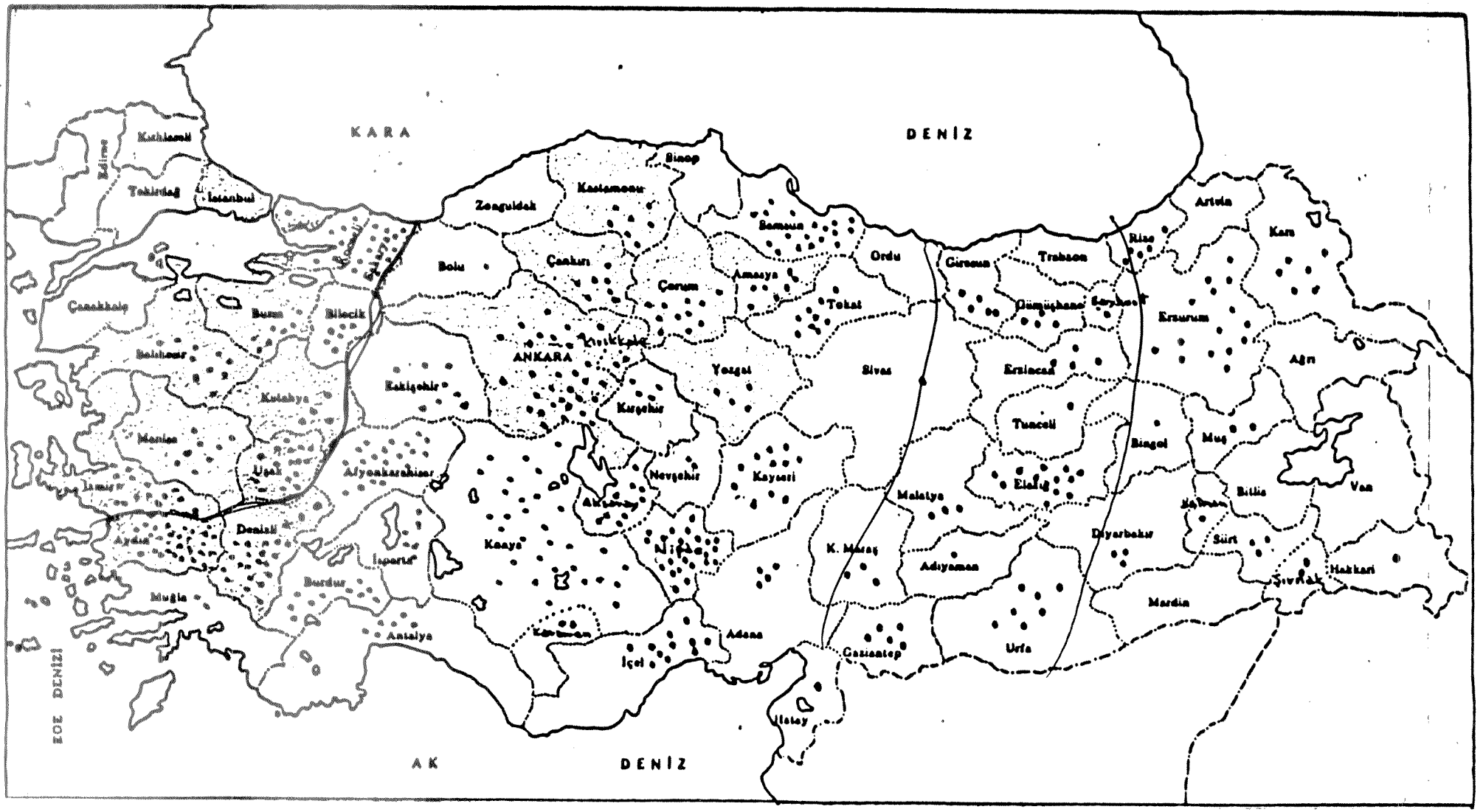


TABLE-4

FMD OUTBREAKS IN TURKEY
JAN.-JUN. 1991

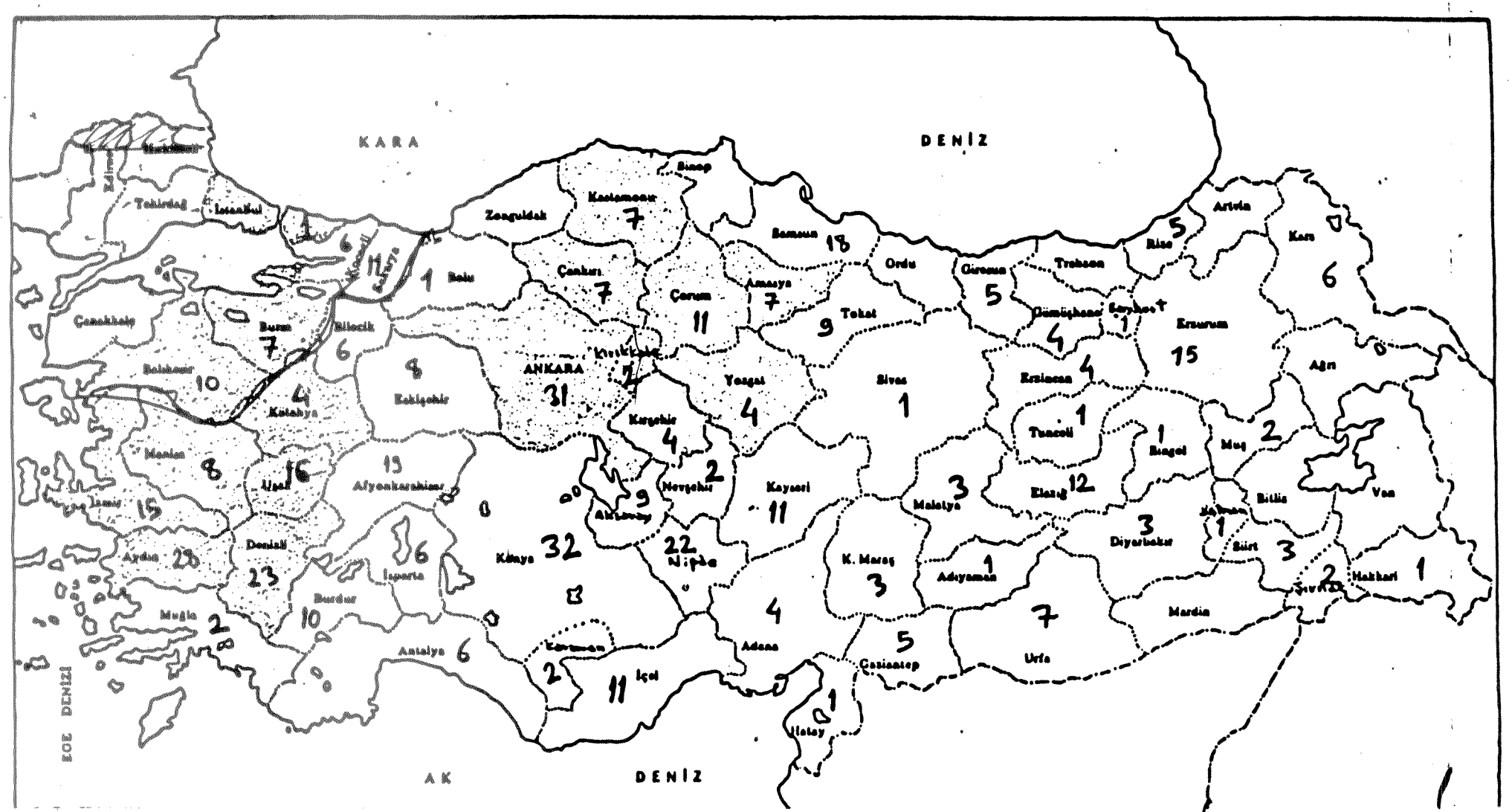


TABLE-5

FMD OUTBREAKS (JAN.-JUN. 1991)
TURKEY

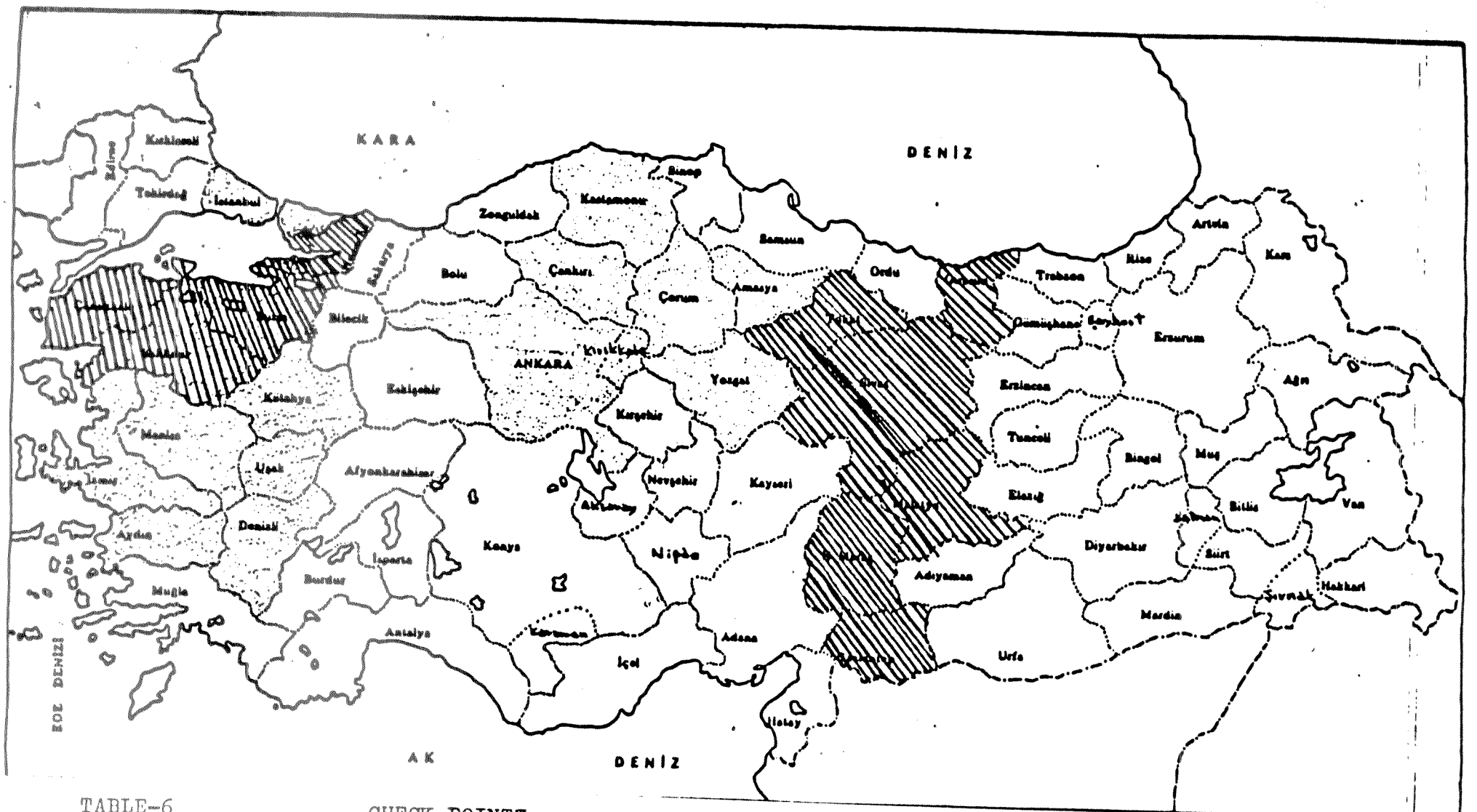
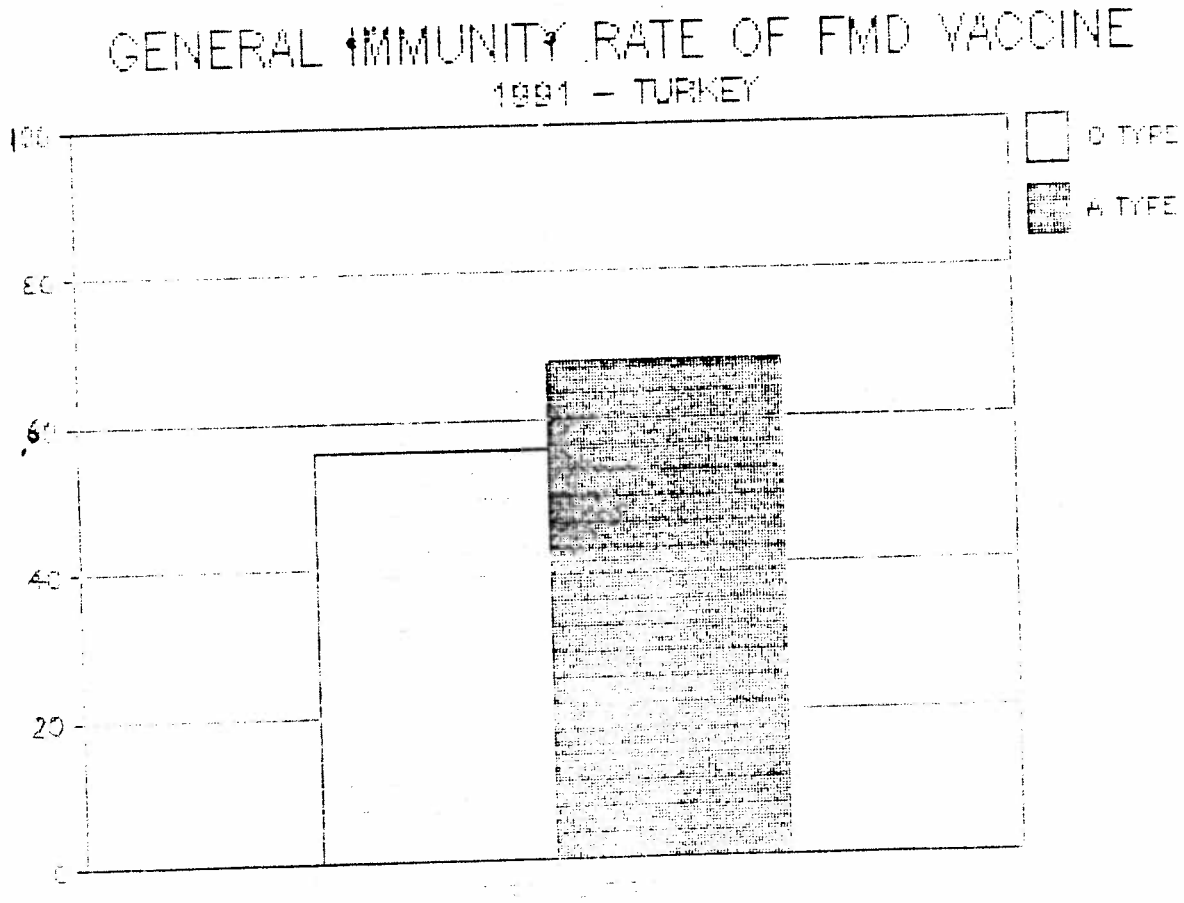


TABLE-6

CHECK POINTS OF THE ANIMAL MOVEMENTS

GENERAL IMMUNITY RATE OF FMD VACCINE
1991 - TURKEY

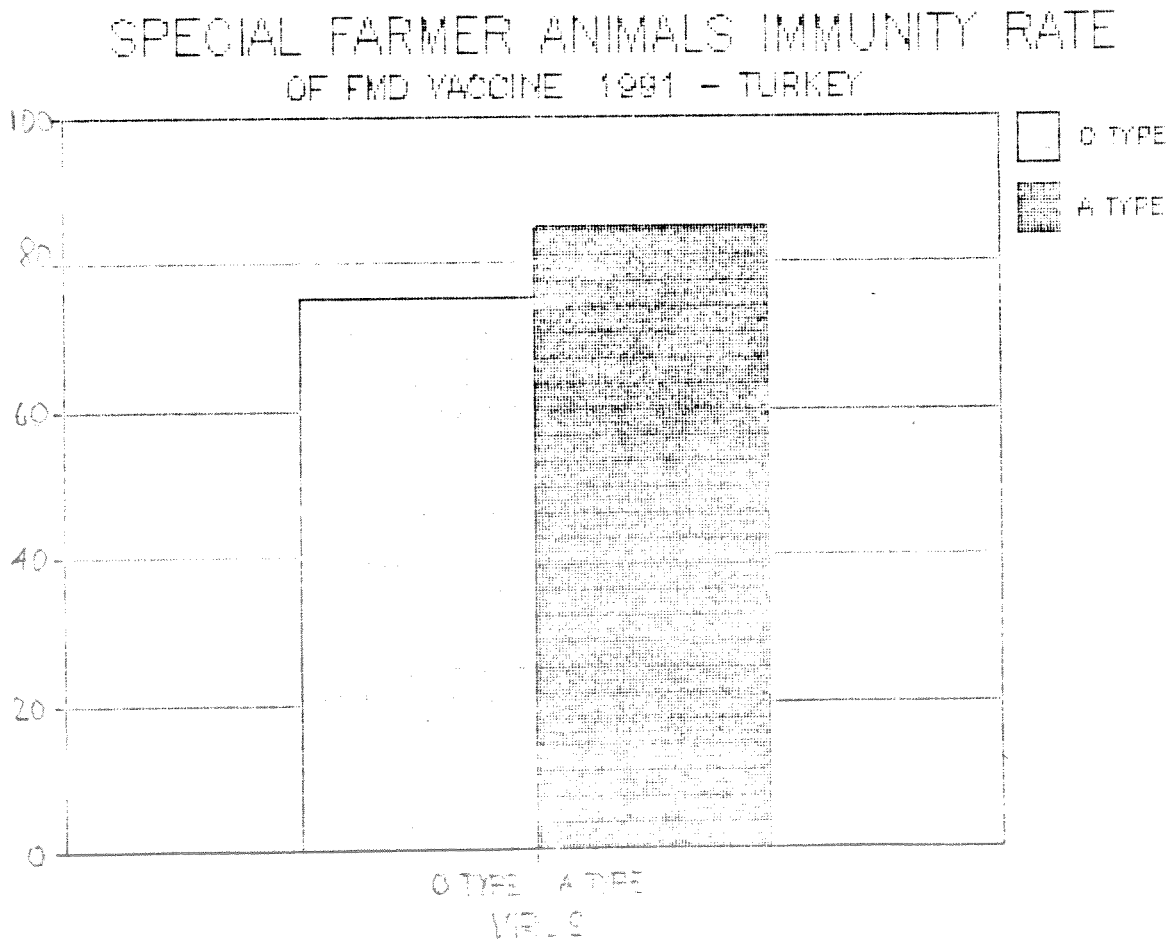
Total Serum :	Protection against to FMD O type virus % :	Protection against to FMD A type virus % :
852	56	68



Graphic-2

SPECIAL FARMER ANIMALS IMMUNITY RATE OF FMD VACCINE
1991 - TURKEY

Total Serum :	Protection against to FMD O type virus % :	Protection against to FMD A type virus % :
464	75.5	85



Graphic-3

TURKEY FIELD STRAINS 1991 r1 VALUES DERIVED BY ELISA :

ANKARA REF.NO:	FMD TYPE :	r1 VALUES :	PIRBRIGHT REF.NO :
172-AFYON	O	0,5	-
115-BALIKESİR	O	1	-
100-ANKARA	O	1	-
198-ÇANKIRI	O	0,5	-
247-ERZURUM	O	0,5	-
306-AFYON	O	0,5	-
414-YOZGAT	O	1	-
525-SİİRT	O	1	-
690-BURSA	O	0,5	-
610-KIRŞEHİR	O	0,9	-
590-BOLU	O	1,5	-
611-KAYSERİ	O	0,8	-
159-İZMİR	O	1	TUR 3/91
78-BURSA	O	1	TUR 2/91

TABLE-7

ANKARA

TURKEY FIELD STRAINS 1991 r1 VALUES DERIVED BY ELISA :

ANKARA REF.NO:	FMD TYPE :	r1 VALUES :	PIRBRIGHT REF.NO :
73-ANKARA	O	-	TUR 1/91
78-BURSA	O	1	TUR 2/91
159-İZMİR	O	1	TUR 3/91
172-AFYON	O	0,8	TUR 4/91
188-ÇANKIRI	O	-	TUR 5/91
247-ERZURUM	O	-	TUR 6/91
52-TUNCELİ	O	1	TUR 7/91
251-KÜTAHYA	O	1	TUR 8/91
288-AFYON	O	-	-
326-ESKİŞEHİR	O	-	-
349-ADANA	O	-	-
613-UŞAK	O	-	-
651-İZMİR	O	-	-
660-ANKARA	A	-	TUR 14/91

TABLE-8 PIRBRIGHT

Genetic and Antigenic Studies on Foot-and-Mouth Disease Viruses Isolated from Samples Collected in Turkey in 1991

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The World Reference Laboratory (WRL)/Community Reference Laboratory (CRL) for Foot-and-Mouth Disease (FMD) has received 14 samples from suspected outbreaks of FMD in Turkey during 1991. All the samples submitted were collected from cattle between January and June. Foot-and-mouth disease viruses (FMDV) have been isolated and typed by ELISA from all of these samples (Table 1). Initially FMDV type O virus was identified, following passage in cell cultures, from both TUR/9/91 and TUR/12/91. However, following subsequent passages on IB-RS-2 cells and determination of nucleotide sequences at both ends of the VP1 gene, type A virus was identified. The presence of both type O and type A FMDV in the original epithelium of one of the samples (TUR/12/91) was confirmed by re-isolation of both viruses types following incubation of the epithelium suspension with either type O or type A antisera. The approximate geographical locations of the 1991 FMD outbreaks are shown in Figure 1.

Five of the FMD type O virus isolates were examined for reactivity with reference bovine vaccinal sera (BVS) against O_1/BFS 1860/UK/67, O_1/Manisa/Turkey/69 and O_1/Dalton/Israel/88 (ISR/2/88) in one-way ELISAs (Table 2). Antigenically each of the type O isolates were O_1-like and were closely related to all of the vaccine strains used in the assay. Any of these potent vaccines including that currently in use in Turkey (O_1/Manisa/Turkey/69) should assist in control.

The FMD type A virus isolate, TUR/14/91, was tested against reference BVS against A_{22}/Iraq/24/64, A_{22}/Mahmatli/Turkey/65 and A/Saudi Arabia/23/86 (Table 3). This virus isolate was most closely related to the vaccine strain A_{22}/Iraq/24/64, a potent vaccine which should assist in control.

All of the 1991 virus isolates, except TUR/3/91, have been examined by determining the sequence of approximately 165 nucleotides at the end of the 1D (VP1) gene. These were compared with virus isolates from previous years (see Table 1). The results of this preliminary nucleotide sequence analysis are shown in the form of dendrograms in Figures 2 (type O) and 3 (type A). The principal conclusions are as follows:-

1) The virus isolated during the vaccine trial (TUR/15/91) was found to be identical with the vaccine strain (O_1/Manisa CP14) over the genome region examined.

2) O/TUR/7/91 was significantly different from O_1/Manisa/Turkey/69 (12%) and from all the other 1991 Turkey type O isolates.

3) Viruses closely related to the main 1991 type O group (e.g. TUR/13/91) have been found in Turkey since 1987 and in other parts of the Middle East including Syria (1987), Saudi Arabia (1990) and Israel (1991).

4) Type O viruses isolated in 1990 fall into two groups, one which was O_1/Manisa-like, but neither of which were closely related to virus isolates from 1991.

5) Samples TUR/9/91 and TUR/12/91, both originally typed as FMDV O were found to contain mixtures of type O and type A viruses (see above).

6) The virus isolates A/TUR/9/91, A/TUR/12/91 and A/TUR/14/91 were different from each other (6%), from virus isolates in preceding years (1985-90; 11%) and from the classical A_{22} group, including A_{22}/Mahmatli/Turkey/65 (14.76%).

Further studies are in progress to sequence representative type O virus isolates collected between 1969 and 1990 and type A virus isolates collected between 1965 to 1988 to determine the patterns of evolution of FMD viruses within Turkey and their relationships with other FMD viruses in the region.

Table 1. Foot-and-mouth disease viruses used in this study.

WRL No.	Type	Province	District	Village	Date collected
TUR/5/79	A	Sakarya	Adapazari	Erenler	16/02/79
TUR/9/85	A	Trabzon	Tonya	Karsular Mahallesi	23/10/85
TUR/1/87	A	Malatya	Merkez	Kullu Magara	14/08/86
TUR/2/87	O	Eskisehir	Merkez	A.Caglan	18/05/87
TUR/2/88	O	Aydin	Cine	Karpuzlu	06/01/88
TUR/4/88	A	Yozgat	Cekerek	Kiyili	19/02/88
TUR/7/88	A	Sinop	Boyabat	Sariagar Cayir	11/04/88
TUR/8/88	O	Gaziantep	Oguzeli	Altinyurt	11/05/88
TUR/9/88	A	Afyonkarahisar	Emirdak	Adayazi	09/06/88
TUR/1/89	A	Erzurum	Oltu	Guzelsukisla	31/01/89
TUR/3/89	A	Ankara	Güdul	Boyall	13/02/89
TUR/6/89	O	Aydin	Sultanhisar	Rekmezmah	20/03/89
TUR/1/90	A	Çorum	Iskilip	Saraycik	28/03/90
TUR/2/90	O	Kirsehir	Kaman	Bayramozu	28/03/90
TUR/3/90	A	Tokat	Merkez	Ortaören	29/03/90
TUR/4/90	O	Erzurum	Pasinler	Yesolöz	03/04/90
TUR/5/90	O	Adana	Seyhan	Karayusuflu	08/05/90
TUR/9/90	O	Tokat	Pazar	Derekoy-Kababasi	28/05/90
TUR/14/90	O	Amasya	Merkez	Deqirmendereköyu	19/06/90
TUR/21/90	O	Denizli	Honaz	Haydar Meukii	24/09/90
TUR/1/91	O	Tunceli	Mazgirt	Kayaci	14/01/91
TUR/2/91	O	Ankara	Kalecik	Karahuyuk	17/01/91
TUR/4/91	O	Izmir	Kemalpasa		20/02/91
TUR/5/91	O	Afyon	Emirdoy	Kirkpinar	20/02/91
TUR/6/91	O	Cankiri	Merkez		05/03/91
TUR/7/91	O	Erzurum	Merkez		18/03/91
TUR/8/91	O	Kutakya	Saphane		18/03/91
TUR/9/91	O+A	Afyon	Emirdoy	Hamzahacili	01/04/91
TUR/10/91	O	Eskisehir	Mahmudiye		22/04/91
TUR/11/91	O	Adana	Seyhan	Gokcelar	29/04/91
TUR/12/91	O+A	Usak	Merkez	Yapalilar	18/06/91
TUR/13/91	O	Izmir	Odemis	Cumhuriyet. Mah.	28/06/91
TUR/14/91	A	Ankara	Cubuk	Kutuoren	28/06/91
TUR/15/91	O	vaccine trial isolate			July 91
O_1/Manisa CP14 (vaccine strain, cattle passage 14)	O	Manisa	Korukkoya		01/04/69

Table 2. Relationship ('r') values of the type O virus isolates to reference bovine vaccinal sera by one-way ELISA.

WRL No.	O_1/BFS 1860 UK/67	O_1/Manisa Turkey/69	O_1/Dalton Israel/88
TUR/9/91	0.5	0.75	1.0
TUR/10/91	1.0	>1.0	0.5
TUR/11/91	1.0	>1.0	1.0
TUR/12/91	0.75	1.0	1.0
TUR/13/91	1.0	>1.0	1.0
TUR/15/91	1.0	>1.0	1.0
O_1/Manisa CP14	0.5	>1.0	1.0

Table 3. Relationship ('r') values of the type A virus isolate to reference bovine vaccinal sera by one-way ELISA.

WRL No.	A_{22}/Iraq 24/64	A_{22}/Mahmatli Turkey/65	A/SAU/23/86
TUR/14/91	0.5	0.35	0.25

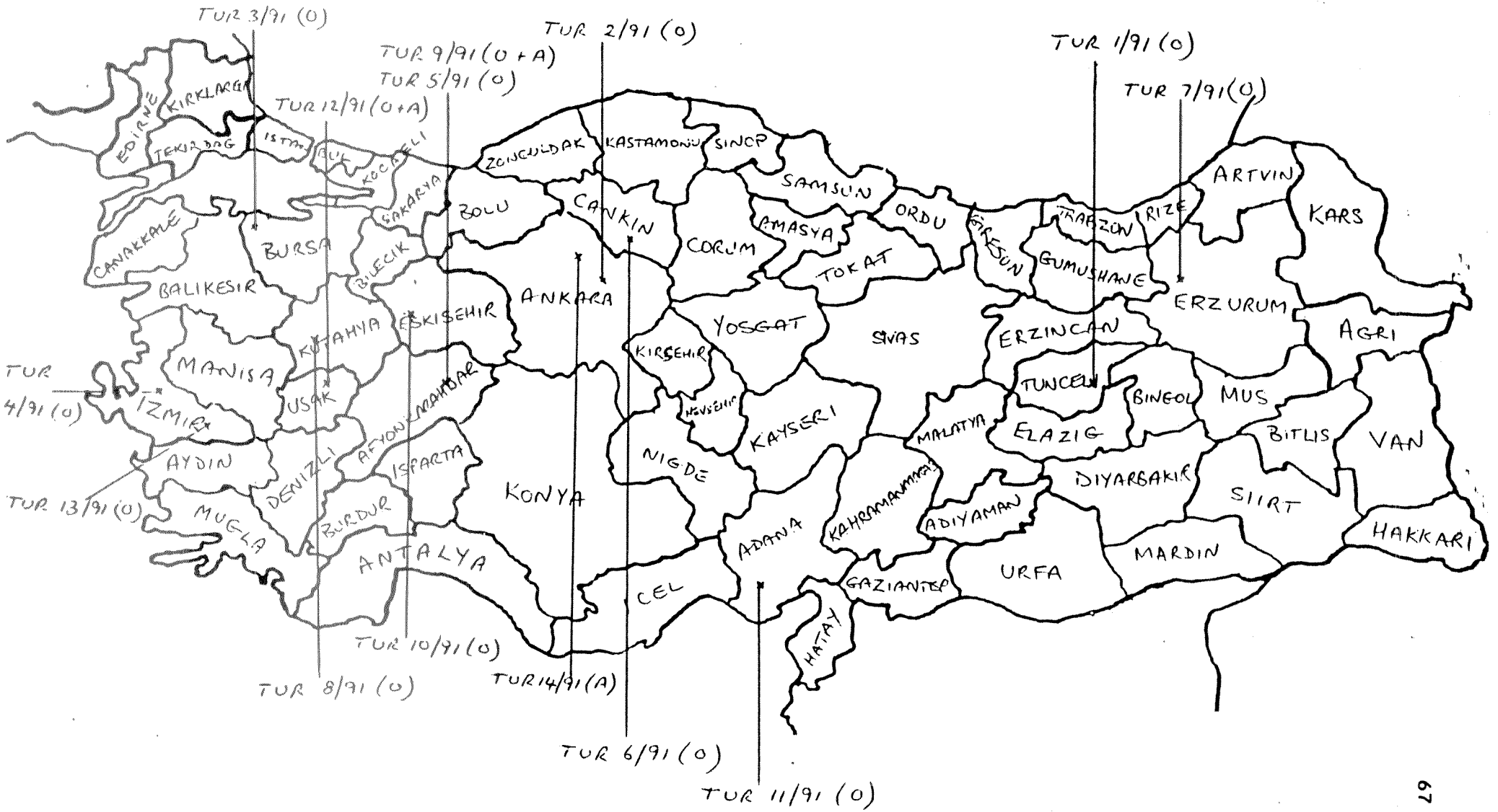

Figure 1. Map depicting the approximate location of FMD outbreaks from which samples were sent to the WRL/CRL during 1991

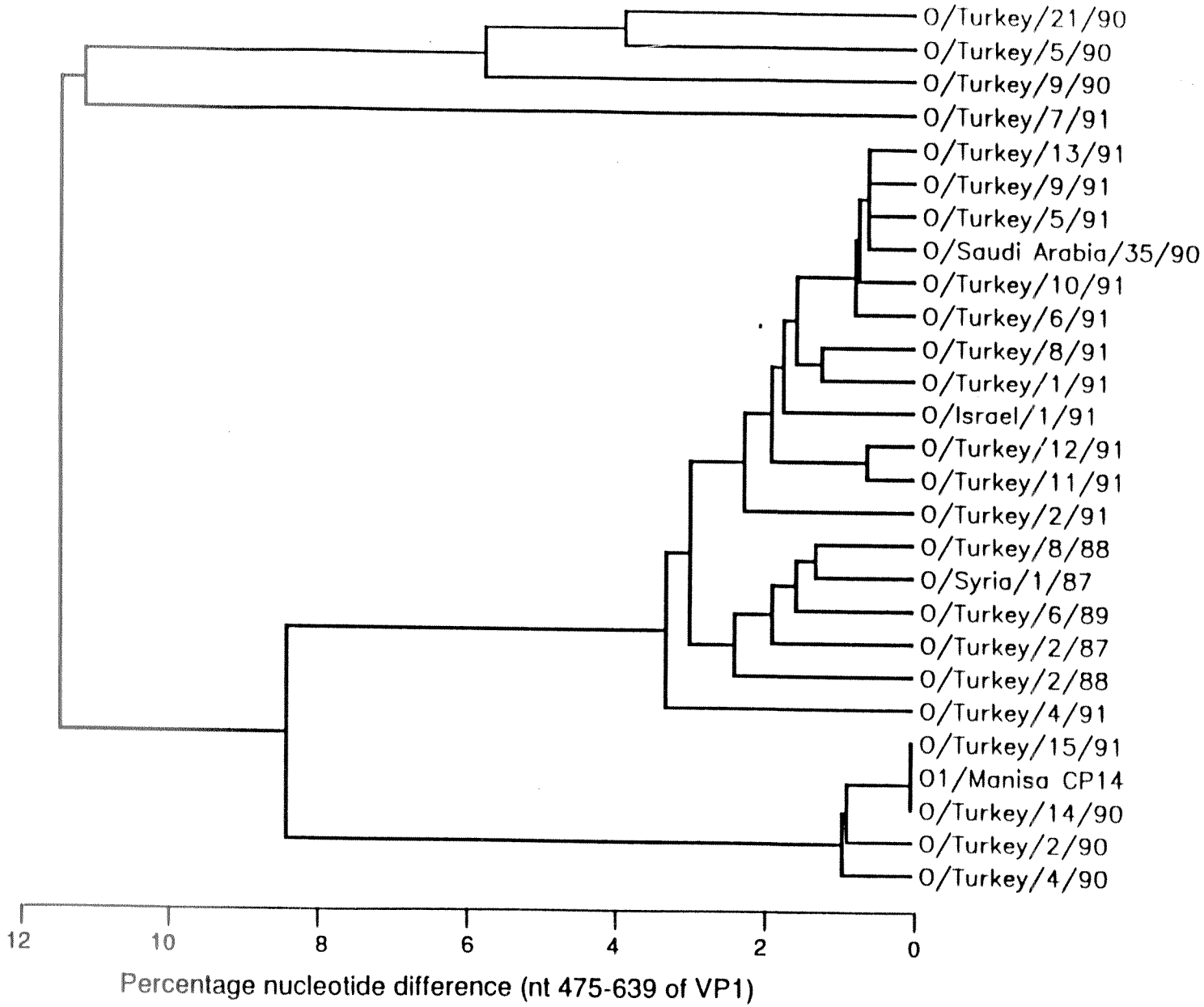

Figure 2. Dendrogram depicting the relationships between type O viruses isolated from outbreaks of FMD in Turkey

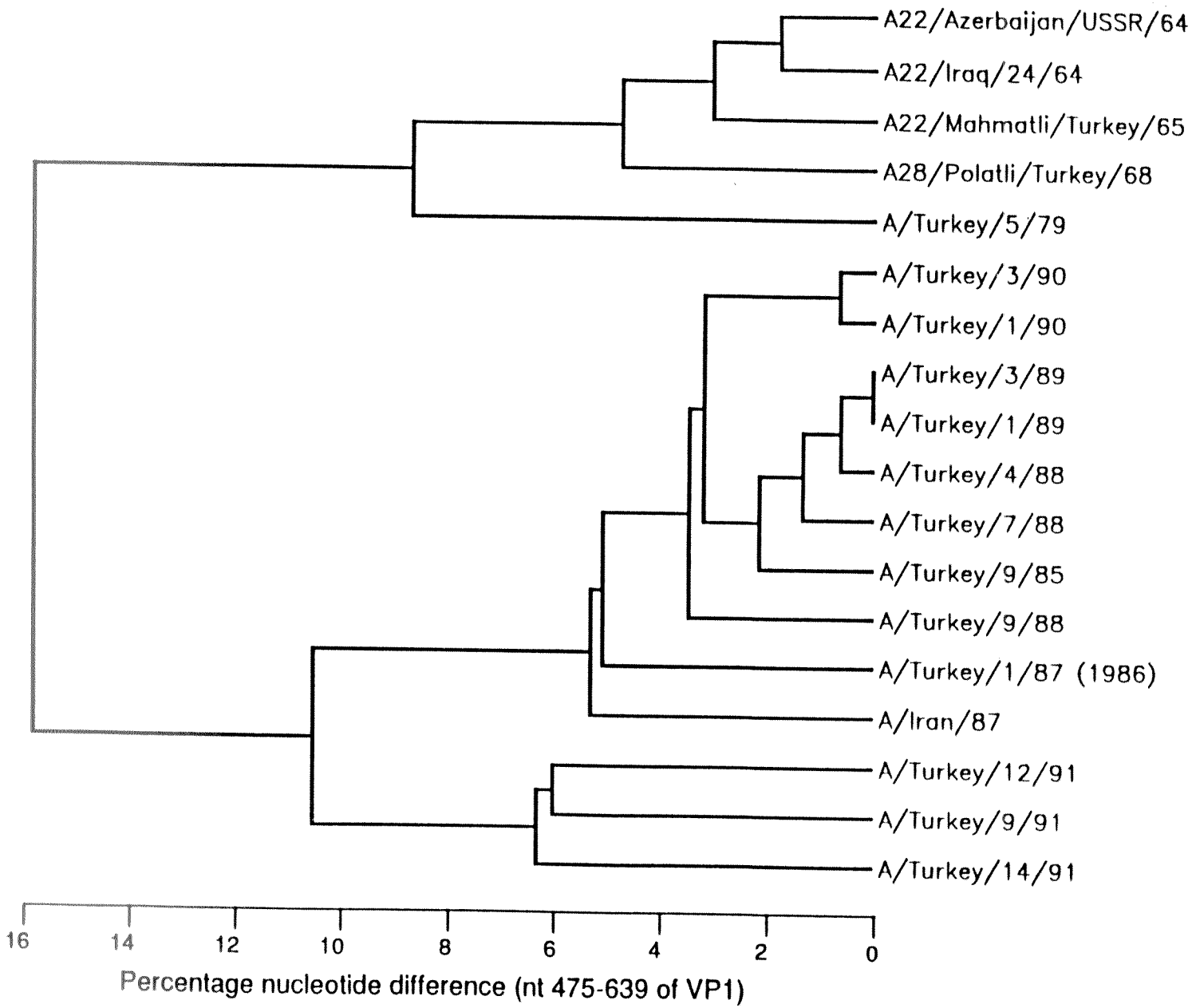

Figure 3. Dendrogram depicting the relationships between type A viruses isolated from outbreaks of FMD in Turkey

Examination of Bovine Semen Samples for Recovery of FMD Virus

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Bulls experimentally infected with foot-and-mouth disease virus (FMDV) may shed the virus with semen as early as 12 hours after infection, and excretion may continue for more than 10 days (Cottral et al., 1968; Donaldson and Sellers, 1983). Although bulls in artificial insemination (AI) stations are carefully supervised and FMD-vaccinated bulls have never been reported to be virus carriers it is a requirement for semen export to non-vaccinating EEC member states that a sample of diluted semen comprising up to 10 percent of an ejaculate is examined for freedom from FMDV (EEC directive 88/40/EEC of 14.06.1988).

Because information on recovery of FMDV from semen at a low level of contamination is scarce we examined small samples of semen destined for export to which a defined amount of virus had been added in a cell suspension plaque assay in BHK-21-CT cells. It had previously been shown that these cells are very sensitive to FMDV of type O, A, and C. Mainly virus of strain O_1 Kaufbeuren was used at a dilution that we could expect about 30 PFU in 0.1 ml in control dishes (6 cm diameter). To simulate field conditions, 0.1 ml portions of semen to which diluted virus had been added were immediately mixed with 0.9 ml of cell suspension and plated. Virus prediluted in buffer and a virus titration were included in each test as controls. Incubation took place at 37 °C for two days. Plaques were counted and percent recovery calculated. Results are shown in Tables 1 and 2.

Most studies were performed with semen from two bulls, Manitu and Voya. It can be seen that recovery was never 100 percent as deduced from controls with virus diluted in buffer. In general, recovery was higher from semen diluted 1 in 2 before addition of virus. This procedure is now preferred over detection of virus in undiluted semen, although expenditure of work is increased. A few semen samples from other bulls indicate that the percentage of recovery depends on the individual animal. We were informed that the extender buffer systems varied only little among different AI stations. The pH values were always in the physiological range. Surprisingly, virus strain A_5 Bernbeuren was more sensitive to inactivating factors in semen than O_1 Kaufbeuren. This was confirmed in a more detailed experiment with semen from bull Manitu (Table 2). Virus O_1 K and A_5 B, respectively, was added at three different dilutions to semen diluted 1 in 2. Recovery of virus O_1 K was in the range expected, but from virus A_5 B only few PFU were recovered. In this experiment, detection of virus specific RNA by polymerase chain reaction (PCR) was examined in parallel. Specific RNA was only detected in semen samples containing virus at the lowest dilution used. It should be considered that this was only a preliminary experiment, and technical details of PCR may well be improved.

In summary, it has been shown that the cell suspension plaque assay with BHK-21-CT cells is a sensitive method to detect a low level of contamination of semen samples with FMDV and that extended semen has an inactivating effect on this virus.

References:

1. Cottral, G.E., Gailiunas, P. and Cox, B.F. (1968). Arch. ges. Virusforsch. 23, 362-377.

2. Donaldson, A.I. and Sellers, R.F. (1983). Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and- Mouth Disease, Lelystad. FAO, Rome.

Table 1. **Examination of bovine semen samples for recovery of FMD virus**

Date	Bull	Recovery in % semen undil. +FMDV 0,K	semen 1:2 +FMDV 0,K	PFU/0,1 ml in test	virus titre log/0,1 ml
19.07.89	Manitu				
		49	61	41	8,09
		48	65		
		46	65		
02.08.89	Manitu				
	89281	57	53	55	8,22
	89282	47	63		
	89291	51	61		
	89292	50	62		
	89301	59	61		
14.08.90	Manitu				
	90281	34	75	34	8,01
	90282	44	72		
	90292	35	73		
	90301	32	62		
	90302	27	67		
24.09.90	Manitu				
	90351	64	71	30	7,95
	90352	63	74		
	90361	57	72		
03.01.91	Manitu				
	90501	54	70	20	7,79
	90511	69	72		
27.09.89	Voya	11	27	12	7,51
26.06.90	Voya				
	9-262	49	54	11	7,45
	9-268	41	46		
	9-271	20	14		
	9-275	33	41		
27.06.90	Voya				
	9-282	55	54	10	7,50
	9-296	62	54		
	9-298	45	36		
03.07.90	Voya				
	9-286	46	63	19	7,75
	9-289	26	43		
	9-300	18	45		
	9-303	29	38		
04.07.90	Voya				
	9-305	21	34	20	7,78
	9-307	35	51		
	9-320	41	67		

Table 1 (contd.)

Date	Bull	Recovery in % semen undil. +FMDV 0_1K	semen 1:2 +FMDV 0_1K	PFU/0,1 ml in test	virus titre log/0,1 ml
17.07.90	Voya				
	9-310	55	62	38	8,06
	9-312	55	59		
	9-314	54	49		
	9-328	58	66		
	9-331	58	59		
	9-333	54	61		
	9-335	49	53		
24.07.90	Voya				
	9-338	83	79	36	8,04
	9-340	81	95		
	9-345	68	78		
	9-347	64	81		
	9-352	68	78		
	9-354	61	63		
	9-356	60	67		
27.04.89	Erz	34	46	12	7,51
24.04.90	Steger				
	7-177	57	59	32	7,97
	Vetter				
	7-036	65	79		
	7-040	63	84		
07.08.90	Sixtus				
	90179	28	88	27	7,89
	90183	15	75		
	90200	29	85		
	90204	30	69		
	90207	29	61		
20.09.89	Schnipp	3	63	36	7,73
	Tober	3	56		(A_5 Bern-beuren)

Table 2. **Detection of FMDV in semen of bull "Manitu"**

1. Virus O_1K, titre $10^{9.00}$ per ml

a)	at virus dilution	1: 3,000,000	expected	33	PFU/dish	
		1: 9,000,000		11	"	
		1:30,000,000		3	"	
b)	virus diluted in buffer	1: 3,000,000	recovered	30	PFU/dis	= 90 %
		1: 9,000,000		10.8	"	= 97 %
		1:30,000,000		4.5	"	= 136 %
c)	virus diluted in semen	1: 3,000,000	recovered	24	PFU/dish	= 72 %
		1: 9,000,000		8.4	"	= 76 %
		1:30,000,000		2.3	"	= 68 %

2. Virus A_5B, titre $10^{8.88}$ per ml

a)	at virus dilution	1: 1,500,000	expected	50	PFU/dish	
		1: 4,500,000		17	"	
		1:15,000,000		5	"	
b)	virus diluted in buffer	1: 1,500,000	recovered	>30	PFU/dish	
		1: 4,500,000		18.5	"	= 109 %
		1:15,000,000		5.1	"	= 101 %
c)	Virus diluted in semen	1: 1,500,000	recovered	0.3	PFU/dish	= 0.6 %
		1: 4,500,000		0.3	"	= 1.8 %
		1:15,000,000		0.05	"	= 1.0 %

APPENDIX 8

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