FOOT-AND-MOUTH DISEASE (FMD)
COURSE MANUAL

european commission for the
control of foot-and-mouth disease

PROGRAM- NTC

2011 – EuFMD: NTCs-Approximate program

Day 0 - Sunday Departure for Nakuru -14.00. Short meeting of trainers and co-trainers
Day 1 – Monday
Welcome; intro of participants, Introduction to Course, organisation of the week. Explanation of the 3 Teams and Roles facilitated by Team Leader or EuFMD Rep
Epidemiology /control, of FMD in Kenya
What is special about FMD investigations? Relevance of FMD pathogenesis, clinical signs, lesion ageing, kinetics of infectivity and transmission, to tracing FMD spread
Sampling and Diagnostic procedures for FMD (include probang sampling in field)
Principles of dangerous contact risk assessment
If time Lessons learnt in FMD investigation – UK 2007
Afternoon: planning the field investigations
Setting up laptops for internet. Demonstration of the EuFMD Training Wikispace- Online
Group work exercises **Epi-team**: decide on roles , main lines of investigation, questions, gathering data on the suspect outbreak circumstances **Clinical Team**: decide roles, gathering data, decide on forms, roles, organisation **Informatics Team**:–assist with data required on recent outbreaks, village data
Feedback, Output: agree on outbreak to investigate, summarise tasks and roles, forms to be completed
Biosecurity talk – both optimal (EU) and pragmatic
Day 2 – Tuesday - Travel to field sites
Supervised Clinical team work; Supervised Epi team work
Decide on plans for Wedns/facilitated by Team leader
Day 3 – Wednesday
As decided on Day 2, new investigation or follow up previous one, or work on samples/findings from Day 2;
Day 4 – Thursday
AM1: lab findings reported followed by
Group work – finalising /assembling the Clinical and Epi Report, and Summary
AM2: Present and discuss Clinical and Epi Report
PM: Case Study or Review Lessons Learnt
Facilitated Feedback /evaluation of the Course, tips for improving
Final biosecurity talk/declaration/actions
Day 5 - Friday
Visit to national park Back to Nairobi, arrival time 17.00

Team work and roles – Nakuru real-time FMD Training Course

February 2011

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Harambee

- Participants, both international and Kenyan, will be expected to work together (Harambee) in planning the field work, during field visits, and analysing and reporting results.

The three main teams are:
1) Support teams - Diagnostics and informatics
2) Clinical investigation team
3) Epidemiology and tracing team

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Support teams

- Diagnostics
  - prepare and assist sampling (materials, equipment)
    - local (Nakuru/Embakasi) diagnostic laboratory participants
    - provide equipment/materials for sampling
    - undertake and report lab tests (at least Svanodip penside test, NSP ELISA)
- Informatics
  - Department of Vet Services staff –answer enquiries on FMD outbreaks, village demographics, animal movements
    - usually District Veterinary officers
- mapping/GIS, reporting tools
  - mapping: all participants can do – Google Earth
  - collaborative reports: can use the online tool (wikispace)

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Clinical investigation team

- prior work:
  - population structure of farm/village, outbreak history
  - agree clinical/sample recording forms (and BS on paper taken off-site) (who)
  - photographs needed/cataloguing (who)
- at site:
  - select animals for clinical examination, with 2 aims:
  - find the freshest (youngest) lesion - collection of samples to confirm or refute FMD
  - find the oldest lesion/prove infection (it may not be among the presented animals!)
    - define period of virus entry
  - form filling: table of affected animals, sample and test results
  - photographic evidence: link to each case and test result
- output
  - summarize evidence of infection (clin and lab)
  - photographic evidence/catalog, with link to animal case/ear tag
  - define entry period; draft sections of the final report
  - recommendations may include immediate actions

TEAM/roles include: Team Leader/chief Reporter, recorder (at field site), photographer etc

Example: Examination form – records signs, the photograph numbers, lesion age

Clinical Examination Form

Name of the Farmer: Zafer Altay - Room 3 Farm ID: …….
Epidemiological unit: Yagmurcak village Date: 13. 10. 2009

Animal ID	Species	Type	L/S/M/PD/A	Temp.	Vesicular Lesions Y/N	Age of Lesions	Samples Tissue/Blood/Serum	Number of Photos	Comments
TR 25967577, No. 10	bov.	cow	-	n.m.	N		S (No. 4)		Vaccinated in April 09
no eartag, No. 11	bov.	calf	-	n.m.	n.e.		-		
no eartag, No. 12	bov.	calf	L/S	40,5°	Y (M + F)	> 7 d	S (No. 3), Saliva No. 3	SANYO 676, 678	Respiratory symptoms
no eartag, No. 13	bov.	calf	-	n.m.	Y (M)	> 7 d			
no eartag, No. 14	bov.	calf	-	n.m.	Y (M)	> 7 d			Very small lesions

L: … Lameness, S: … Salivation, M: … Drop of Milk Yield, PD: … Perinatal Death, A: ……Abortion

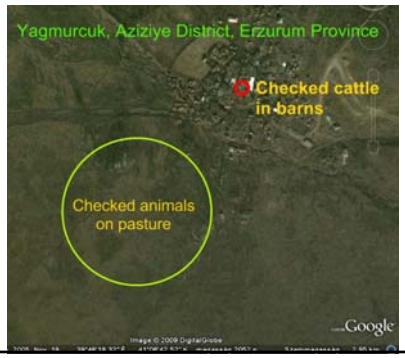
Estimated age of oldest lesion seen: > 7 d

from OLDEST lesion: work out time window when infection entered group

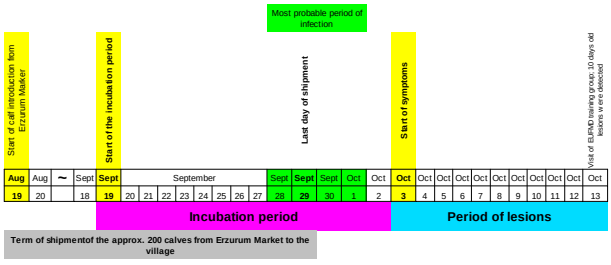
Epidemiology/Tracing Team

- tasks
- pre-visit:
  - identify recent outbreaks of relevance in region (location and time before present)
  - identify procedure on site (team leader, reporter, questions/questionnaires to use)
- at site:
  - undertake group and where required individual interviews
  - use participatory interview methods where needed to identify recent and longer term FMD events and risk factors/entry/spread routes
  - sketch out village entry/exit and boundaries;
  - identify animal groups in village that may be at different risk/history
- after return:
  - make source and spread windows, time-line
  - identify possible and mostly likely source
  - identify possible spread
  - recommend follow-up actions
- output: immediate report (to group), and follow-up written sections for final report
- TEAM/roles include: Team Leader, chief Reporter, interviewer & recorders (at field site), mapper (of field site) and GIS (for report), photographer etc

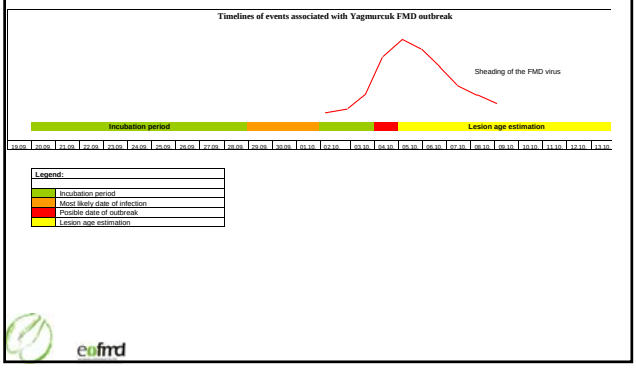
Example: use of Google Earth to illustrate where the clinical team sampled animals



Example - Timeline of the events identified in Yagmurcuk village, Turkey – based on oldest lesion found



Example of time-line of events with in red, a schematic representation of how FMD virus shedding by infected animals built up then declined in the village



FMD-OVERVIEW

FMD-overview

Kenya, 15th-19th November 2010

Nick Juleff, Institute for Animal Health

Overview

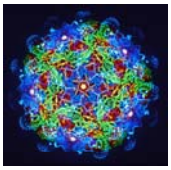
- Introduction to FMD
- Pathogenesis
- Kinetics of infection and transmission
- Clinical signs
- Vaccination

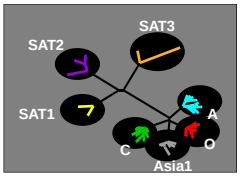
Foot-and-mouth disease

- highly contagious, acute vesicular disease of cloven-hoofed animals
- Economically significant
  - Taiwan 1997: US$1.6 billion
  - UK 2001: £3.1 billion direct, £3.6b indirect
  - UK 2007: no official figure yet; ~£100m estimate (Defra)
  - Japan 2010: ? (~£1.7 billion)
- Direct production losses
- Major barrier to trade in animals and products
- FMD is not considered a public health problem (Acha and Szyfres 1987)

Aetiology

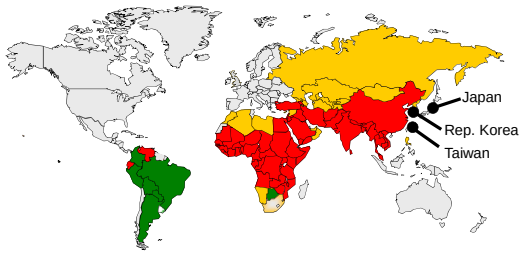
- Foot-and-mouth disease virus (FMDV)
- Small, non-enveloped RNA virus
- Family picornoviridae, genus *Aphthovirus*





Seven immunologically distinct serotypes

Conjectured status for FMD (2009/10)



Don King (IAH)

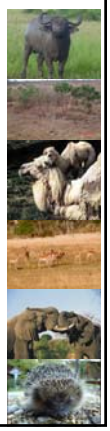
http://www.wrlfmd.org/fmd_genotyping/

Susceptible species

- FMD affects mainly ruminant animals and pigs, but there are over 70 animal species known to be susceptible, to a greater or lesser degree, to the disease.
- Cattle, water buffalo, pig, sheep and goat.

Other species and wildlife animal

- **Yaks** and **wild bovidae** are susceptible. Infection is common in **African buffaloes** but is subclinical. These animals may be a source of infection for cattle.
- **Wild pig** species are also susceptible.
- **Camelidae** (camels, llamas, alpacas, vicunas) have a low order of susceptibility and any infections are likely to be subclinical.
- Many **deer** and **antelope** species are susceptible and UK deer can transmit to farm animals. All such species should be regarded to be susceptible until proven otherwise. In Africa, clinical FMD observed in **impala** and **greater kudu**.
- **Elephants** can be experimentally infected.
- Natural disease has been observed in European **hedgehogs** during past outbreaks in the United Kingdom. Several other **small rodents** and other mammals, e.g. coypu and agouti, have been shown experimentally to be susceptible but probably play no part in the epidemiology of the disease.



Overview

- Introduction to FMD
- Pathogenesis
- Kinetics of infection and transmission
- Clinical signs
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Pathogenesis of FMD

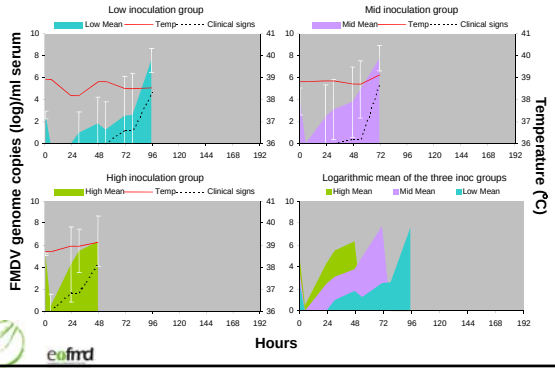
- Incubation periods:
  - 2-14 days in individual animals, but dose related
  - 2-5 days most common
  - but may be as short as 24 hours

Importance of dose – experiment design

FMDV O UKG 34/2001 (PanAsia strain)

- Three groups of 4 pigs
- Each group placed in a separate isolation room
- Intravenous inoculation
  - High inoculation group - $10^{5.9}$ $TCID_{50}$
  - Medium inoculation group - $10^{4.9}$ $TCID_{50}$
  - Low inoculation group - $10^{3.9}$ $TCID_{50}$

Importance of dose – results

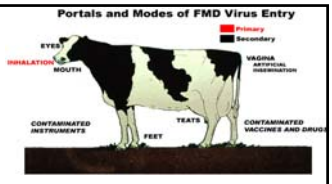


Routes of Infection

- Cattle
- Sheep mainly inhalation
- Goats
- Pigs - inhalation and/or ingestion
- Through skin and mucosae - all species

  Direct or indirect (mechanical/fomites) contact with infected animals, contaminated animal products, or by airborne virus

Virus entry



Respiratory system
- major route of infection in ruminant animals
- **Cattle and sheep: very small doses** of virus can initiate infection. (10-25 TCID50).
- Pigs: require about 600 times more FMDV by this route but are major generators of infected aerosols

NB: aerosols are mainly generated by exhaled breath, but can also result from such things as **splashing**, **exhaust air from milk tanks**, **use of pressure hoses**

Oral infection
- Much higher virus doses are required to infect animals by the oral route
- Ruminant animals are seldom infected naturally by this route (10^5 TCID 50 for cattle).

Pigs: frequent route

Other routes

Virus may also enter through **lesions in the skin or mucosa** (Concrete, grass seeds, feeding on rough fodder, **foot-rot**, trauma from milking machines...)

AI, vaccines and drugs

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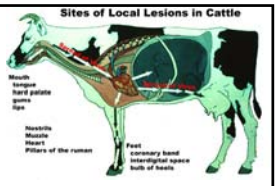
Virus spread in the infected animal



- The **oropharynx** is considered as the usual tissues for initial FMD virus multiplication
- Virus can be detected in the **pharynx for 1-3 days before** the onset of **viraemia** and **clinical signs**

•The virus is then transported in blood/lymphatics?

- Secondary sites of virus multiplication in cornified, stratified squamous epithelium
  - where the vesicular lesions quickly develop
  - viral amplification
  - virus also multiplies in the myocardium

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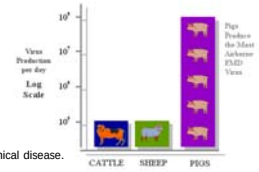
Virus excretion

- Large quantities in expired air
  - Pigs liberate vast quantities of airborne virus in their expired breath.
    - An infected pig : up to 400 million TCID50 /day,
    - ruminant animals excrete a maximum of only 120,000 TCID50/day
- In all secretions and excretions
  - ruptured vesicles (
  - Lacrymal secretions (
  - Saliva (cattle: 10^6 to $10^{8.8}$/ml ID50)
  - Urine (cattle: $10^{2.5}$ to $10^{6.5}$/ml ID 50)
  - Faeces (cattle: 10^2 to $10^{3.3}$/ml ID50)
  - milk (cattle: 10^3 to $10^{4.5}$/ml ID50)
  - Semen



- Excretion of virus can begin up to four days before clinical disease.
- Most virus excretion ceases about 4-5 days after the appearance of virus vesicles, when antibodies develop, except in the oesophogeal-pharyngeal fluid

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FMDV in serum, secretions and body fluids

Cattle infected by indirect contacts with pigs

	Days											
	-4	-3	-2	-1	0	1	2	3	4	5	6	7
Serum						+++						
Pharygeal fluid					+++							
Oral saliva					+++							
Nasal secretion					+++	+++						
Lacrymal secretion						+++	+++					
Urine					+++							
Feaces					+++							

Challenge

Clinical signs

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Development of immunity against FMDV

- Immunity to FMD is primarily mediated by antibodies
- 3 to 5 days from the first appearance of clinical signs, circulating antibodies detected by ELISA
- High levels of antibodies are reached 2 to 4 days later
- The antibody titre normally stays at a relatively high level for many months after infection and may remain detectable for several years in ruminants
- In pigs, especially in fast-growing young animals, antibodies may only be detectable for a few months

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FMDV persistence

- Antibodies appear rapidly and clear virus from most sites
- FMD virus can persist to 28 days and beyond in the oropharynx of ruminants but not pigs – so called "persistently infected" or "carrier" animals
- up to 50% of ruminant animals become persistently infected after clinical recovery
- occurs irrespective of the immune status of the animal
- The length of persistence is species-dependant

  Cattle up to 3.5 years

  Sheep up to 9 months

  Goats up to 4 months

  African buffalo at least 5 years
- Virus "excretion" is intermittent, low level and declines over time (virus recovery from probang/pharynx samples)

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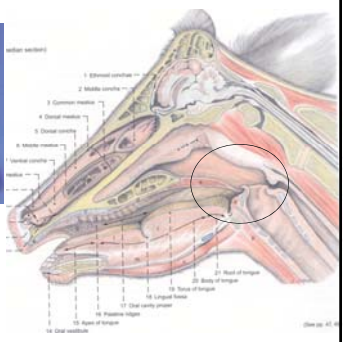
Probang sample



Bovine Anatomy, Budras et al

Significance of Persistently Infected Animals

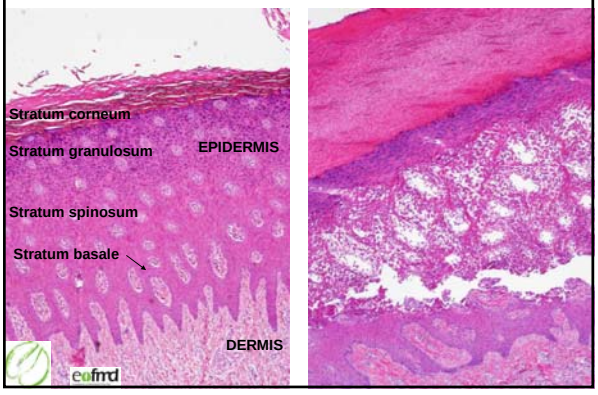
- **Controversial.** Lack of consensus underlies many disagreements on control policies
- Spread from carriers is certainly **rare**
  - it has been documented in the field e.g. in the UK in the 1920's when total stamping out was suspended and from African buffalo
  - It has **not** been reproduced in **cattle** under **experimental conditions**
  - **Carrier African buffalo can transmit FMDV** to naïve cattle under experimental conditions
- FMD-free trading partners adopt the precautionary principle and maintain embargoes because of carriers and their potential risk

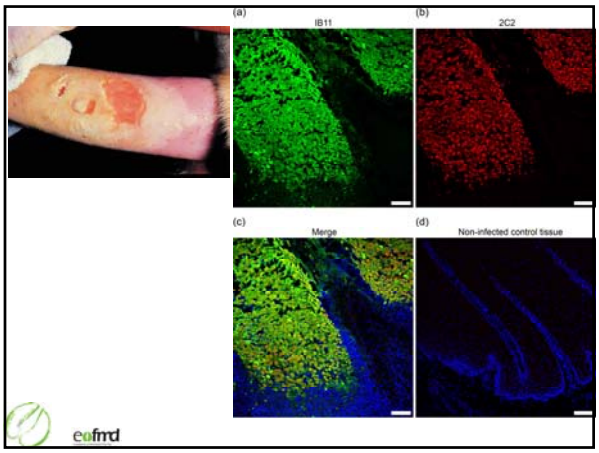
Overview

- Introduction to FMD
- Pathogenesis
- Kinetics of infection and transmission
- Clinical signs
- Vaccination

Diagnosis of FMDV based on epidemiologal and clinical findings

- Highly contagious
- Fever: dull, depressed, anorexia, loss of condition
- Reduction in milk yield
- Nasal discharge (serous to mucopurulent)
- Vesicles:
  - Mouth (+/- salivation)
    - Examination must include gingiva, gums, tongue, buccal cavity and nares
  - Feet (+/- lameness)
    - Examinations must include coronary band, interdigital space, bulbs of heel, accessory digits and pressure points of the limb (important in pigs)
  - Mammary glands
- Death in young animals (abortion, myocarditis)




(a)
IB11
(b)
2C2
(c)
Merge
(d)
Non-infected control tissue
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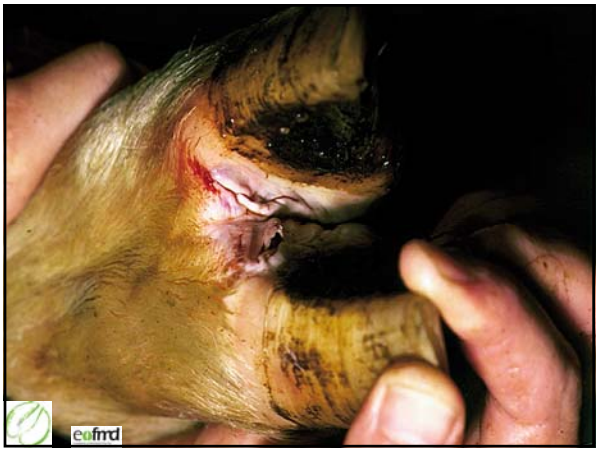
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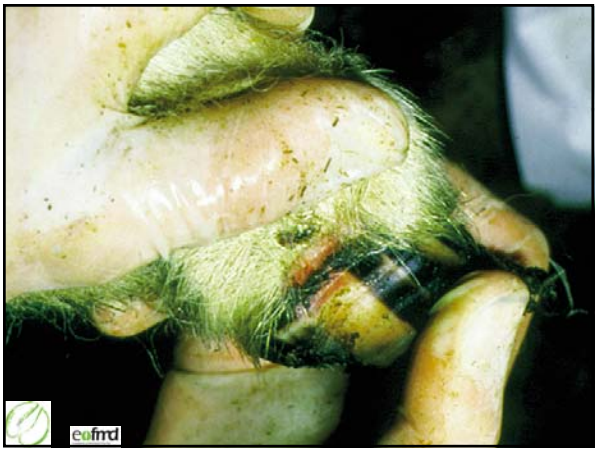
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FMDV O Lausanne
2 days old lesions
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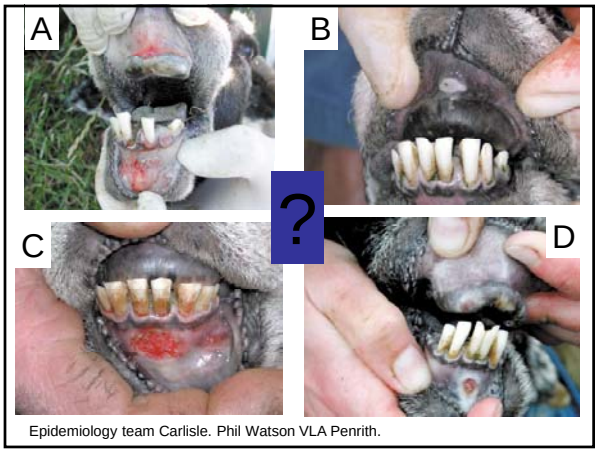
Differential Diagnosis

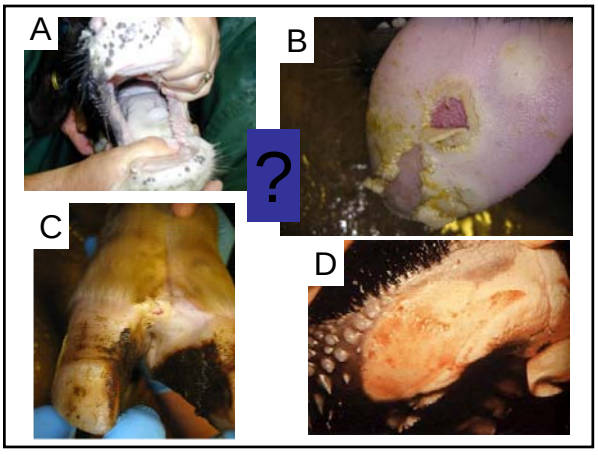
- infectious diseases
  - Swine vesicular disease
  - Vesicular stomatitis
  - vesicular exanthema of swine (calicivirus)
  - Rinderpest
  - Bluetongue
  - Peste des petits ruminants
  - BVD/mucosal disease
  - Bovine papular stomatitis, ORF (parapoxvirus)
  - Bovine ulcerative mammilitis
  - Pseudocowpox
  - Malignant catarrhal fever
  - Contagious ecthyma ('scabby mouth')
  - Infectious bovine rhinotracheitis/infectious pustular vulvovaginitis
  - Dermatophilosus infection

Differential Diagnosis (cont)

- Dermatitis
  - Scalding, wetting, contact dermatitis, photosensitisation
  - Phytophotodermatitis-contact with plants containing furocoumarins
- Trauma
  - Rough grazing (sheep!)
- lameness
  - hoof abscess, footrot, bad flooring, new concrete, mud



Epidemiology team Carlisle. Phil Watson VLA Penrith.



OMAGODS

FMD ?

Rough Grazing

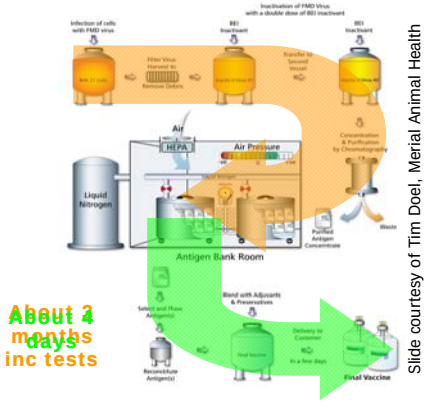
Overview

- Introduction to FMD
- Pathogenesis
- Kinetics of infection and transmission
- Clinical signs
- Vaccination

All licensed FMD vaccines are killed

- Grown in suspension cultures
- Inactivated by binaryethyleneimine
- Purified, remove NSP (mostly!)
- Adjuvant
  - Alhydrogel and saponin
  - or oils to form a single or double oil emulsion
- Allowed for but unlikely to be used for economic reasons
- If vaccination used, it takes longer to regain “FMD-free” trading status (3 months vs. 6 months)

- Specific for each strain and short duration of immunity
- Does NOT prevent infection
- vaccinated animals can be sub clinically and persistently infected
- Policy “uncertainty” is reducing as experience and validation of NSP antibody assays increases



Slide courtesy of Tim Doel, Merial Animal Health

FMD Vaccines – Antigenic Diversity

FMD Type	Extent of diversity
O	2 main lineages: South America and 'Old Europe'; Middle East and Asia; several recognised vaccine strains (Manisa, BFS, Campos). The most prevalent serotype
A	High antigenic diversity. New antigenic variants emerge frequently. Second most prevalent serotype.
C	Limited antigen diversity and very restricted geographical distribution
SAT 1-3	Highly genetically diverse. Limited endemic range with periodic excursions.
ASIA1	Limited antigenic diversity. Middle East and Asia

Acknowledgements

- David Paton
- Eoin Ryan
- Don King
- Colleagues at the IAH

KINETICS OF INFECTION AND TRANSMISSION

Kinetics of infection and transmission

Kenya, 15th-19th November 2010

Nick Juleff, Institute for Animal Health

Overview

- Infection
- Incubation period
- Kinetics of infection
- Virus emission
- Transmission windows

Routes of Infection

- Cattle
- Sheep — mainly inhalation
- Goats
- Pigs - inhalation and/or ingestion
- Through skin and mucosae - all species

  Direct or indirect (mechanical/fomites) contact with infected animals, contaminated animal products, or by airborne virus

Estimated minimum doses* for various species and routes of exposure

Species	Inhalation	Intradermal	Intramuscular	Nasal instillation	Oral
Cattle	10	100	10^4	10^4-10^5	10^3-10^6
Sheep	10	100	10^4	10^4-10^5	10^3-10^6
Pigs	>800	100	10^4	Unknown	10^6-10^7

From Alexandersen S. *et al.* 2003

* Estimated minimum doses reported to cause clinical disease in TCID50

Overview

- Infection
- Incubation period
- Kinetics of infection
- Virus emission
- Transmission windows

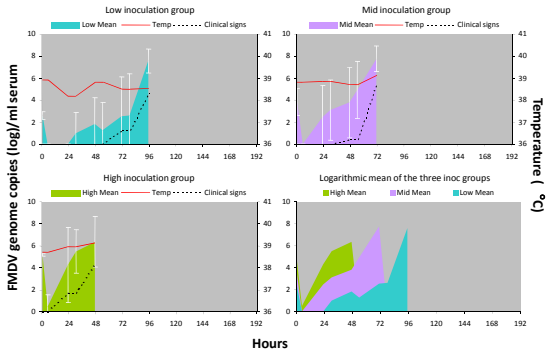
Incubation period

- Incubation period: 1–14 days
  - most common 2-5 days
- Dose-related: low dose → longer incubation
- Importance of dose – experimental design

FMDV O UKG 34/2001 (PanAsia strain)

- Three groups of 4 pigs
- Each group placed in a separate isolation room
- Intravenous inoculation
  - High inoculation group - $10^{5.9}$ $TCID_{50}$
  - Medium inoculation group - $10^{4.9}$ $TCID_{50}$
  - Low inoculation group - $10^{3.9}$ $TCID_{50}$

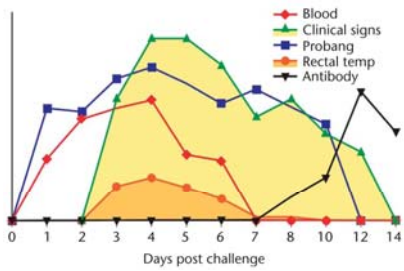
Importance of dose – results



Overview

- Infection
- Incubation period
- Kinetics of infection
- Virus emission
- Transmission windows

Kinetics of infection



- Pictorial representation of data accumulated from a number of cattle challenge experiments
- Clinical signs; 2 to 3 days post contact challenge
- Peak rectal temperatures of approx 41°C
- Animals are debilitated for 4 to 5 days, usually from 7 days onwards lesions are healing
- Viraemia for approx 7 days. Typical peak value 10^7 to 10^8 TCID50/ml
- Probang; typical peak value 10^7 to 10^8 TCID50/ml. May be detectable in carriers for months!
- Antibody detected in first week post infection. Neutralizing antibody from day 7

Overview

- Infection
- Incubation period
- Kinetics of infection
- Virus emission
- Transmission windows

Virus emission

- Vesicular lesions and skin
- Breath, milk; faeces; semen
- All secretions and excretions; all tissues during acute phase
- Bone marrow and lymph nodes in carcass meat (drop in pH after death inactivates virus in skeletal muscles)
- Change in pH in pig meat is variable and has not been documented for sheep meat.

Virus emission

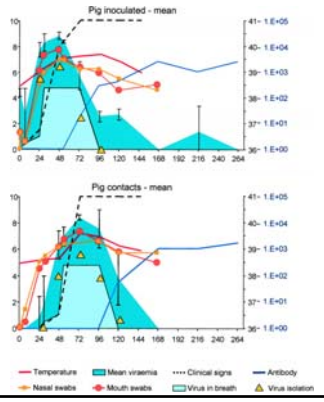
- Ruptured vesicles (> 10^9 /ml ID50)
- Lacrymal secretions (cattle: $10^{6.1}$ to $10^{7.0}$/sample ID50)
- Saliva (cattle: 10^6 to $10^{8.8}$/ml ID50)
- Urine (cattle: $10^{2.5}$ to $10^{5.5}$/ml ID 50)
- Faeces (cattle: 10^2 to $10^{3.3}$/g ID50)
- Milk (cattle: 10^3 to $10^{4.5}$/ml ID50)
- Semen: variable in quantity and in duration

Excretion of virus can begin up to four days before clinical disease

Virus emission

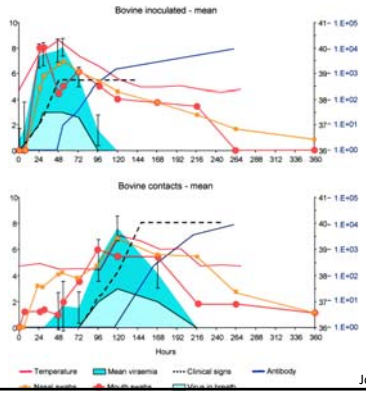
- Airborne virus mainly from the exhaled breath of infected animals as droplets and droplet nuclei (pigs)
- Originates initially from the upper and later from the lower respiratory tract (?)
- The precise mechanism of release has not been determined
- Also potential for generation of aerosols from virus containing fluids or dried material, for example from milk tankers, urine, faeces
- quantitatively a minor role compared to breath

Virus emission – pigs



John Gloster IAH

Virus emission - cattle



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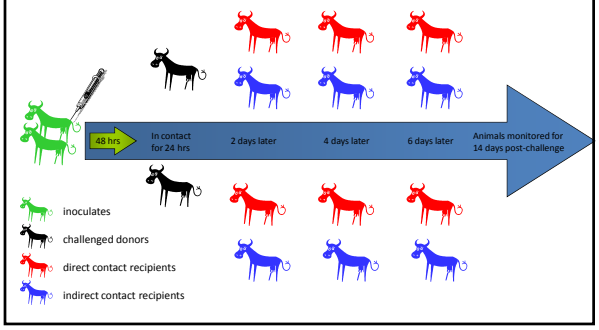
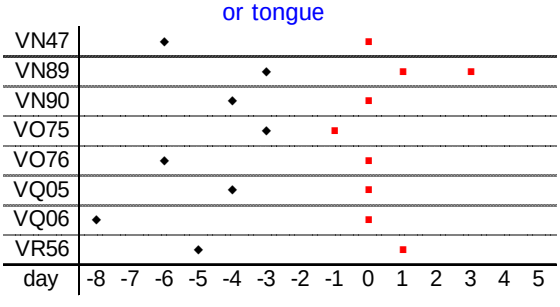
Overview

- Infection
- Incubation period
- Kinetics of infection
- Virus emission
- Transmission windows

Defining and quantifying the period of infectiousness in cattle experimentally infected with type O FMDV

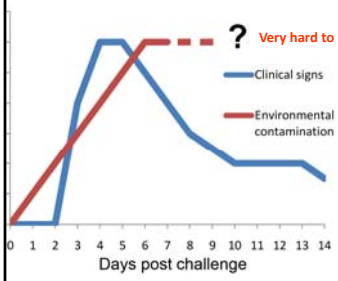


Challenge and transmission events in relation to the first clearly visible lesion in oral cavity, nose or tongue



Day 0 – first day clinical signs were observed
◆ – day of challenge, ■ - transmission event

Infected premises



Up to:
- 20 weeks on hay/straw
- 4 weeks on cow's hair 18 – 20°C
- 14 days dry faeces
- 39 days in urine
- 6 months in slurry in winter
- 3 days on soil in summer & 28 days in autumn
- Snow-covered soil: More than 185 days
- survival decreases with increasing heat and decreasing humidity

Bartley LM, Donnelly CA, Anderson RM, Review of foot-and-mouth disease virus survival in animal excretions and on fomites. Vet Rec 2002 Nov 30;151(22):667-9

Acknowledgements

- John Gloster
- David Paton
- Colleagues at the IAH

AGEING OF LESIONS

Ageing of lesions

Kenya, 15th-19th November **2010**

Nick Juleff, Institute for Animal Health, Pirbright

Why is this important?

- Age range of lesions-particularly the oldest lesion
  - Essential for case history and epidemiological report
  - Pre-requisite to determine origin of infection
  - Duration and weight of virus excretion
  - Prediction of further spread

Estimating the age of lesions

Day of Clinical Disease	Appearance of lesion
Day 1	Blanching of epithelium followed by formation of fluid filled vesicle
Day 2	Freshly ruptured vesicles characterised by raw epithelium, a clear edge to the lesion and no deposition of fibrin
Day 3	Lesions start to lose their sharp demarcation and bright red colour. Deposition of fibrin starts to occur.
Day 4	Considerable fibrin deposition has occurred and regrowth of epithelium is evident at the periphery of the lesion.
Day 7	Extensive scar tissue formation and healing has occurred. Some fibrin deposition is usually still present.

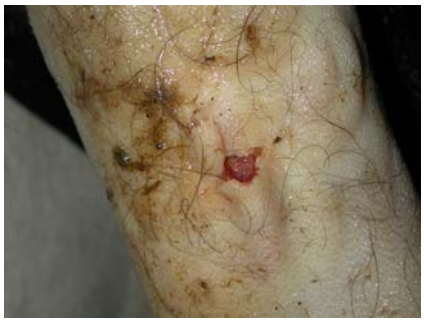
Estimating the age of lesions

- Photographs of contact exposure – field and experimental
- Clinical manifestation may vary between virus strains –
  - Different affinity
  - especially sheep (lesions may be too transient for gauging time of infection)
- Complicated by secondary infections
- Between day 0 and 5 – one day margin of accuracy

Cattle - Day 1

Unruptured tongue vesicle, fluid filled, blanching of epithelium

Cattle - Day 1



One day old vesicle, ruptured when tongue drawn from mouth

Cattle - Day 2

Note raw epithelium, clear edge to lesion and no deposition of fibrin

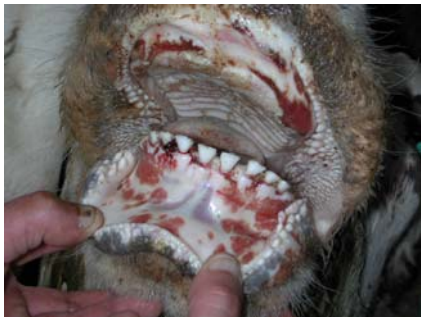
Cattle - Day 2

Field example. Note raw epithelium, clear edge to lesion and no deposition

Cattle - Day 2

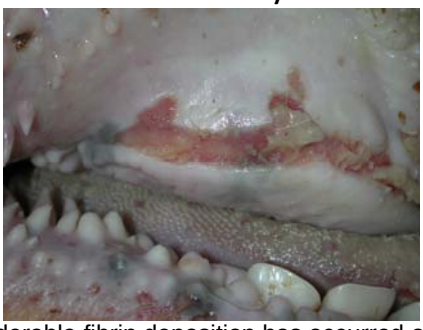
No raw epithelium, clear edge to lesion and no deposition of fibrin

Cattle - Day 3



Lesions start to lose their sharp demarcation, fibrin deposition starts

Cattle - Day 4



Considerable fibrin deposition has occurred and regrowth of epithelium is evident at edge of lesion

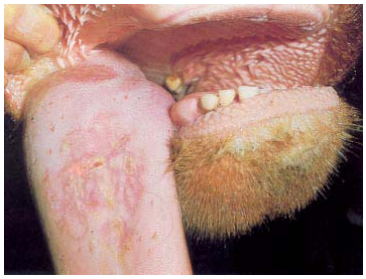
Cattle - Day 5

Note progressive loss of lesion margination and extensive fibrin infilling

Cattle - Day 7

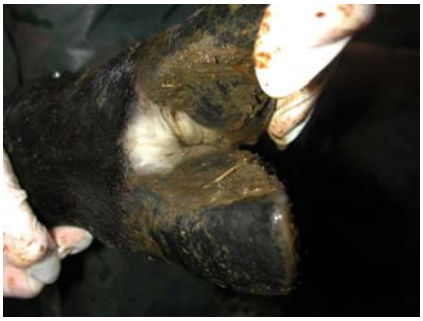
Field example. Extensive scar tissue formation and healing has occurred. Some fibrin deposition is usually still present

Cattle - Day 10



Scarring and indentation at site of lesion, fibrous tissue proliferation, loss of papillae

Cattle - Day 1



Unruptured vesicle, fluid filled, blanching of epithelium

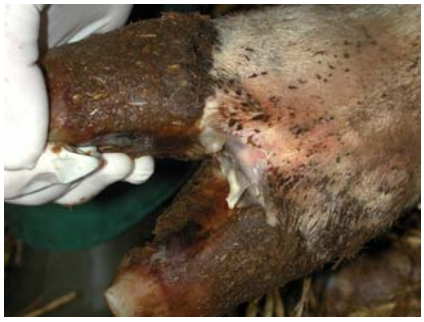
Cattle - Day 2

Ruptured vesicle, note raw epithelium, clear edge to lesion and no deposition of fibrin

Cattle - Day 3

Note friable epithelium, deposition of fibrin starting to occur

Cattle - Day 4

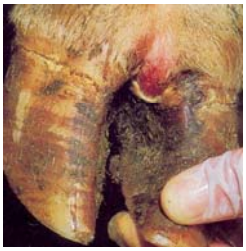


Considerable fibrin deposition

Cattle - Day 4

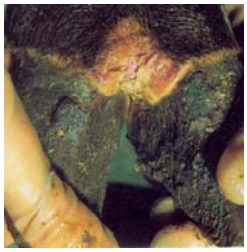
Considerable fibrin deposition and regrowth of epithelium

Cattle - Day 5



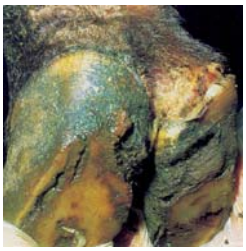
Granulation/regrowth of epithelium

Cattle - Day 7



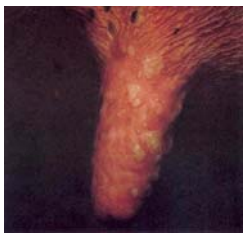
Healing progressing under the necrotic epithelium

Cattle - Day 11



Note healing and under-running of horn tissue

Cattle - Day 1



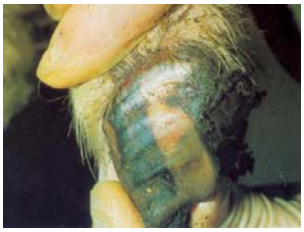
Sheep - Day 1

Note necessity to reflect hair to view the lesion

Sheep - Day 2

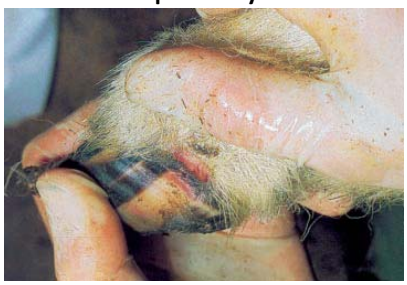
Ruptured and unruptured coronary band vesicle

Sheep - Day 3



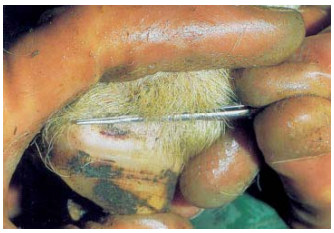
Sero-fibrinous exudate and swelling

Sheep - Day 4



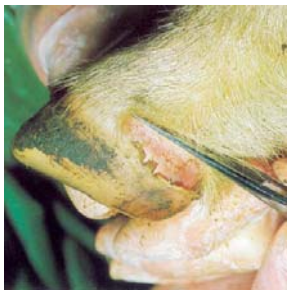
Signs of early healing

Sheep - Day 6



Scab formation and healing

Sheep - Day 10



Note under-running of horn tissue

Sheep - Day 1

Sheep - Day 2

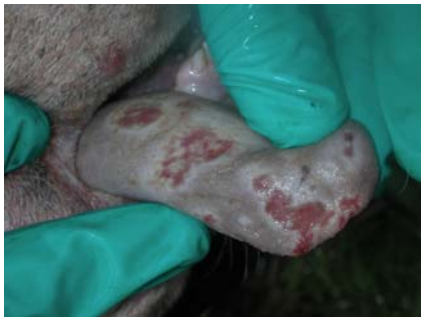
Note sharp lesion margins

Sheep - Day 3

Note rapid loss of edge definition of lesion

Sheep - Day 4

Sheep - Day 6

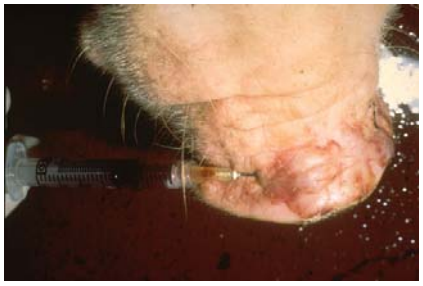


Sheep - Day 7

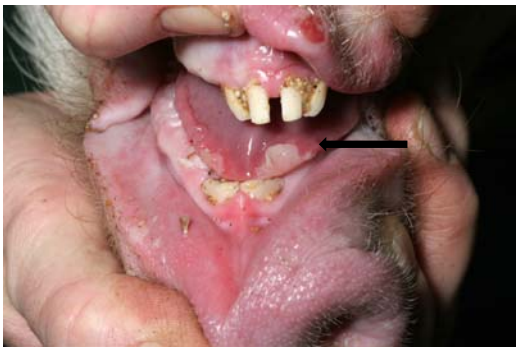
Pigs

- Most information obtained from foot lesions
- If lesion is at coronary band < 1 week old
  (7 days for lesion to mature and new horn growth to begin)
- Thereafter measure distance from coronary band to lesion
- horn grows at 1mm per week-adults
- horn grows at 2mm per week-weaners

Pigs – Day 1

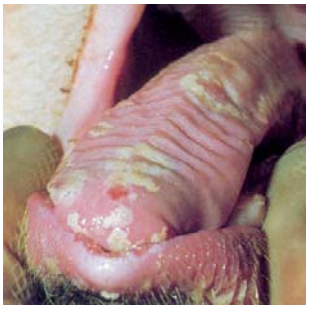


Pigs – Day 3



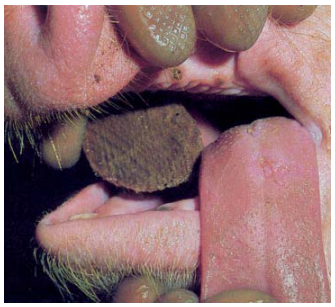
Note deposition of fibrin

Pigs – Day 4



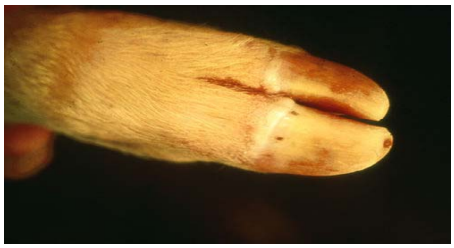
Note extensive fibrinous in-filling

Pigs – Day 8

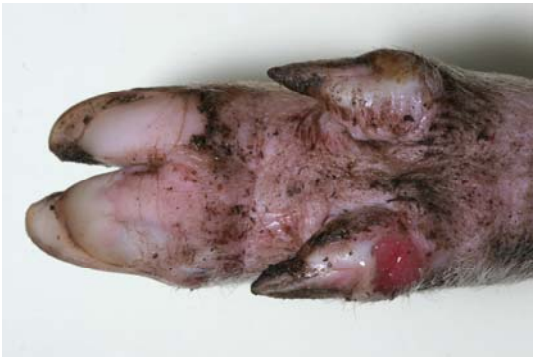


Healing lesion

Pigs – Day 1

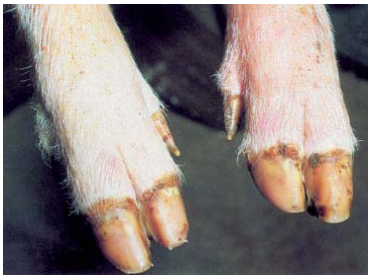


Pigs – Day 3



Supernumerary digits

Pigs – Day 9



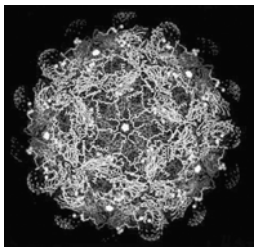
Acknowledgements

David Paton

Colleagues at the Institute for Animal Health

DIAGNOSTIC AND SAMPLING PROCEDURES FOR FMD

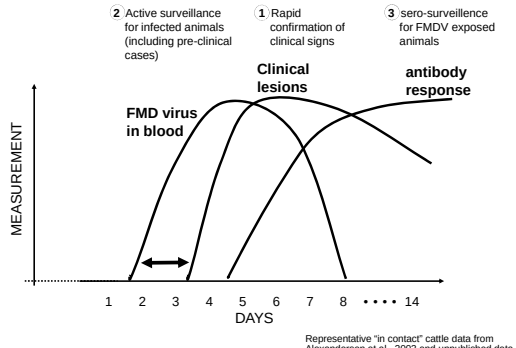
Diagnostic and Sampling procedures for FMD



Nick Juleff IAH

EuFMD training
Kenya,
15th-19th November **2010**

Diagnostic windows

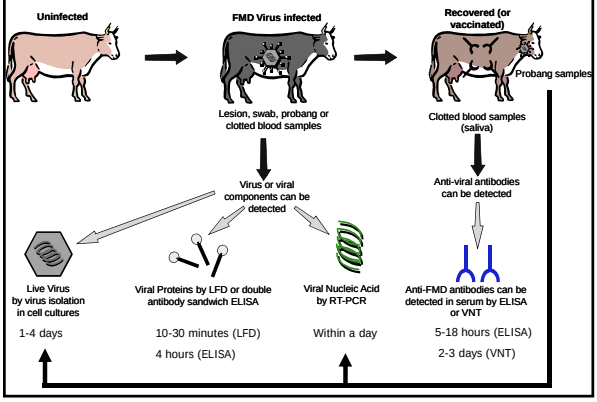


Representative "in contact" cattle data from Alexandersen et al., 2003 and unpublished data from IAH

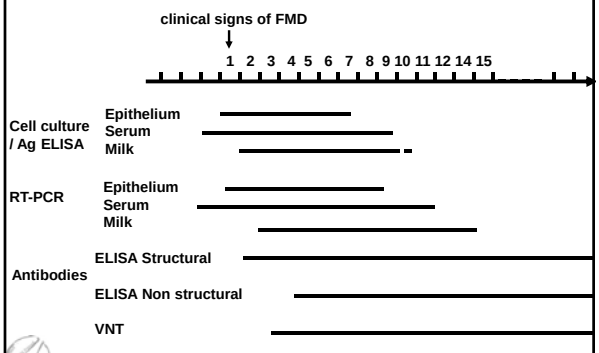
Laboratory diagnosis of FMD

- Confirms clinical diagnosis
- Supports but does not replace the need for accurate clinical diagnosis
- The quality of the diagnosis is only as good as the quality of the sample submitted
- Requires full epidemiological information on samples submitted for rational interpretation

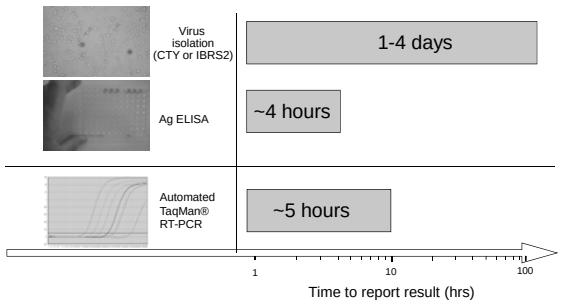
Principals of FMD Diagnosis



FMDV diagnosis: Window of detection by different techniques



Current assays for FMDV detection



Virological Samples

- Urgent
  - send as soon as possible, by most direct route
  - always give advance warning to lab and ETA
  - correct external package label to identify urgency
  - do not package together with other samples of less urgency
- Hazardous
  - package and label properly
- Fragile
  - keep cool but not frozen, except by prior arrangement, if long delay
  - avoid extremes of pH therefore use buffered media
- Adequate quantities
- Separate tube for each animal
- Correct forms

Sampling from lesions

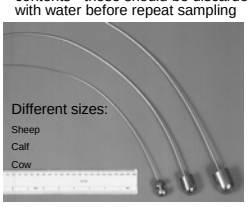
- Lesion material is the richest source of FMDV and the sample of choice for diagnosis
- Samples from ~ 4 animals with obvious lesions should be sufficient to confirm a diagnosis
- The most suitable materials are
  - Vesicular epithelium, vesicular fluid, heart muscle from myocarditis cases
  - For tissues
    - At least 2 cm^2 of epithelium from unruptured or freshly ruptured vesicles
    - Transport medium - equal amounts of glycerine and 0.04 M phosphate buffer pH 7.2-7.6
  - For vesicular fluids
    - Plain, small volume tube

Sampling in absence of lesions

- For diagnosis select ~ 6 animals, prioritizing those with suspicious clinical signs
  - Fever, depression, lameness, hot feet
- Collect clotted blood samples to obtain serum to detect viraemia or antibodies
- Collect probang and/or oronasal swabs

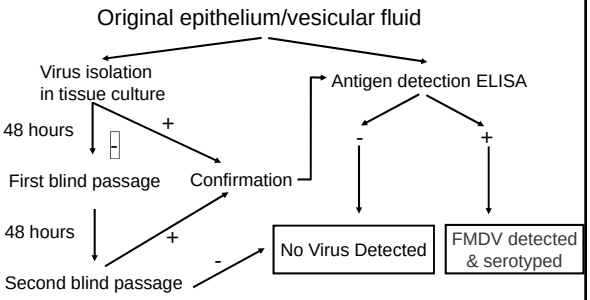
Probang samples

- Aliquot 2-3 ml 0.08 M PBS with 0.01% BSA, 0.002% phenol red and antibiotics, adjusted to pH 7.2 per animal to be sampled.
- Cattle: pour probang sample from the cup into a wide-necked bottle & examine visually for quality. Add 2ml, including visible cellular material, to equal volume of buffer and mix. The final pH of a normal sample should be ~pH 7.6.
- Sheep: insert probang cup directly into a disposable container into which has been dispensed 3 ml of buffer solution and gently mix
- Samples taken from some animals may be heavily contaminated with ruminal contents - these should be discarded and the animal's mouth should be flushed with water before repeat sampling



3 bucket "system"
- Water
- 4% Na^2CO^3 or 0.2% citric acid
- Water

WRL procedure for FMDV antigen detection

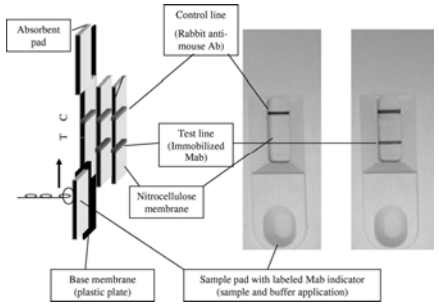


'Pen-side' test for antigen detection

Lateral Flow Device (LFD)

- Not serotype specific
- Based on technology used in pregnancy test kits
- Similar sensitivity to Ag-ELISA
- High specificity
- Used to test epithelium or vesicular fluid
- Result within minutes
- Used on-farm in UK 2007
- Used in regional lab in Turkey in 2009
- Relatively low cost per test

FIG. 1. Diagram of LFA



Oem, J. K. et al. 2009. Clin. Vaccine Immunol. 16(11):1660-1664

Clinical and Vaccine Immunology

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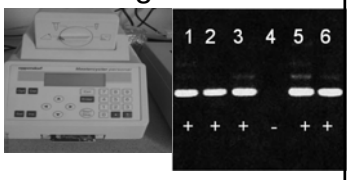
FMDV molecular diagnostics

Multiplex RT-PCR

- Very sensitive
- Simple
- Takes 4h
- Ability to serotype



Automated RT-PCR

- 2 pan-serotype assays in routine use
- Automated RNA extraction
- 84 samples ~5hours
- Highest demand: 311 samples/day

Rapid detection of FMDV in the field: Portable PCR platform

Smiths Bio-Seeq™

- Non-specialist user
  - Nucleic acid extraction
  - PCR set-up
  - Analysis
- 5 independent modules
- Battery operated
- Decontaminate by immersion
- Field trial (Turkey)
- Platform for other livestock diseases

Serology

- Clotted blood – one tube per animal
- Do not need refrigeration unless delayed/ very hot weather
- Separate forms and packaging from virological samples

Tests for antibodies to structural proteins of FMDV (SP tests)

- Detect antibodies to the virus capsid or shell
- SP antibodies are induced by both infection and vaccination
  - But usually stronger and more long-lasting antibody response to infection
- Relatively serotype specific
- Include
  - Virus Neutralisation Tests (VNT)
  - Various ELISAs
    - Solid Phase Competition ELISA
    - Liquid Phase Blocking ELISA
    - Ceditest FMDV type O (Prionics)
    - Isotype-specific tests for IgM and IgA
- SP antibodies appear around 5 days after infection and usually within 2-3 days of appearance of lesions

Tests for antibodies to non-structural proteins of FMDV (NSP tests)

- Detect antibodies to the non-structural proteins of FMDV involved in virus replication, e.g. 3ABC
- NSP antibodies are induced by infection but not by immunisation with purified vaccines
  - Multiple vaccination increases the likelihood of inducing NSP antibodies
- Pan serotype specific
- Several commercial ELISA test kits, some of which are species-specific and some work for all species
  - Ceditest FMDV-NS (Prionics)
  - Bommeli
  - *SVANOVIR™ FMDV 3ABC-Ab ELISA*, Svanova Biotech AB
  - UBI
- NSP antibodies appear around 7 days after infection and usually within 3-4 days of appearance of lesions
- NSP response may be reduced and delayed in case of subclinical or mild clinical infection following vaccination

Sampling in Kenya

- Epithelium samples: gly-iso buffer
- Vesicular fluid: collect using a syringe and needle. No buffer.
- Blood: whole blood in EDTA/Trizol, clotted blood in plain vacutainer
- Probang samples: buffer

FMD EPIDEMIOLOGY AND TRACING DANGEROUS CONTACTS

FMD epidemiology and tracing dangerous contacts

Kenya, 15th-19th November 2010

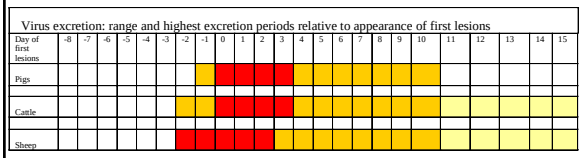
Nick Juleff, Institute for Animal Health

Routes of infection

- Direct contact with infected animals
(ban on animal movements)
- Contaminated animal products
(ban on meat/milk from infected areas)
- Airborne virus
(use wind records to estimate spread)
- Mechanical transmission of virus on people, vehicles, etc → **BIOSECURITY**

Incubation and excretion periods

- Incubation period: 1–14 days
  - most common 2-5 days
- Dose-related: low dose → longer incubation
- Virus excretion: may occur *before* onset of clinical signs



Why is FMD so contagious?

- WIDE host range
- HIGH Morbidity/LOW mortality
- Infection dynamics
- Patterns of viral shedding
- FMD virus can survive in environment for long periods

Key epidemiological factors

- Animal movements

UK 2001: best estimate is that >100 farms were already infected before first diagnosis

- Role of markets & abattoirs

UK 2001: 10 infected sheep in Longtown market exposed 24,500 sheep

- Pigs: high level excretion, airborne spread
- Sheep: subclinical infection, easily missed
- Persistence of epidemic through "local spread": stringent biosecurity reduces risk

Principles for controlling a FMD outbreak

Prevent transmission from infected to susceptible animals

↓↓ production of virus
Cull infected and in-contact animals, ± dangerous contacts if high-risk

↓↓ potential for direct contact between animals
Movement restrictions

↓↓ virus survival time in the environment
Biosecurity, cleansing & disinfection of infected farms

↓↓ number of susceptible animals
Emergency vaccination or contiguous culling if situation v. severe

DEFRA - Initial control measures

- Establish temporary control zone
  - Stop animal movements
  - Size – prevent spread of disease
  - Supplementary movement control zone (2007)
- "Stamping-out" on infected premises (IP)
  - 24 hours
- Cull dangerous contacts
  - 48 hours
- Establish 3km protection and 10 km surveillance zones

- Protection zone (3km radius around IP)
  - No animal movement except to emergency slaughter under licence
  - Movement of animal products, feed and bedding under licence
  - Requirement for increased on/off farm biosecurity
  - Animal products treated to destroy FMDV
  - Footpaths closed
  - census
  - surveillance - regular inspections, sero-surveillance
  - tracing at start
  - *For early / index cases, the EU requires tracing of animals and products moved from the zone since 21 days before the probable introduction of infection*
- Surveillance zone (10km radius around IP)
  - As for protection zone except:
    - Footpaths remain open
    - Licensed animal movements are possible

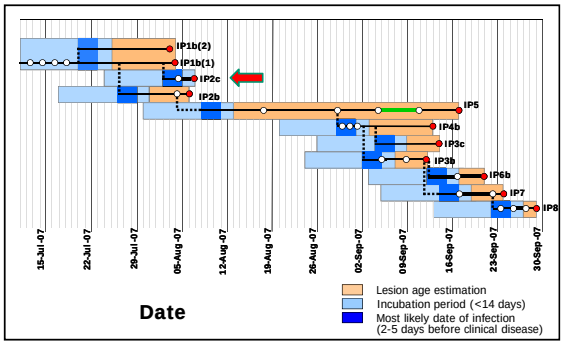
Additional control strategies

- Scientific and veterinary advice
- Cull other susceptible livestock exposed to disease
  - e.g. Premises under viral plumes, adjoining premises
  - Extensive sampling, clinical examination (2007 pre-clinical diagnosis)
- Pre-emptive or "firebreak" culling of animals

Establishing Timelines

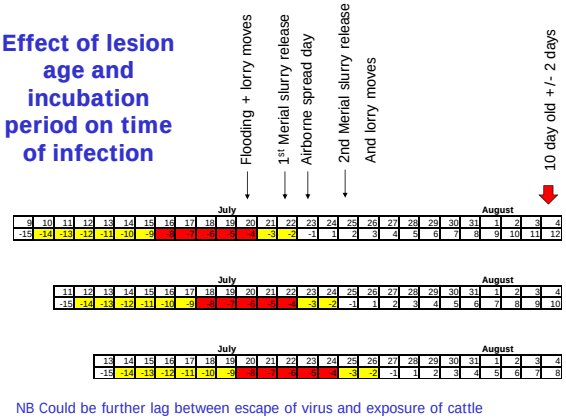
- Estimate age of **oldest lesion** on farm
  → Determine date lesions first appeared (NB: error margin)
- Subtract incubation period **1-14** days → window for **introduction of virus**
- Prioritise "onto farm" contacts in most likely incubation period of 2-5 days before lesions appeared

Timeline example: UK 2007



Data from Cottam et al., 2008

Effect of lesion age and incubation period on time of infection



NB Could be further lag between escape of virus and exposure of cattle

Lessons from the field

- Epidemiological benefits of lesion ageing, extensive sampling, sequencing virus isolates in real time
  - 2nd phase of outbreaks (IP3 – IP8) shares all the unique changes common to 1st phase
  - Therefore outbreaks are linked and not due to independent sources
  - IP5 (farm with FMD serology positive cattle and sheep) bridges gap between two phases of the outbreak
- Diagnosis of preclinical viraemic animals using real-time PCR
- Hobby/part-time farmers with inadequate handling facilities: implications for neighbouring farmers?

Tracing dangerous contacts (DCs)

Methods:

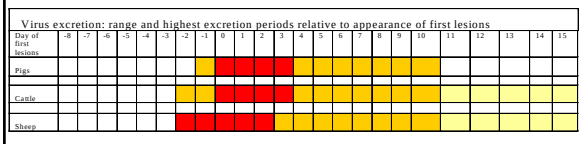
- Interview farmer & farm staff
- Examine written records (milk tanker, feed deliveries, AI visits, etc)
- Walk boundaries of farm
- Estimate age of FMD lesions
- Contact dairy, vets, AI techs etc

Key information:

- Estimated age of oldest lesion
- Animal movements
- Personnel visiting farm
- Farm personnel visiting other livestock holdings
- Vehicle/equipment movements
- Security of farm boundaries

Risk periods

- *Time* of contact is very important
- Tracings ***onto*** farm: incubation period 1-14 days before lesions appear; most likely 2-5 days
- Tracings ***off*** farm: refer to **virus excretion period**

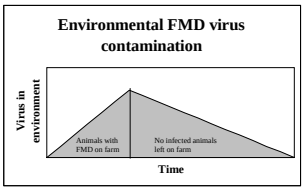


NB: virus can be excreted *before* clinical signs appear

Milk: can contain virus **4 days** before disease appears

Risk periods

- Levels of environmental FMD contamination increase from first case until after final case on farm has recovered
- Slow decrease in virus levels thereafter, related to temperature and humidity
- Fomite transmission contacts: relationship between time of contact and degree of risk



Type of contact

Animal movements most critical contact

- Pigs: high level of virus excretion
- Sheep: mild to sub-clinical disease; easy to miss

Local spread

- Check farm boundaries
- Nose-to-nose contact with neighbouring herds/flocks possible?
- Fences stock-proof?
- Shared streams at boundary – close proximity possible?
- Common grazing?

Tracing animal movements

• Trace source of animals moved ***onto*** farm during incubation period

•Trace destination of animals moved ***off*** farm during virus excretion period (from 4 days prior to disease appearance onwards)

•Identify any markets/stopovers en route, incl. pick-ups at other farms

Method: Use animal movement databases, farmer interview, records, etc

Action: restrict all implicated premises, initiate investigations

Tracing contiguous herds & common grazing

- Determine if neighbouring stock were in fields adjacent to IP; evaluate fencing/waterways/natural features between premises.
- Determine which other farms had stock grazing land also used by IP stock, and relevant timelines.

Method: Maps of contiguous farms, VI to walk farm boundary, telephone enquiries, local knowledge

Action: Issue restriction notices and initiate investigations on any farms where stock grazed commonage at same time or after stock from IP, subsequent to estimated earliest date of infection introduction, and any contiguous farms.

Type of contact: Personnel

- Close interaction with animals? Vet, AI tech, shared labourer, foot-parers, sheep shearers, etc
- Interaction with affected stock or non-affected stock?
- Visits by farmer & staff to other livestock holdings: contact with animals at other premises?

Tracing personnel contacts

- Identify workers, especially those in contact with animals, on the IP who also work on other farms
- Identify itineraries of vets, AI techs, other technicians who were on IP during risk periods

Method:

- Interview farmer and farm staff, any other relevant personnel
- Interview vets, AI techs etc, examine calls book
- Relate farmer names to herd numbers, identify on map
- Relate contact dates to risk periods, prioritise accordingly

Action:

- Evaluate degree of risk of each contact
- Prioritise accordingly; restrict highest risk ones
- As a minimum, first 3 farms visited by vets/AI techs after IP require investigation as a priority

Type of contact: Vehicles/equipment

- Milk tankers: can leak virus-containing milk at pipe connection
- Trailers which contained animals: gross contamination with virus possible
- All vehicles: wheels, wheel arches, undercarriage contaminated with faeces/muck with virus
- Key question: was vehicle/equipment in contact with animals or in yard/lane contaminated with faeces?

Tracing milk tankers

- Identify premises visited following collection of milk from IP during risk period.

Method:

- Contact dairy by phone, request records of tanker itineraries during risk period; verify with driver (may vary route)
- For tracings *off* the IP, the risk period is from 4 days prior to the appearance of clinical signs, although tracings which occurred during clinical disease should be prioritised

Action:

- Identify premises visited
- Prioritise farms with large numbers of stock and farms visited immediately after IP.

Tracing other vehicles/equipment contacts

- Identify nature of contacts
- Estimate time in relation to risk period
- Ascertain origin or destination of vehicles/equipment
- Ascertain degree of contact with infected animals on IP
- Evaluate risk level and prioritise contact investigation accordingly

Method:

- Interview farmer and farm workers and other relevant personnel.
- Phone premises which were origin/destination of contact vehicles and establish number and species of stock on farms
- Establish dates of contacts and relate to risk period

Action: investigate priority contacts first

Risk of airborne spread

- Virus emission profile estimated by number of animals with FMD and age range of lesions
- Meteorological agency (e.g. Met Office) will combine this with meteorological data
- Model output: map with areas showing relative risks of airborne FMD spread
- Farms in "high risk" category: investigate first
- Virus can spread long distances by air, but this is not as common as other methods of spread (animal movement, fomites)

Summary:

• Latest UK Met Office model, including GIS output in operational service

Assessment of Biosecurity on IP

- Level of biosecurity in operation on IP *prior* to diagnosis can hugely influence onward spread
- Difficult to assess after diagnosis
- General level of farm hygiene can provide rough estimation

Biosecurity on dangerous contact: also critical risk factor, but impossible to assess without inspection, unless prior knowledge available

Reasons for prioritisation

- The number of contacts to be traced can become very large
- Resources for investigations not unlimited
- Time can be critical

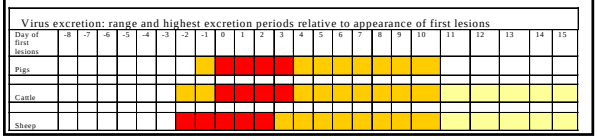
→ **Need to prioritise "hot" contacts**

Prioritising Dangerous Contacts

Three factors to consider:

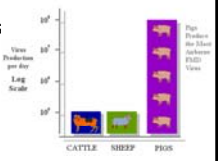
1. Species: pigs > cattle > sheep
2. Type of contact: animal movement > people in direct contact with FMD animals > vehicles in direct contact etc.
3. Time of contact in virus excretion window

NB: may get multiple waves of infection in herd/flock → continual virus excretion as new animals fall ill



Prioritising DCs: Likely result of virus spread

- Pigs: if infected, will generate huge amount of virus → tracings to pig farms a priority
- Number of animals on DC: as numbers increase, so does chance of infection and significance of outbreak
- Type of enterprise:
  - Markets
  - Abattoirs
  - Farms owned by dealers



Categorising risk posed by DCs

Very high risk:

- Animal movements during risk period
- Farms owned/worked on by workers from IP
- Contiguous herds with possibility of nose-to-nose contact
- Animals grazing common land with IP stock
- Any market or abattoir connected to IP during risk period

Categorising risk posed by DCs

High risk:

- Farms visited by vets/AI techs after IP during risk period
- Farms visited by milk tanker after collection at IP
- Contiguous farms where nose-to-nose contact is less likely but stock are in adjacent fields

Categorising risk posed by DCs

Medium risk

- Shared equipment/trailers/vehicles in direct contact with infected animals on IP
- Neighbouring/nearby farms with some distance between animals on IP and DC
- Personnel in contact with animals on IP and DC

Categorising risk posed by DCs

Low risk:

- Vehicles/equipment shared between farms but not in contact with animals
- Personnel shared between farms but not in contact with animals
- Personnel visiting the IP and then other farms but not in contact with animals

Acknowledgements

- Eoin Ryan, Dept of Agriculture, Ireland
- Dónal Sammin, Dept of Agriculture, Ireland
- Colleagues in the Institute for Animal Health, Pirbright, UK

THE 2007 UK FMD OUTBREAK

The 2007 UK FMD outbreak: field investigation perspective

Nick Juleff, Institute for Animal Health

Slides prepared by Eoin Ryan

UK 2007 FMD Epidemic

- Virus escape from Pirbright site (IAH and Merial vaccine plant)
- 8 infected premises, most consisting of multiple holdings
- Mainly extensive beef production
- Most farms in semi-urban areas
- Part-time/hobby farmers
- 1578 animals on IPs culled
- 278 animals infected

IP1: 3 holdings

- 29th July: farmer notices an animal "off colour"
- 2nd August: several cattle lame & drooling. PVP tells farmer to contact Defra directly.
- 3rd August: Defra vet examines & takes samples; +ve for FMDV
- 4th August: cattle at all 3 holdings killed; virus identified as O1 BFS 1860 (used at Pirbright site)

IP1: main holding

- 38 cattle, all infected
- Lesion ages: 3 to 10 days old
- Beef store cattle grazing on open pasture 4km from Pirbright site
- No handling facilities: Defra brought in gates & straw bales to make corral
- Shot with rifles then pithed

IP1: two other holdings

- 4 cattle at home farm: no FMDV
- 22 cattle on open pasture: no FMD lesions, [illegible] PCR +ve (viraemic)
- Only link between premises: farmer
- First time preclinically viraemic animals detected using PCR in an outbreak
- Logistical problems again: straw bales, rifles
- Carcases transported by sealed lorry to incinerator 70 miles away

IP2: three holdings

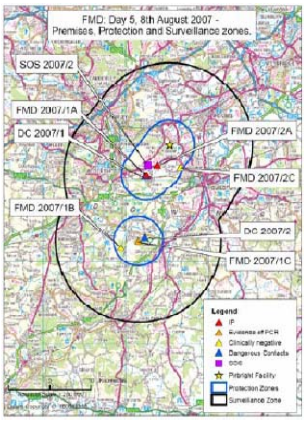
- 49 cattle 1km from IP1; 58 at home farm; 12 on third holding
- 6th August: farmer notifies Defra, samples taken from holding with 49 cattle: +ve
- 25 killed that night, the rest on next day
- Cattle examined & bled post-mortem

IP2

- 49 cattle: 44 had lesions, 2-7 days old
- Logistical probs: no handling facilities, no water, rifles, journalists
- 58 cattle on 2nd farm: no FMD lesions BUT
- 15 of 58 viraemic by PCR/VI
- 12 cattle at 3rd holding: no FMD

Phase 1: August

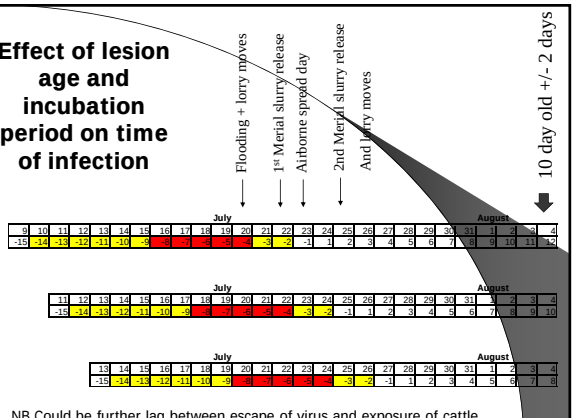
- 3 Aug: IP1 (3 locations)
- 6 Aug: IP2 (3 locations)
- 3 contact herds culled
- 24 Aug: PZs lifted
- 8 Sep: SZ lifted
- Origin: contamination from Pirbright site



Likely times of lab escape of virus from Pirbright site in 2007

- 20th July: flooding
- 23rd July: best airborne spread day
- 22nd July: 1st centrifuge waste discharged
- 25th July: 2nd centrifuge waste discharged
- 20th July: 4 lorries via Westwood Lane
- 25th July: 2 lorries via Westwood Lane

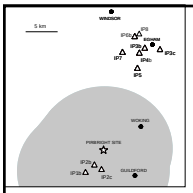
Effect of lesion age and incubation period on time of infection



NB Could be further lag between escape of virus and exposure of cattle

Difficulties

- Assuming lesion of 12 days and longish incubation then likely release times too late
- Flooding period fits best as time of release most consistent with lesions and incubation
- But flooding and first lorry movements precede likely virus release through drains
- Second period of lorry movements more likely associated with viral contamination, but too late to have infected farm

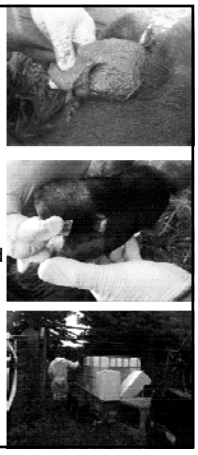


Phase 2: September

IP3

- Confirmed 12th Sept
- 8 locations: 2 FMD+ve
- 36/47 cattle had FMD lesions, 1-5 days old
- 9/29 cattle had lesions at 2nd holding, 1-5 days old
- Other holdings all FMD negative
- Culling completed 16 Sep (4 days later)
- Logistical probs on out-farms:

No handling facilities, escapees, police

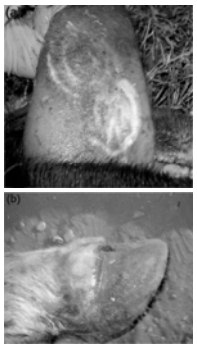


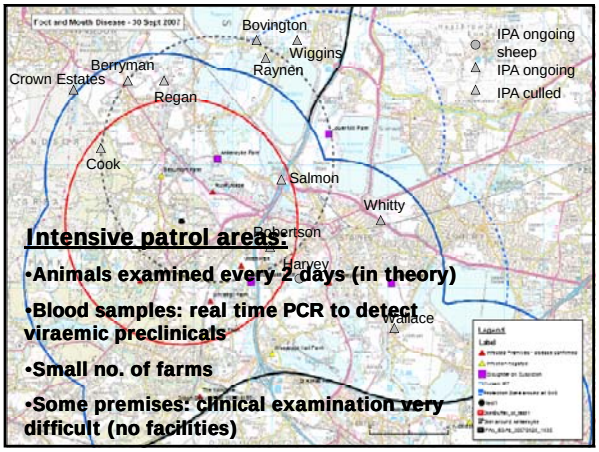
IP4

- Separated from IP3 by narrow stream
- Diagnosed Sept 13th (farmer report)
- 54/54 beef cattle had FMD lesions, 5-10 days
- 800 pigs kept 500m away: no FMD
- Farmer had been on holidays for 10 days – unclear who checked cattle
- Excellent handling facilities – fast cull

IP5: the missing link

- After IP3 & 4, 3km PZ implemented
- All sheep bled, cattle & pigs "clinically inspected" – often just observed over fence
- 17th Sep: IP5 sheep tested seropositive
- IP5: 16 sheep, 22 cattle, 2 pigs
- Farmer retired & unwell; animals were pets
- FMD lesions ~ 3 weeks old (large error margin)
- Virus detected in probangs – 10/16 sheep carriers



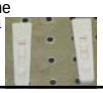


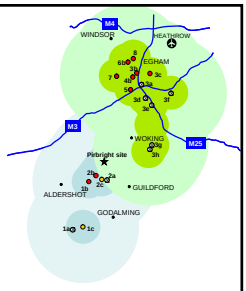
IP6

- 21st Sept, Defra inspection
- 34 cattle, 2 locations
- 2/32 cattle with 3-4 day lesions
- Another 5 viraemic

IP7

- 24th Sept, Defra inspection
- 16 Dexter cattle, wild & unhandled
- Hobby farmer: retired architect
- 14 with 1-5 day lesions
- All FMDV +ve
- Use of LFD on farm
- Logistical probs: xylazine darts, rifles; urban area

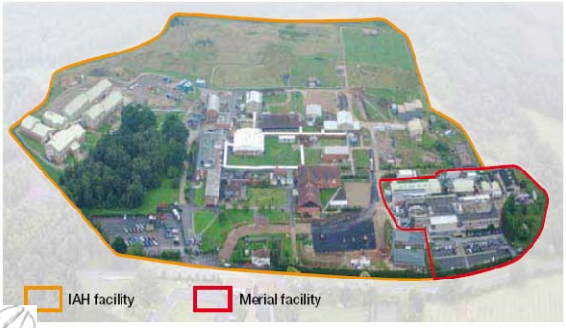




IP8

- 29th Sept, Defra inspection
- 54 beef cattle at infected site
- 1 with lesions, 2 days; 15 viraemic
- 134 cattle, 16 sheep
- 4 locations, 134 cattle, 16 sheep
- Other sites FMDV -ve

Shared IAH / Merial Facility



Source of virus: Pirbright site

- Virus: O1 BFS1860 (1967 UK outbreak)
- 3 strains used on Pirbright site:

IAH1 (v. small quantities, Institute for Animal Health research)

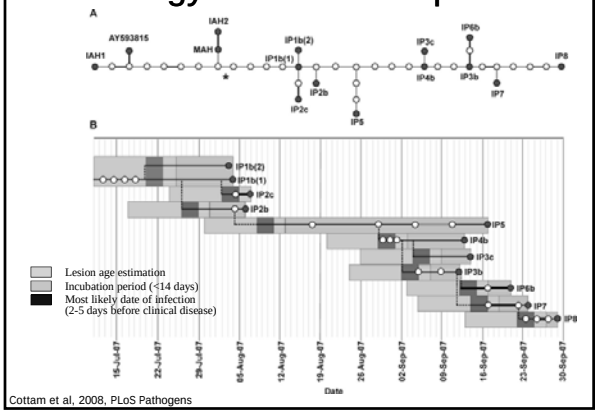
IAH2 (v. small quantities, Institute for Animal Health research)

MAH (6,000 litre batches for vaccine production, Merial Animal Health)

- Molecular epidemiology reveals transmission pathways

(Cottam et al, 2008, PLoS Pathogens 4(4): e1000050)

Genealogy and infection profiles



Cottam et al, 2008, PLoS Pathogens

- Merial vaccine waste inactivation method not validated for large volumes
- Permitted by Defra (UK Govt) – MAH did not breach their licence
- Dispute over responsibility for pipes
- Unresolved questions:

How did virus get from Pirbright to IP1?
How credible is the pipe/soil/lorry theory?
How did it get from IP1/2 to IP5?

Lessons from the field

- Epidemiological benefits of lesion ageing, extensive sampling, sequencing virus isolates in real time
  - 2nd phase of outbreaks (IP3 – IP8) shares all the unique changes common to 1st phase
  - Therefore outbreaks are linked and not due to independent sources
  - IP5 (farm with FMD serology positive cattle and sheep) bridges gap between two phases of the outbreak
- Diagnosis of preclinical viraemic animals using real-time PCR
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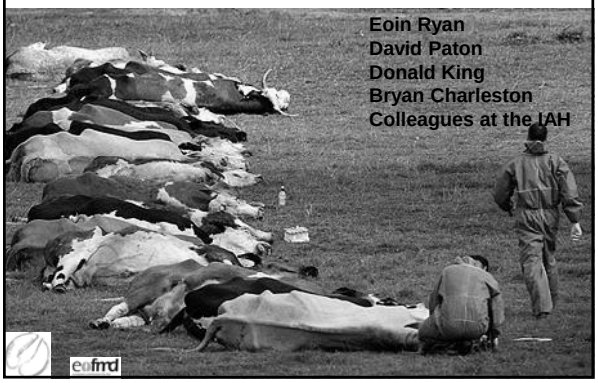


Eoin Ryan
David Paton
Donald King
Bryan Charleston
Colleagues at the IAH

BIOSECURITY AND FMD

Biosecurity and FMD

EuFMD training
Kenya,
15th-19th November **2010**

Nick Juleff, Institute for Animal Health

Biosecurity & FMD

- Real risk of transmission associated with personnel
- Veterinary surveillance, farm visits etc → **High risk?**
  (2001 UK outbreak - 2 day veterinary surveillance 3km protection zone)
- New sequencing techniques allow detailed analysis of transmission pathways → "nowhere to hide"
- Vital to "lead by example"; if vets do not observe biosecurity properly, very difficult to persuade other staff & farm visitors

Adapting to Circumstances

- Achievable level of biosecurity depends on the circumstances
- Apply general principles using veterinary judgement
- During the EuFMD training course: may not always be possible to achieve highest levels of biosecurity
- Every effort should be made to maximise biosecurity even if local farmers are not observing it
- Essential to avoid any feeling among farmers that we may be spreading disease

Biosecurity talk

- General biosecurity principles
- Biosecurity & containment protocols used in UK
- Specific instructions for Kenya

Why is FMD so contagious?

- WIDE host range: cattle, sheep, pigs, goats, other cloven-hoofed animals
- HIGH Morbidity/LOW mortality: lots of animals affected but few deaths in adults
- Infection dynamics: rapid, high levels of virus/transmission
- Patterns of viral shedding: FMD virus shed in saliva, breath, milk, vesicles, urine, faeces (all excretions and secretions)

Routes of infection

- Direct contact with infected animals
(ban on animal movements)
- Contaminated animal products
(ban on meat/milk from infected areas)
- Airborne virus
(use wind records to estimate spread)
- Mechanical transmission of virus on people, vehicles, etc → **BIOSECURITY**

Physical and chemical properties of the virus

- Relatively stable/resistant in the environment

⇒ importance of **cleaning and disinfection** in control

- Particularly susceptible to small pH changes

⇒ the use of "mild" **acidic or alkaline reagents** as disinfectants

Virus survival

- Inactivated below pH 6.5 or above pH 9, most stable at pH 7.2-7.6

Documented examples in the UK:

- Survival: 14 days in dry faeces, 39 days in urine and up to 6 months in slurry in winter
- 3 days on soil in summer and 28 days in autumn
- Up to 20 weeks on hay/straw or up to 4 weeks on cow's hair at 18 to 20ºC
- Survival is dependant on pH, temperature, humidity and initial concentration

Biosecurity principles

- Minimise contact between farms
- Do not enter or bring anything onto or off a farm unless necessary
- Carry out cleansing and disinfection **before and after** visiting any farm
- Strict segregation between "dirty" and "clean" areas are essential
- Quarantine period: if you have been in a "dirty" area, avoid any premises with livestock for at least 5 days
- NB: *Risk reduction at every step*

Disinfection principles:

- Clean *before* disinfection
  - dirt and organic matter can protect virus from disinfectant
- Disinfect surfaces fully and completely
  - splashing disinfectant on something is not enough
- Ensure adequate contact time
  - disinfectants need time to work
- Use approved disinfectant
  (http://www.defra.gov.uk/animalh/diseases/control/testing_disinfectants.htm)

Virus	Washing Soda Na^2CO^3	Citric acid + 0.005% NP40	FAM 30	Virkon
Foot-and-mouth disease	4%	0.2%	1:240	1%

Personal Biosecurity 1

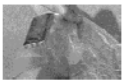
Inner layer:

- Tyvek suit underneath waterproof outer-wear
- Inner layer of gloves in case outer layer tears

Outer layer:

- Latex/nitrile Gloves
- Wellingtons with plastic over-boots to reduce dirt on boots, facilitating cleaning
- Waterproof outer-wear
- Hat/hood

Personal Biosecurity 2



When leaving farm via disinfection point:

- Clean and disinfect items to be removed, e.g. mobile phones in ziplock bags, samples, etc, and place in bag in clean area
- Clean and disinfect outer layer of clothing
- Discard boot covers and outer gloves in bag on dirty side; waterproof overalls in bag on clean side
- Clean and disinfect wellies, then place in bag on clean side with overalls
- Discard paper suit and inner gloves in bag on dirty side
- Clean and disinfect hands, arms, fingernails, etc.
- Double-bag everything being removed: samples, wellies, outer waterproof suit.

Personal Biosecurity 3

- Do not bring lighter/cigarettes onto farm, unless prepared to leave them there.
- If you wear glasses, these must be submerged in disinfectant when leaving
- Remember: if something is exposed on farm, it must be disinfected prior to removal

Vehicle biosecurity for visits

- Empty all non-essential items from car
- Arrange a "clean" area (e.g. back seat) and a "dirty" area (e.g. boot); line both with plastic bags
- Do not drive onto farm; park outside premises
- Put all necessary kit into bag to take onto farm; (returning to car will require a change of outer-wear-scrubs)

Vehicle biosecurity: Cleaning and disinfecting vehicles

- 2001 example
- Apply principles of cleaning and disinfection
- Clean exterior using power-washer or hose and disposable sponge, remove all visible dirt
- NB: wheel arches and tyres
- Spray with disinfectant over exterior
- Interior: dispose of all rubbish, clean all dirt
- Wipe steering wheel, gearstick, pedals, handbrake, footwell, etc with cloth dipped in disinfectant
- Assess risk in rest of vehicle and act accordingly

Establishing a disinfection point at entrance to farm: 1

- Requires bucket/container, supply of water, disinfectant, brush, plastic sheeting, plastic bags
- Best location is at gate/fence: visible divide between "clean" and "dirty" sides is important
- Lay down plastic sheeting on clean side
- Plastic bag for contaminated rubbish on dirty side
- Plastic bags for wellies, samples etc on clean side

Establishing a disinfection point at entrance to farm: 2

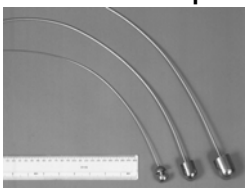
- Bucket/container with disinfectant and brush on clean side; a second disinfectant bucket with brush on dirty side
- NB – disinfectant must be kept fresh, replenished and at proper concentration
- Water supply essential: if not available near disinfection point, arrange for supplies to be delivered
- Dirty side: wellies, bags containing samples & paper records, mobile phones in bags etc cleaned and disinfected
- Clean side: second disinfection of above; C&D of hands, arms, glasses etc

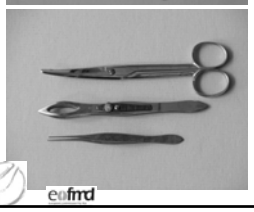
Establishing a disinfection point at entrance to farm: 3

When clearing up disinfection point

- Have all items on dirty side put in plastic bag, sealed and put in safe place (e.g. shed) on farm for subsequent disposal
- C&D items (e.g. brush, bucket) on clean side, put in plastic bag, seal, and put in "dirty" area of your vehicle.

Sample biosecurity





3 bucket "system"

- Water
- 4% Na^2CO^3
- Water

Kenya: specific instructions

- Make every effort to maximise biosecurity, even in challenging circumstances
- Laboratory: Work on FMD virus samples inside safety cabinet; clean & disinfect hood before and after use
- Prior to returning home, **submerge all clothes worn on farms** or **in lab** in Virkon for 30 minutes in bath, then have hotel clean them (Virkon will be provided)
- **Avoid** farms, zoos, vet schools etc for **7 days**

Acknowledgment

- Eoin Ryan, Irish Dept. of Agriculture
- Colleagues at the Institute for Animal Health

EMERGING TRENDS IN FMD SAT1 OCCURRENCE IN KENYA

EMERGING TRENDS IN FOOT AND MOUTH DISEASE SAT1 OCCURRENCE IN KENYA

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Introduction

In the recent past, Kenya has witnessed an upsurge of Foot and mouth Disease (FMD) serotype SAT1 outbreaks, with grave consequences including mortalities among adult cattle herds and severe teat lesions that had not been observed before. The epidemic has swept across the country indiscriminately including farms that have had regular and stringent vaccination regimes using trivalent vaccines incorporating SAT1. The first witnessed cases were in June 2009 in Trans-mara district located in the south-western region of the country. This later spread to the Rift valley, Central and Eastern provinces and seems to be spreading further across the country.

FMD SAT1 had been having low and sporadic incidences in Kenya for several years since 1962 when it was first recorded. The highest reported outbreaks in the history of the serotype in the country was in 1979, where 46 outbreaks occurred in 8 different districts. From 1980 to 1999, incidences had reduced, but up-surged in 1999 and since then has remained low until 2009 and 2010 (DVS Kenya reports). The overall trends of reported FMD outbreaks during the last 15 years in Kenya are illustrated in Figure 1 while trends for SAT1 over the last 48 years are shown in figure 2.

SAT1 outbreaks have successfully been controlled since the 1979 epidemic through ring vaccinations using the current vaccine strain. Following the observed alarming spread and apparent failure of ring vaccinations, we report records of SAT1 outbreaks observed in the recent past in Kenya.

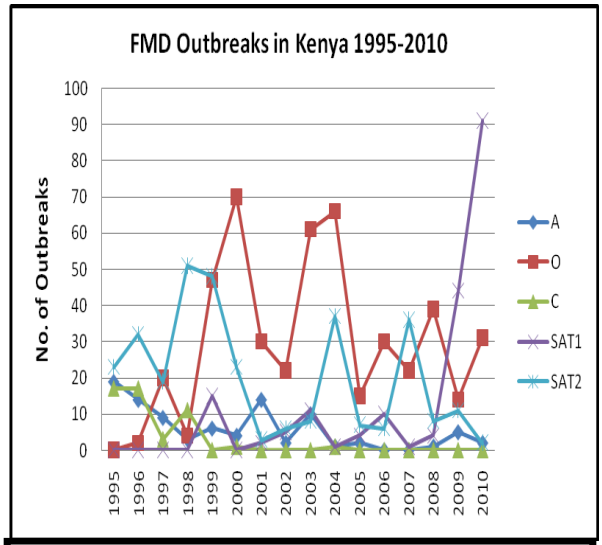

FIG1: FMD Outbreaks in Kenya from 1995 to 2010

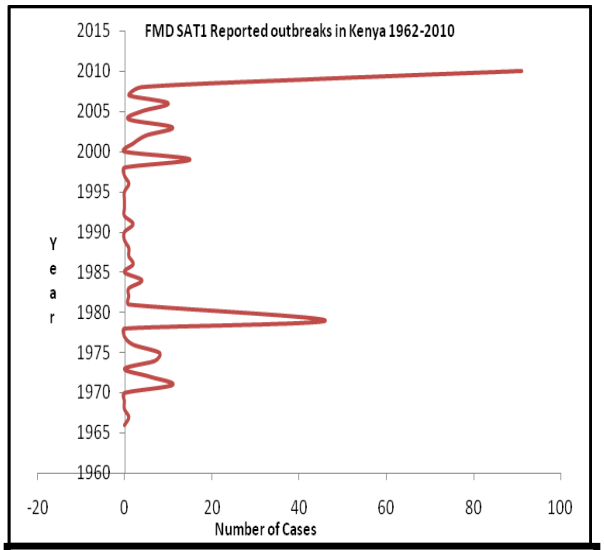

FIG2: FMD SAT1 Outbreaks in Kenya from 1962 to 2010

Materials and Methods

Epithelial samples from clinical cases were routinely submitted to the National FMD laboratory, Embakasi by various District Veterinary Officers (DVOs) across the country for serotype identification. The samples were accompanied by forms that gave information on morbidity and mortality rates among herds affected. A total of 184 samples had reached the laboratory from various districts by the end of August 2010 compared to 105 by the same period in 2009.They were tested using virus isolation on tissue culture and antigen detection ELISA (Anonymous, 2004). Parallel samples were submitted to the World Reference Laboratory (WRL), Pirbright for comparative serotyping and sequencing.

Furthermore, investigation teams comprising of officers from the National laboratory and the field veterinary services visited some of the affected farms in response to reports by farmers and field staff of suspected FMD outbreaks characterized by severe clinical signs in previously vaccinated herds. Clinical examinations were undertaken and photos of lesions recorded. Various samples including epithelial tissue were taken for laboratory diagnosis at Embakasi and WRL.

Results

Serotyping results by the Embakasi laboratory and the WRL showed that most of the outbreaks were caused by FMDV SAT1. By the end of August 2010, SAT1 outbreaks comprised 70% (91/130) of the total positively confirmed laboratory submissions and only 30% (39/130) were due to the other serotypes (O and SAT2). So far, all the eight provinces have reported FMD outbreaks with higher occurrences recorded in the Rift valley, Central and Eastern regions. Out of the 80 districts countrywide that have so far reported the disease, 52 have had SAT1.

All the farms affected by SAT1 reported typical FMD clinical signs as described in Kitching, (2002). The most prominent signs observed in affected cattle are represented by the photos in Figure3. A morbidity rate of 100% was observed in all the visited farms. Mortality rates in some farms went as high as 50%, but on average, 15% across all ages. One model farm with very stringent vaccination schedule had an average of 15.8% (90/570) mortalities of which 64.4% (58/90) were adult milkers.

Genotypic analysis by WRL revealed that these 2009/2010 Kenyan SAT1 FMDV field strains were genetically divergent from the vaccine strain by as much as 10% nucleotide difference within topotype I (NWZ), (Figure 4).

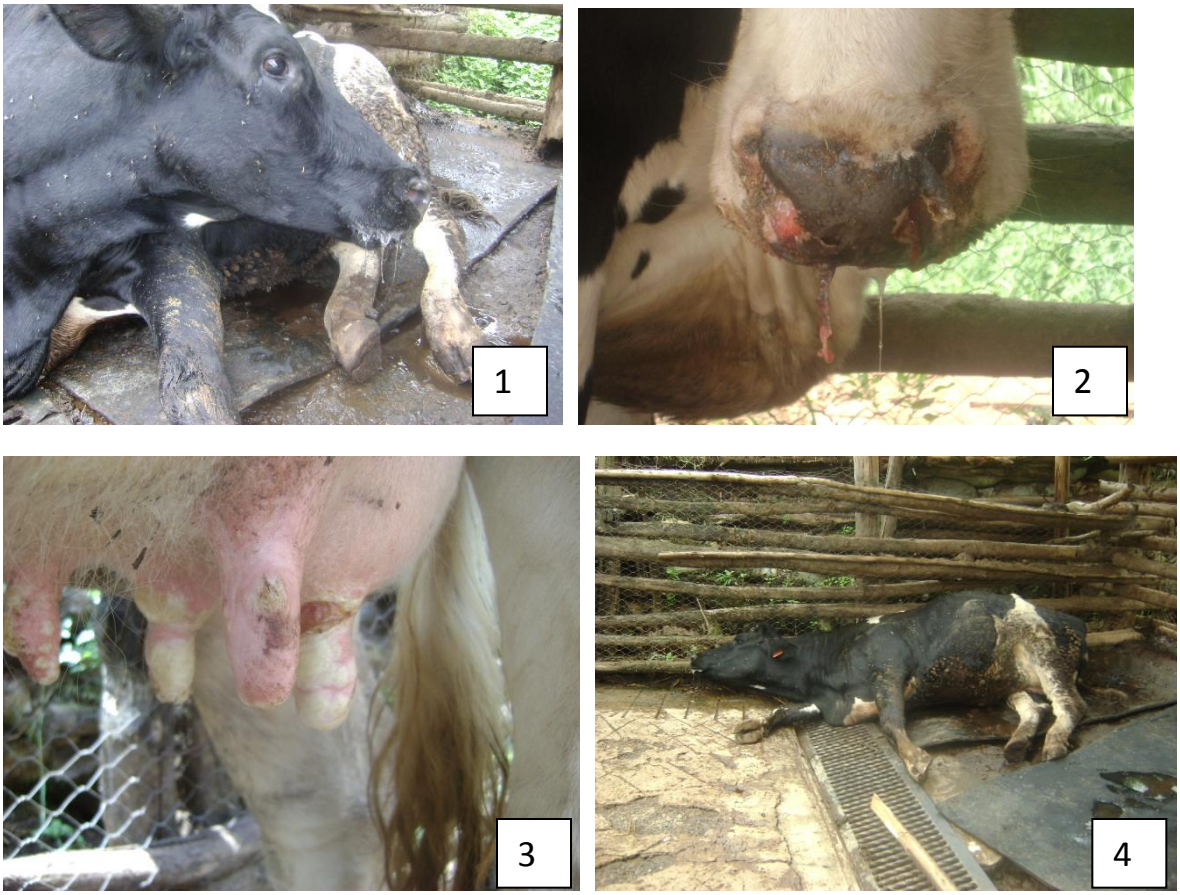

FIG 3: Severe FMD Clinical signs in one of the dairy farms: 1-frothing and recumbency;
2-Ulceraions and peeling muzzle; 3- Teat blisters and Ulcerations; 4 – Death of an adult animal

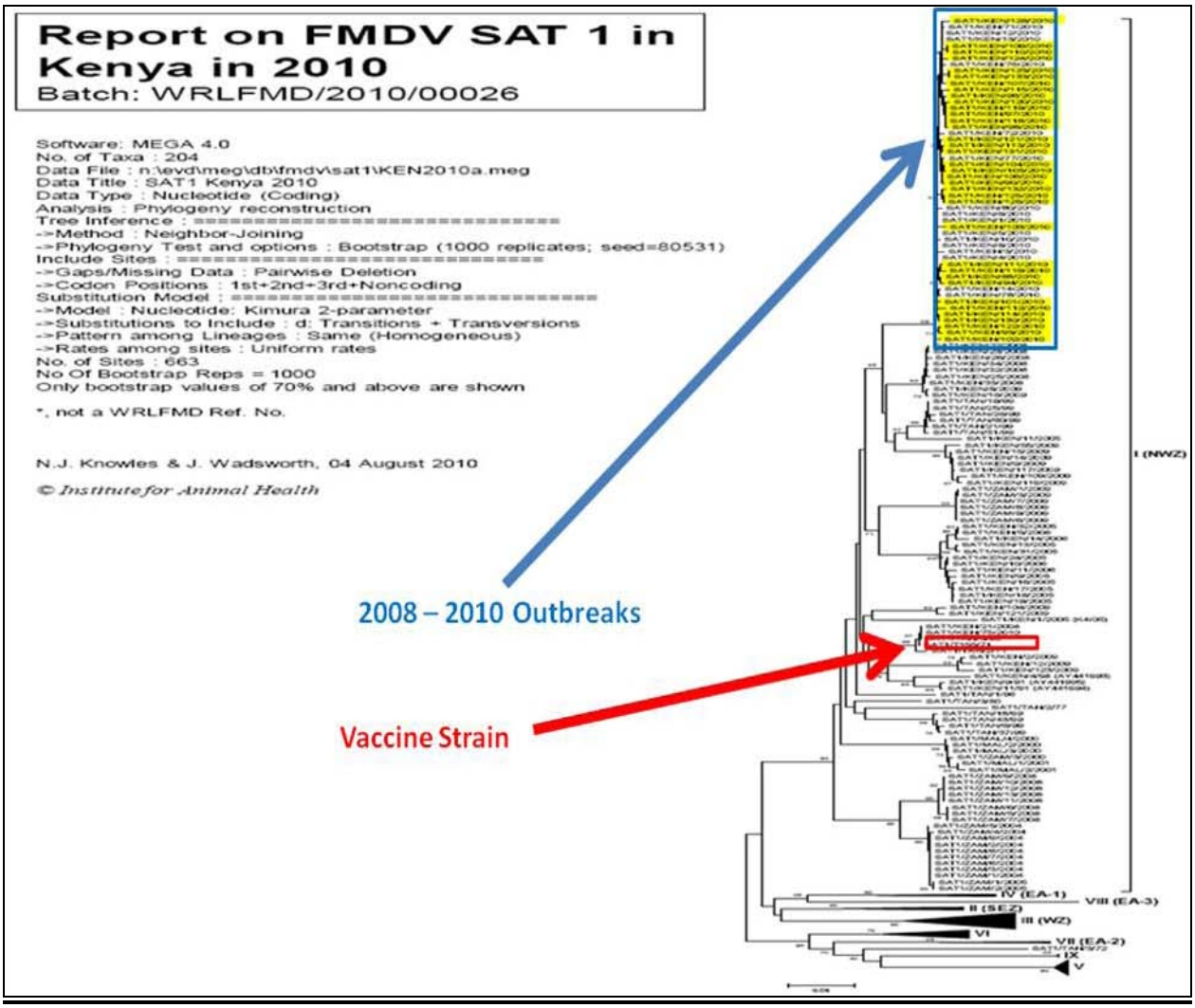

Fig. 4: Neighbour-joining tree of FMDV SAT1 viruses (© Institute for Animal Health)

Discussion

The serotyping reports have shown that SAT1 has early this year emerged as the most prevalent serotype in Kenya. In the recent past, the most prevalent serotypes have been O and SAT2 meaning that most of the readily available vaccine stocks have been against the prevailing strains of these two serotypes (DVS Kenya reports). The emergence of SAT1 as the prevalent serotype implies that sufficient stocks of vaccine relevant to the current strains had to be availed to the farming community within a short time. This can proof to be a challenge where routine characterization of strains is not being undertaken and there is only a single vaccine strain in use (Kitching et al., 1989). The clinical picture of 100% morbidity rates and as high as 50% mortality rates gave an indication of naivety of the populations affected by this SAT1 strains. This was further evidenced by the WRL revelation of genetically divergent strains to the current vaccine strain (WRL report). It is interesting that although belonging to the same topotype, it is apparent

that there was significant antigenic difference between these outbreak strains and the vaccine strain as demonstrated by the lack of protection observed in the field. The Kenyan 2009/2010 SAT1 outbreak strains were also closely related to viruses causing outbreaks in neighboring Tanzania implying regional spread of these strains. Though the genetic diversity may not necessarily imply that there is significant antigenic difference with the vaccine strains, it is an indicator of a change that calls for immediate antigenic matching and enhanced vaccine performance monitoring. Effective vaccine matching should be part of routine FMD vaccine production and quality control processes.

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3. Kitching R.P. 2002. Clinical variation in foot and mouth disease: cattle. *OIE Scientific and technical Review*, 21(3): 513-517.

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Acknowledgements

The authors thank the Director of Veterinary Services (DVS), Kenya for facilitating this work, all the District Veterinary Officers and the Kenya Wildlife Services for providing the samples. Much gratitude goes to all laboratory staff members at the FMD Embakasi laboratory for their support and hard work. The WRL is thanked for genetic characterization and serotyping reports.

VADEMECUM FOR FMD OUTBREAK DETECTION AND INVESTIGATION

european commission for the control of foot-and-mouth disease

Vade mecum for FMD outbreak detection and investigation

Example (prepared for Lebanon, 12/2009)

Foreword:

This document is prepared by the EuFMD Secretariat and is intended to assist veterinary services with the investigation and response to suspect FMD outbreaks. It should be adapted for application by field officers, and updated after changes in diagnostic capacity or experience.

- **Protocol for outbreak detection:**

In case of confirmation of the suspicion, suitable samples should be taken in order to confirm infection.

The investigation of suspicions should be made by competent persons under authority of the vet services, who understand the type of samples required for laboratory confirmation.

Penside tests (Svanodip – FMD) can be used on site or at the laboratory, but only with suitable samples from early cases.

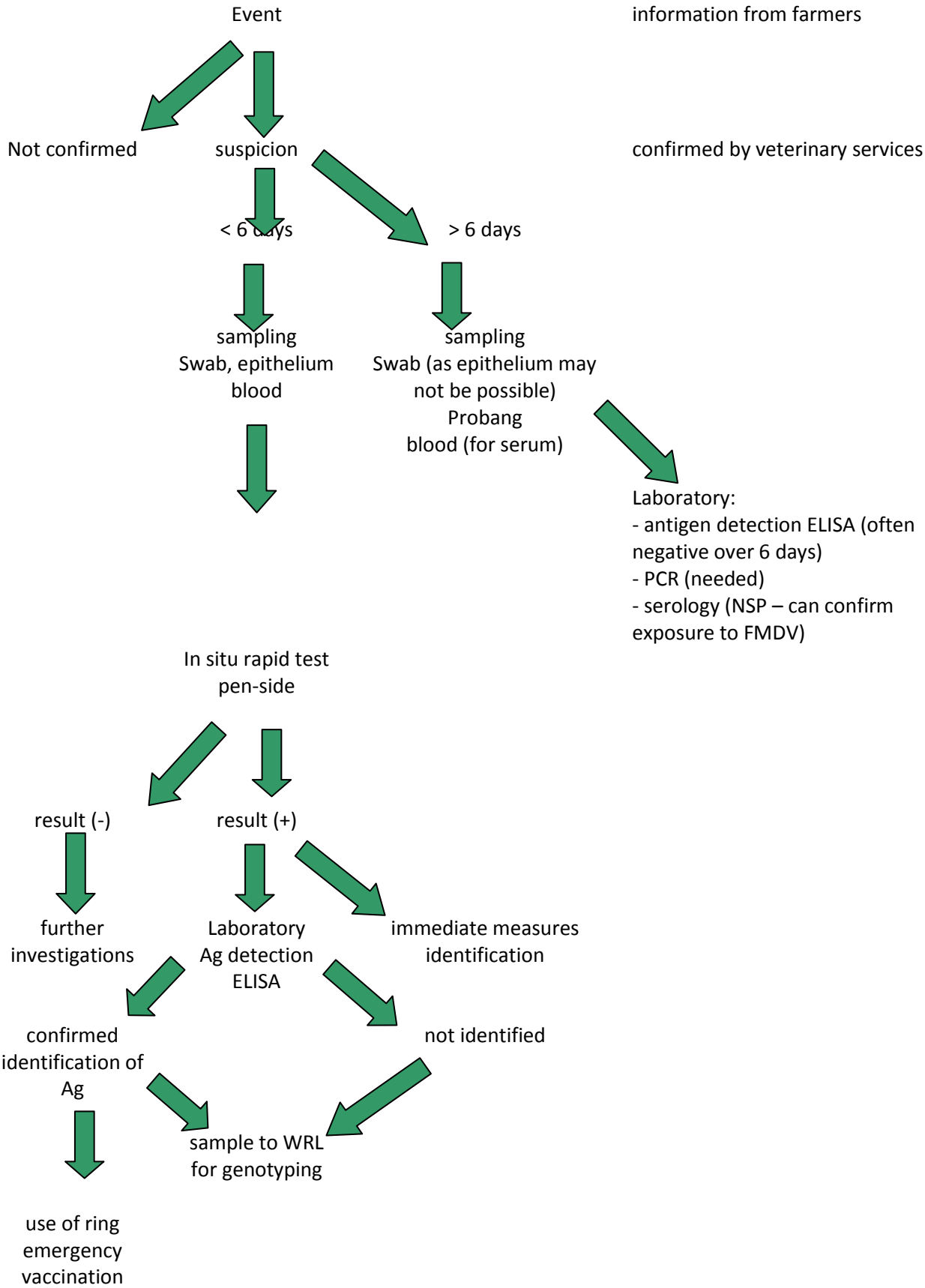
A range of samples, including whole blood for serum, are advisable if early lesions (<6 days) are not abundant.

The veterinarians collecting samples should complete tables for each animal, supplying information (table 1).

Table 1. Information collected on animals examined and sampled.

no	animal ID	species and sex[1]	age[1]	clinical signs						type of lesions						samples taken[5]	vaccination status[1]	estimated age of the oldest lesions
				lameness	fever[2]	salivation	foot[3]	mouth[4]	teats	intact vesicle	recently rup-tured vesicle	raw eroded area	ulcer with fibrinous scab	ulcer with fibrosis	indistinct break coro-nary band			

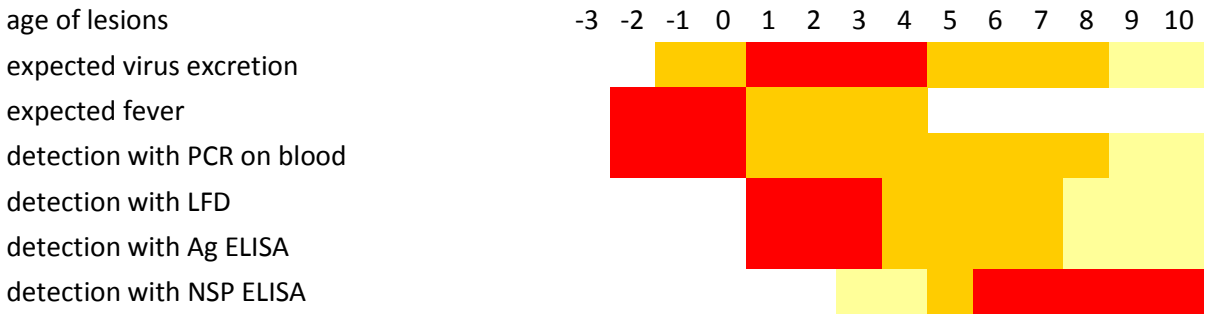
The flow of activities to arrive at a confirmation of FMDV is given in the following decision tree:



Decision tree for outbreak detection and investigation

The basis for use of tests is given below, and information collected at sampling used to compile tables such as shown in Tables 2 and 3.

Progression of disease as related to expected signs of disease and diagnostic detection:



Red = most likely time frame of detection
Yellow = likely time frame of detection
Pale yellow = less likely time frame of detection

In case of positive result, the immediate measures previewed in the CP should enter into force. These measures should include the treatment of animals and the disinfection of the premises.

- **<u>Official investigation to identify possible source and risk of spread</u>**

The official investigation should try to identify the oldest lesion in the epidemiological unit; the date of entry of infection is up to 14 days previously. The investigation should identify which events in this 14 day period may explain the entry of infection, such as entry of contaminated animals, or vehicles, and the most likely sources identified.
Further analyses should be done in order to identify the serotype involved in the outbreak, on an urgent basis with the aim to identify type within 24 hours and if the national lab cannot do this, by an international lab within 5 days. The authorities should apply a ring vaccination with a vaccine containing the involved serotype. For this purpose, a suitable monovalent type, or at least a bivalent vaccine A/O should be available for the national vaccination campaign.

The reporting of the investigation should provide the following information (example)
Information concerning the epidemiological unit:

- Province:
- Village (s):
- Name of owner (owners):
- Animal population susceptible to FMD, identification, type of husbandry...
- Maximal capacity:
- Maps – GIS code:

Information concerning the outbreaks (or epizootic):

- Timeline (from earliest to last event)
    1. Start of possible window of introduction of FMDV (date and possibly risk factor of introduction of FMDV, dissemination of the disease) (1-14 days before first signs)
    2. First observation of clinical signs (date)(=day 0)
    3. Notification of suspicion to veterinary services (date)
    4. Date of sampling
    5. Date of official investigation
    6. Date confirmed

- Clinical examination and sampling of the animals
    1. Describe (table 3)
    2. Clinical signs (table 3)
    3. Aging of lesions (table 1)

- Sampling
    1. Blood sampling
    2. Tissue sampling
    3. Pen side test
    4. Bio-security & disinfection
    5. Sending samples to the lab

- Vaccination history: Describe (date, vaccine, animals...)
- Results of the laboratory analysis (table 2)
- Source and spread of infection:
    1. most likely event that introduced infection in the unit
    2. time period when spread of infection most likely to occur from the epi-unit (= from 2-4 days before signs depending on species)

Table 2. Results of the laboratory investigation of the samples taken in the village (example of compilation)

animal ID	LFD	NSP	Ag ELISA	clinical signs	conclusion[3]
	+[1]	+	inconclusive	+ (2-3 d)	infected
1234567	+[1]	+	type O	+ (2-3 d)	infected
6789234	+[2]	+	-	+ (2-3 d)	infected

[1] epithelium and saliva

[2] epithelium and vesicle fluid

[3] conclusion based on references in table 3

Table 3: Animals examined and sampled in the village (example of compilation).

no	animal ID	species and sex[1]	age[1]	clinical signs						type of lesions						samples taken[5]	vacci-nation status[1]	estimated age of the oldest lesions
				lameness	fever[2]	salivation	foot[3]	mouth[4]	teats	intact vesicle	recently rup-tured vesicle	raw eroded area	ulcer with fibrinous scab	ulcer with fibrosis	break coro-			
1	12344567	bov / M	7 months	-	NT	-	-	-	-	-	-	-	-	-	-	B - S	not reported	
2	12324567	bov / M	not known	-	NT	-	-	-	-	-	-	-	-	-	-	B - S	not reported	
3	12234567	bov / M	7 months	-	NT	-	-	-	-	-	-	-	-	-	-	B	not reported	
4	12234567	bov / F	1.5 year	-	NT	+	-	LTD	-	-	-	-	+	+	-	B	not reported	6 to 7 days
5	12334567	bov / F	1.3 year	+	NT	+	C	G	-	-	-	-	+		-	B	10.2008	5 to 6 days
6	12344567	bov / F	1.3 year	+	NT	+	I	LG	-	-	+ (I)	-		+ (M)	-	B - E	10.2008	7 days
7	12345567	bov / F	1.5 year	+	NT	+	-	MLD	-	-	-	-	+		-	B - S - E	not reported	5 to 6 days
8	12345667	bov / F	1.3 year	+	NT	+	I	L	-	-	-	-	-	+	-	B	10.2008	7 days
9	12345677	bov / F	1.3 year	-	NT	+	-	ML	-	-	-	-	-	+	-	B	10.2008	7 days
10	12345678	bov / F	1.3 year	+	NT	+	I	LTD	-	-	-	+ (I)	+ (T)	+ (D)	-	B	10.2008	7 days
11	not identified	bov / M	not known	-	NT	-	-	-	-	-	-	-	-	+?	-	B - P	not reported	7 to 10 days

[1] information retrieved from the livestock information system

[2] NT: not tested (animals did not appear to have fever)

[3] foot: **C**oronary band – **I**nter-digital space / [4] mouth: **M**uzzle - **L**ips - **G**ums - **T**ongue - **D**ental pad / [5] samples: **B**lood - **S**aliva - **V**esicle fluid - **E**pithelium - **P**robang sample

NTC2-NOVEMBER2010

EuFMD training course
– Nakuru, Kenya – November 2010

Executive Summary

A five day course was convened in Nakuru, Kenya supported by FAO and the EuFMD Commission. Participants included veterinarians from Kenya, France, Latvia, Switzerland, Australia, New Zealand and United States. The course was organized with a one day symposium in Nakuru led by FAO and an FMD technical expert from the Institute on Animal Health Pirbright. Instruction was provided on aging of FMD lesions, biosecurity, sample collection and investigation technique. This was followed by two days of field visits in which three outbreaks were investigated and samples were collected and tested immediately in the Regional Veterinary Laboratory. The remainder of the course consisted of report writing, discussion of laboratory results and the epidemiological information that had been gathered.

Laboratory results yielded serotype O from Farm 2 and 3 visited. Non-structural protein antibody ELISA results were also obtained during the course. These results are presented in Appendix A and discussed in the relevant sections. This document contains laboratory results as of December 1, 2010.

Appendix B displays a map of farms visited and areas discussed in the epidemiology investigation.

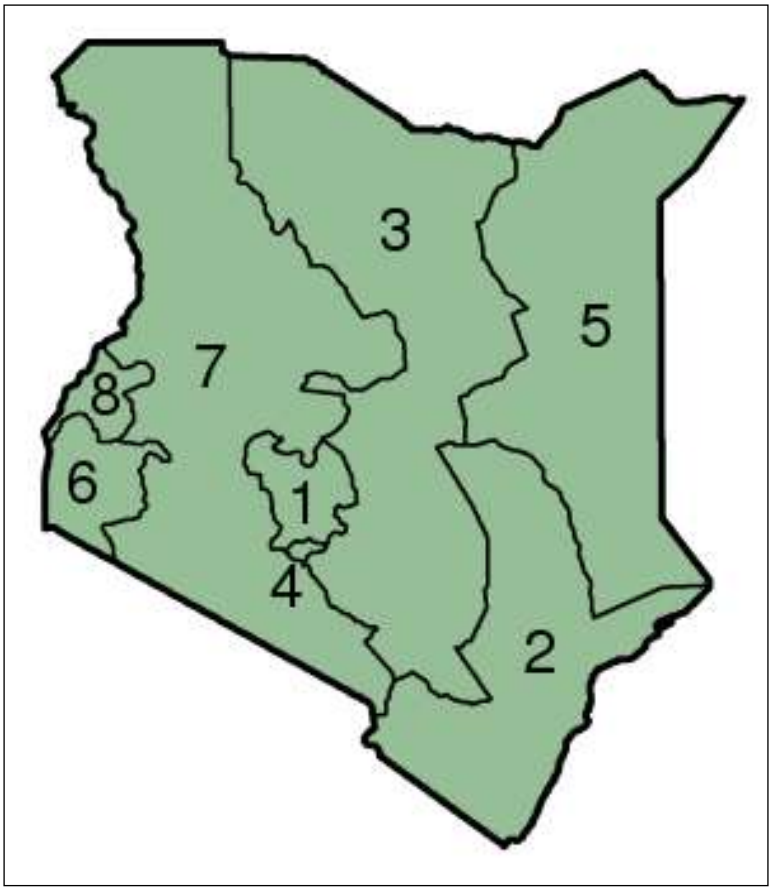
Participants had the opportunity to observe clinical cases of FMD, perform sample collection, conduct interviews with farmers, collate epidemiological information, practice biosecurity principles and asses laboratory results. This unique opportunity was made possible by the laboratory staff in Nakuru and Nairobi, farmers, district veterinarians and the EuFMD team.

Background

Veterinary services

The Department of Veterinary Services (VS) in Kenya undertakes disease control, investigation and disease surveillance. Kenya is divided into 8 provinces (Figure 1) each headed by a Provincial Director of Veterinary Services. The private sector performs the clinical services and artificial insemination.

Figure 1: Map of Kenya showing the 8 provinces



KEY:

1. **Central**
2. **Coast**
3. **Eastern**
4. **Nairobi**
5. **North Eastern**
6. **Nyanza**
7. **Rift Valley**
8. **Western**

The Rift Valley Province has 64 districts. Nine of these districts (Nakuru, Naivasha, Nakuru North, Rongai, Molo, Njoro, Kuresoi, Subukia and Gilgil) form the Nakuru county where the investigation took place. Each district is headed by a District Veterinary Officer (DVO) who performs the departmental mandate of disease control, vaccination, meat inspection and extension service. The DVO is supported by livestock officers and animal health technicians who are based in the divisions and locations. The regional veterinary laboratory in Nakuru performs disease surveillance and submits samples to the national laboratory in Embakasi, Nairobi where all FMD analysis is performed.

FMD Situation

FMD is endemic in Kenya and most outbreaks are reported in the Rift Valley Province which is the largest of the 8 provinces. To date, serotypes O, A, C, SAT1, and SAT2 have been detected in Kenya. Most common are serotype O and SAT2, however since 2008 SAT1 outbreaks have increased dramatically. Serotype C was last documented in 2004. The situation in wildlife is not well studied, but buffalos are known to harbour SAT serotypes. A random countrywide survey was conducted in 2009/2010 to obtain a better picture of FMD prevalence in the different animal species. Results will be available soon.

In recent years sporadic FMD outbreaks have been reported in all districts. In 2010 FMD, outbreaks appear to be more frequent and spreading faster than before. For the current calendar year, twenty three FMD outbreaks have been confirmed by the national FMD laboratory (Table 1). The main serotypes responsible for these outbreaks are SAT1 and O. Information of number of infected farms and species are not available, thus morbidity and mortality in the Rift Valley province cannot be estimated.

Table 1: FMD outbreaks confirmed by national FMD laboratory in Nakuru county and close neighbour districts from 01.01.2010 to 15.11.2010

serotype	date	District
O	02.08.2010	Nakuru Central
O	02.08.2010	Nakuru Central
O	30.08.2010	Laikipia West
SAT1	18.06.2010	Laikipia North
SAT1	24.06.2010	Laikipia Central
SAT1	28.06.2010	Laikipia East
SAT1	10.07.2010	Nakuru North
SAT1	16.07.2010	Njoro
SAT1	26.08.2010	Koibatek
SAT1	26.08.2010	Koibatek
SAT1	10.09.2010	Naivasha
SAT1	10.09.2010	Nakuru Central
SAT1	30.09.2010	Gilgil
SAT1	01.10.2010	Mogotio
SAT1	07.10.2010	Marigat
SAT1	08.10.2010	Nakuru North
SAT2	16.08.2010	Naivasha
NVR	15.01.2010	Naivasha
NVR	08.06.2010	Naivasha
NVR	22.06.2010	Naivasha
NVR	22.09.2010	Nakuru Central
NVR	30.09.2010	Nakuru North
NVR	30.09.2010	Gilgil

NVR= No virus recovered

Livestock distribution and movement

In Kenya there are 1.5 million beef cows, 1.25 million dairy cows, 9 million sheep, 12 million goats, 1 million camels and 30000 pigs. The numbers of animals in Nakuru County can be seen in Table 2.

a) Animal movements associated with trade:
   1. Laikipia → Rongai → Baringo
   2. Baringo → Nakuru → Naivasha → Nairobi
b) Animal movements associated with grazing:
   1. Naivasha → Nakuru → Laikipia;
   2. Narok/Kajiado → Navaisha → Nakuru → Rongai /Laikipia

Table 2: Livestock population in Nakuru county (data from 18.11.2010)

District	Cattle	Sheep	Goat
Naivasha	86161	120667	48915
Nakuru North	53000	23000	17000
Molo	23550	12580	1756
Njoro	32970	33550	23564
Rongai	126950	50847	70155
Nakuru	11500	2830	5480
Subukia	10000	14000	12000
Gilgil	n.a.	n.a.	n.a.
Kuresoi	n.a.	n.a.	n.a.

In Nakuru county there are 4 main livestock routes and about 10 livestock markets where animals move for trade or pasture (nomadic herds) from one district to another.

FMD diagnostics

FMDV antigen is detected using Ag ELISA , virus isolation using baby Hamster kidney cells (BHK) or calf thyroid cells (CTY) or the lateral flow device LFD as a penside test. All 5 serotyes (O, A, C, SAT1, SAT2) circulating in Kenya are screened. For PCR, samples for FMDV antigen detection are sent to the FMD world reference laboratory in IAH, Pirbright, UK. However, it would be useful to establish the PCR method in the local lab in the future to strengthen the diagnostic power in the affected region.

FMDV antibodies are detected using a test for structural proteins (SPs) for example virus neutralisation test (VNT) or liquid phase blocking ELISA (LBPE) which cannot differentiate between vaccinated and naturally infected animals. To be able to differentiate between those two antibodies non-structural proteins tests (the 3ABC and 3D ELISA) are used.

Probang, tissue, saliva and vesicular fluid samples are analysed using Ag ELISA and its results confirmed with samples which show a cytopathogenic effect (CPE) when passaged on BHK or CTY cells.

Serum is analysed using mostly VNT or otherwise LBPE. Animals which are known to be vaccinated, animals which come from an area where vaccination takes place and animals which are going to be exported are tested using 3-ABC or 3-D.

The most likely time for detection of virus is within the first 4 days after clinical lesions appeared, but could be possible up to 7 days and for PCR detection up to 10 days after the first clinical signs. Antibodies can be detected most likely after 7 days post infection (p.i.), and possibly already after 5 days p.i. The figure below represents the window of detection for various techniques or tissues.

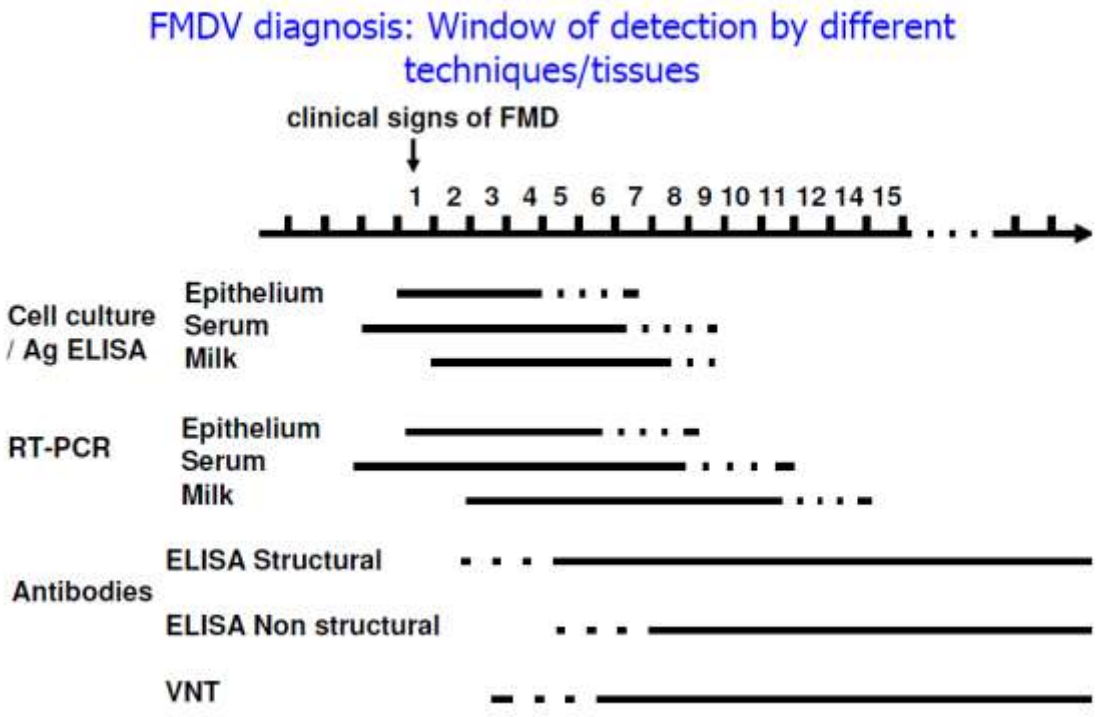


Figure 2: This figure depicts the window of detection by different techniques or tissues. *From: IAH*

FMD control history and vaccination

Until the early 1980s, FMD was controlled in designated areas of Kenya, including parts of the Rift Valley province with compulsory and free vaccination for cattle. Small ruminants were not vaccinated. This vaccination service achieved 80% vaccination coverage which enabled Kenya to access international trade markets. However, a change in government policy from 1986 introducing cost sharing with the farmers and an unavailability of government subsidised vaccines meant the vaccination coverage decreased below 10%. This resulted in more frequent FMD outbreaks. Currently, farmers can access more expensive vaccines from the Kenya Veterinary Vaccines Production Institute (KEVEVAPI) to supplement the DVO's supply. Vaccinations available for use in Kenya are multivalent formulations and contain either 3 or 4 of the serotypes O, A, SAT1 and SAT2.

When an outbreak is declared, quarantine is imposed on a location level. Movement may occur within the location but is restricted into and out of the location. Restrictions are placed on milk and other animal products. Ring vaccination around the outbreak is performed and nearby markets is closed. There are also restrictions on movement of animal feed out of the affected farm. The official in charge of the administrative unit assists with enforcement of quarantine.

Recommendations for further control and investigations of the outbreaks are provided below for each farm. These recommendations are based on the local situation and farm-level biosecurity measures. In contrast to an endemic country, control measures in an FMD free country vary per country and could range from culling all infected animals to vaccination to live based on rate of disease spread and other economic and political factors. Furthermore, all animal movements would cease and all possible movements of animals, animal products, humans and vehicles in the risk period would be traced back and forward to find all farms were the disease might have spread to. Ring vaccination, whenever possible, as well as a vaccination to live strategy, should be considered to control the outbreak. Policy varies per country for disease control measures yet the ultimate goal is to become disease free by OIE standards as rapidly as possible.

The investigation team

The NTC2 EU-FMD team was supervised by Nick Juleff (UK), Keith Sumption (FAO), Nadia Rumich (FAO) and consisted of local veterinarians (Eunice Chepkwony, Sabenzia Wekesa, Veronica Wanjohi, Abraham Sangula, Gitnui Kaba, Bernard Rono, William Birgen and Fredrick Mukendi), as well as veterinarians from other countries (Mary Vanandel , NZ; Jennifer Davis, AU; Laurie Fromberg, US; Mara Uzule-Springe, LA; Yann Villagi, FR; Silke Bruhn, CH). Roles were switched between a clinical investigation team taking care of the sampling on the farm as well as aging lesions and an epidemiological team who did interviews of the farmers and DVOs to gather epidemiological relevant information. Animals to sample were chosen based on looking for the oldest lesions on the farm to be able to trace back the highest risk period of FMDV introduction as well as for the youngest lesions to be able to still detect virus. Selection took place relying on the information provided by the owner as well as the appearance of clinical signs.

The epidemiological investigation was performed using a questionnaire developed by the group (Appendix C) aiding in the establishment of the probable timeline of important events. The possible period of introduction of the virus was estimated by subtracting 14 days from the day of the oldest known lesion. The high risk period was estimated at 2-5 days within the incubation period. The period of highest shedding was determined to be day 1 to 4 after the first and last animal displayed clinical signs.

Biosecurity measures

On arrival at each of the 3 farms visited, the investigating team vehicles were parked outside the infected area near the farm entrance. Protective clothing was donned by all team members. This consisted of disposable overalls, latex gloves (double gloving with taping at the wrist to the protective suit) and rubber boots.

On departure, the team cleaned the boots thoroughly with a brush with 0.5% Sodium Lauryl Ethyl Sulphate and a second washing with Dettol®. Finally the boots were disinfected by dipping them into 4% sodium bicarbonate solution. The car tyres of the vehicles were washed with 4% sodium bicarbonate solution.

At the end of each day the clothes worn on the farms were soaked overnight in a 0.2% citric acid solution and then laundered by the hotel.

Case farm 1 – Tere Estate

Farm history

Location

The farm is located in the Nakuru North district, Bahati division, Bahati location and Wanyororo B sublocation.

Production system

There were five cattle on the farm, along with approximately 15-20 chickens and 10 dogs. Cattle are dairy cattle and milk is sold.

Animals graze on the farm only. They do not share grazing or water with any other animals. However, they are fed a supplementary feed, a compounded product purchased from Mairu kumi shopping centre. The feed is purchased monthly and carried home from the shop in a bag. The last feed was purchased in late October. Mairu kumi is also in the Bahati division and has been experiencing outbreaks of FMD.

Farm biosecurity

The farm is relatively isolated with coffee plantations surrounding the area where the cattle are kept. However, no biosecurity measures were taken on the farm.

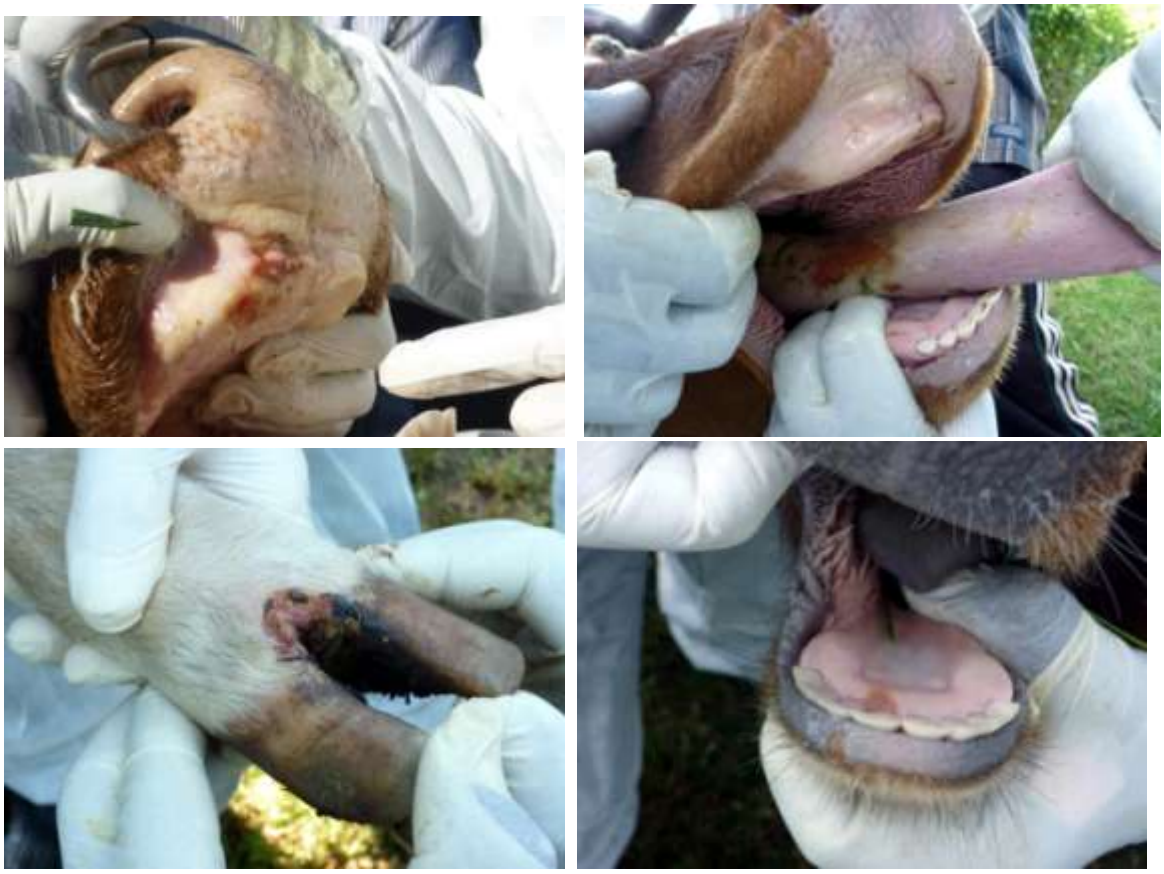
According to the owner of the cattle, there is no contact with wildlife.

Clinical examination and sampling

Only two animals are reported by the farmer to be showing clinical signs—Animal A and Animal C. Four animals were sampled- one cow, one bull, and two calves. Animals displayed clinical signs such

as salivation, lameness, and decreased milk production. All cattle displayed mouth lesions while three of the four cattle had feet lesions, one of which had a secondary infection. One cow also displayed possible scarring on the teats. Age of the mouth lesions ranged from 7 days to greater than 10 days. Serum samples were taken from all four of the cattle. A probang sample was taken on one of the calves. Epithelial tissue was taken from one of the cows and a lateral flow device (penside test) was conducted. The epithelial sample was too old yielding negative results to the penside test. All animals sampled were treated with multivitamins and antibiotics. Further details are provided in Appendix A: Clinical examination form – Farm 1. Serotype for this farm at the time the report was written was unknown.

Preliminary laboratory results yielded negative antigen ELISA results from the epithelial tissue from Animal A as well as negative cell culture from the epithelial tissue from Animal A. These results should be interpreted with caution as the epithelial sample obtained was likely too old. NSP test results yielded negative results from Animal A and weak positive results from Animal B, Animal C and Animal D. Since these lesions were dated between 7-10 days, the weak positives could possibly be due to this outbreak of FMD or due to previous vaccination (impure vaccine containing NSPs), previous exposure to any serotype of FMD or improperly aging the oldest lesions.



Top Left: Animal A (cow) dental pad lesion aged to about 8 days old
Top Right: Animal C (calf) tongue lesion aged to about 7 days old
Bottom Left: Animal C (calf) foot lesion
Bottom Right: Animal D (calf) lower lip and lingual periodontal lesion aged to greater than 10 days old.

Epidemiological findings

FMD & vaccination history

The animals have no history of vaccination as far as the farmer is aware. Two animals—Animal A and B—were purchased in July 2009 whilst two others were born on the property. The district veterinarian reported that vaccination had not been practiced in this area as there had been no outbreaks in the area.

Current outbreak

The farmer reported only two animals showing clinical signs.

Animal A, the first animal to show clinical signs, was observed to be limping on Wednesday 10th November. By Friday November 12th she was salivating and the farmer reported the potential outbreak to a private veterinarian. The outbreak was reported to the district veterinarian on Sunday November 14th; the district veterinarian visited the property on Monday 15th November. A second animal, Animal C, was seen to be limping on Saturday 13th November.

Animal movements and other contacts

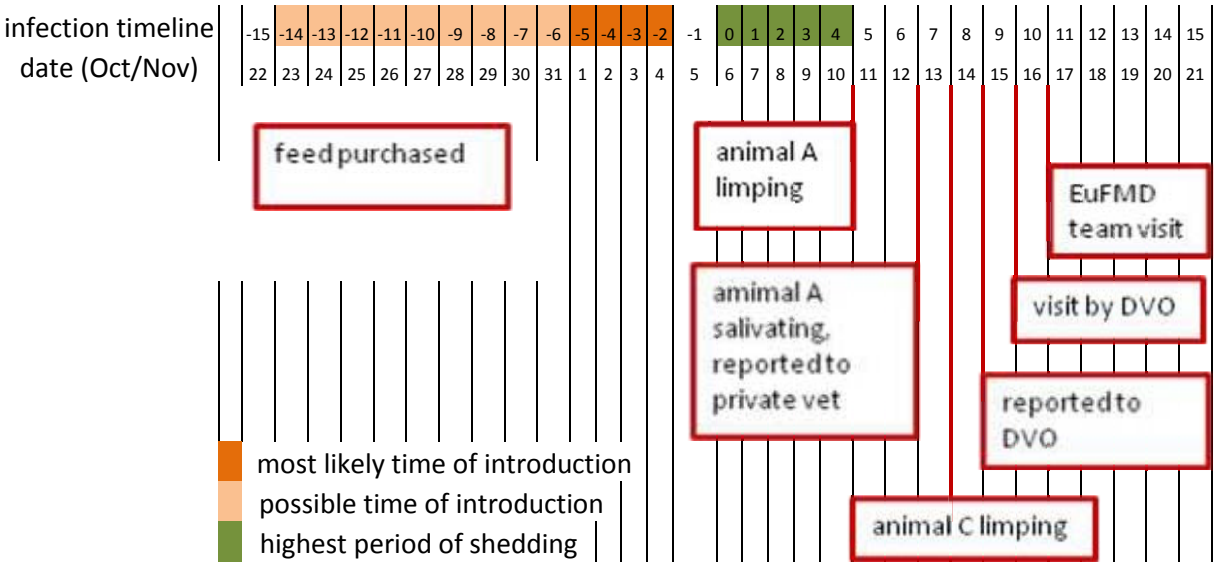
There have been no recent animal movement on or off the farm. No animal products have been moved onto the farm. Milk was moved off the farm prior to the outbreak however since quarantine has been imposed it has not been longer sold (also decreased volume due to production drop).

Temporary workers enter the farm daily and walk along a path shared by cattle. Workers come from surrounding neighbourhoods some of which have been experiencing FMD disease outbreaks.

The last visit by a veterinarian was in April/ May 2010 when a private veterinarian was called out to treat mastitis.

The monthly feed was last purchased in late October from Mairu kumi, where there have been FMD outbreaks in the surrounding area.

Timeline



Farm summary

Most likely source of introduction:

The purchased feed is considered a highly likely source of introduction due to the known FMD outbreaks in the area that it came from and the direct pathway to the animals.

Introduction by manure/ infectious material on shoes of workers coming onto the farm is considered possible but not the most likely.

Recommendations:

More information is required on farms in the locality. Although FMD has not been reported this does not rule out possible cases.

It is recommended that the farm remains under quarantine until the cases are resolved. Animals should be moved off the pathway shared with workers to try and prevent spread to other farms.

The farmer could implement basic biosecurity measures such as separating pathways for workers and trucks coming onto the farm from animals. Routine vaccination of animals should also be practiced against known circulating serotypes.

It is also recommended that feed retailers take measures to minimise spread of disease by avoiding purchase of feed from known infected areas. Farmers purchasing feed should also be aware and purchase feed locally or from areas not experiencing outbreaks of FMD.

Additional information that could be of value in this instance would include a map of the farm showing fences, farm boundaries, extent of resident animal ranges and location of water points. A graphical representation of roads and paths used by workers and stock could clarify local conditions and this information could clarify entry and exit pathways for disease.

Tracing information needs to be obtained if available for milk collection and feed purchases. This could allow neighbouring properties to be prioritised for examination.

Estimated risk of spread:

The farm is isolated and very small, so that virus shedding as well as possible routes to spread disease are minimal.

Case farm 2 – Solai Arus

Farm history

Location

The farm was located in Ruiyabei location and Arusito sublocation in Solai division.

The owner of the farm was Wilbert Mameti. The owner was not present for the visit and his wife answered the questions of the EuFMD team.

Production system

Farm had mixed beef and dairy production with approximately 50 cattle (30 adults and 20 calves/yearlings). There were also dogs and chickens on the farm.

The whole herd grazes together on the farmer's property only. The animals share a common watering point, on the property, with animals from neighbouring farms.

Farm biosecurity

There is no biosecurity on the farm. Animals from approximately six neighbouring farms enter the property daily for water from the common watering point. Most neighbouring farms have 30-50 cattle and some also have sheep and goats. However, the sheep and goats do not share the watering point.

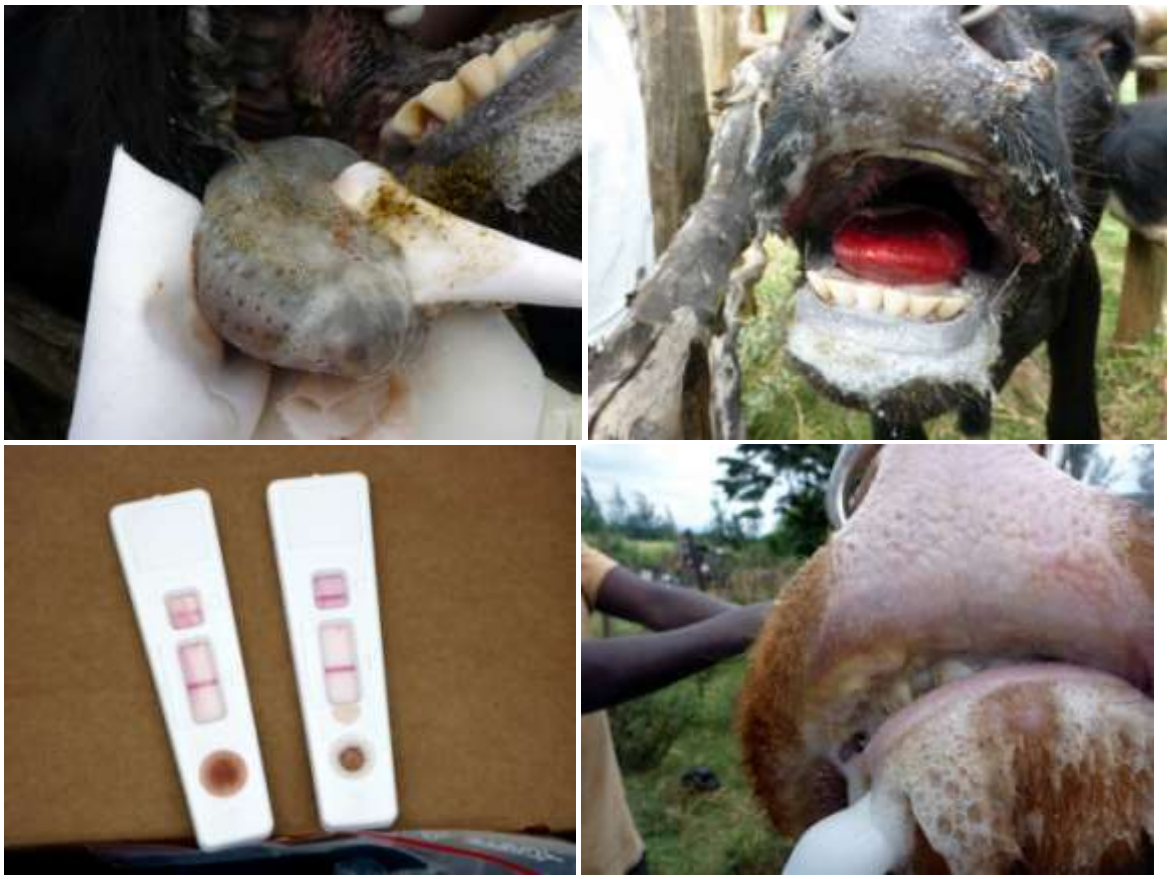
Clinical examination and sampling

Animals displayed clinical signs such as salivation, nasal discharge, swollen protruding tongue, and lameness. Two animals were sampled including one cow (Animal E) and one bull (Animal F). As most of the herd was pregnant, the farmer was concerned that the stress of examination and sample collection would further compromise the animal's condition. As typical lesions were noted in the examined animals and LFD penside testing yielded a positive result, it was decided that further examination was not in the best interest of the herd.

The farmer gave information that first clinical signs were two weeks ago thus it was not possible to examine the oldest lesions.

All animals sampled displayed mouth lesions. Due to being in a crush, foot lesions were not able to be safely visualized. Age of the lesions was estimated to be around one day old without including a margin of error. Serum samples were taken from all animals sampled. The cow sampled had a swollen protruding tongue with a large ruptured blister. While trying to grasp the tongue, the distal end was exfoliated and an epithelial sample was obtained. A Penside test was run with this epithelial tongue sample and yielded positive results. A probang sample was taken from the bull. An epithelial sample was also taken from the bull, but a penside test was not conducted. All animals were treated with multivitamins and antibiotics. Further details are provided in Appendix A: Clinical examination form – Farm 2.

Preliminary laboratory results yielded positive antigen ELISA results from the epithelial tissue from Animal E and Animal F as well as positive cell culture from the epithelial tissue from Animal E and Animal F. The virus was typed as serotype O. NSP test results yielded weak positive results from Animal E and Animal F. Since these lesions were aged at Day 1, the weak positives could possibly be due to previous vaccination with vaccine containing NSPs, previous exposure to any serotype of FMD or improperly aging of the oldest lesions.



Top *Left: Animal E (cow) with one day old ruptured vesicles on the tongue*
Top Right: Animal E (cow) with raw epithelium after outer layers of epidermis was removed during examination
Bottom Left: Positive Penside Test from Animal E
Bottom Right: Salivation and blanched vesicle on dental pad of Animal F (bull) aged to one day.

Epidemiological findings

FMD and vaccination history

The animals were vaccinated against serotypes O, SAT1 and SAT2 on the 11th September 2010 due to an outbreak of SAT1 in Nakuru and the proximity of this farm to a livestock market (approximately 1 km). Animals from many areas, including Nakuru come to the market to be sold.

Prior to this the last routine vaccination was approximately 18 months ago.

Some animals on affected neighbouring farms had been vaccinated and did not show clinical signs of FMD.

Current outbreak

Disease was first noticed in neighbouring farms approximately 3 weeks ago.

The first case on this farm was noticed by the farmer two weeks ago. One calf showed lameness and salivation. Disease then spread through the herd with the majority of cases noticed on Saturday 13th November. The farmer estimates approximately 20 animals appear to be affected.

The date that the outbreak was reported is unknown.

Animal movements and other contacts

Provisional quarantine was imposed on the whole administration unit when the market was closed. We do not know when this occurred.

No animals have recently been purchased or moved off the farm. There is daily movement of animals from neighbouring farms to the shared watering point. Local veterinarians suggested that the farmer may be trading animals and the procedure for return of unsold animals from market was unknown.

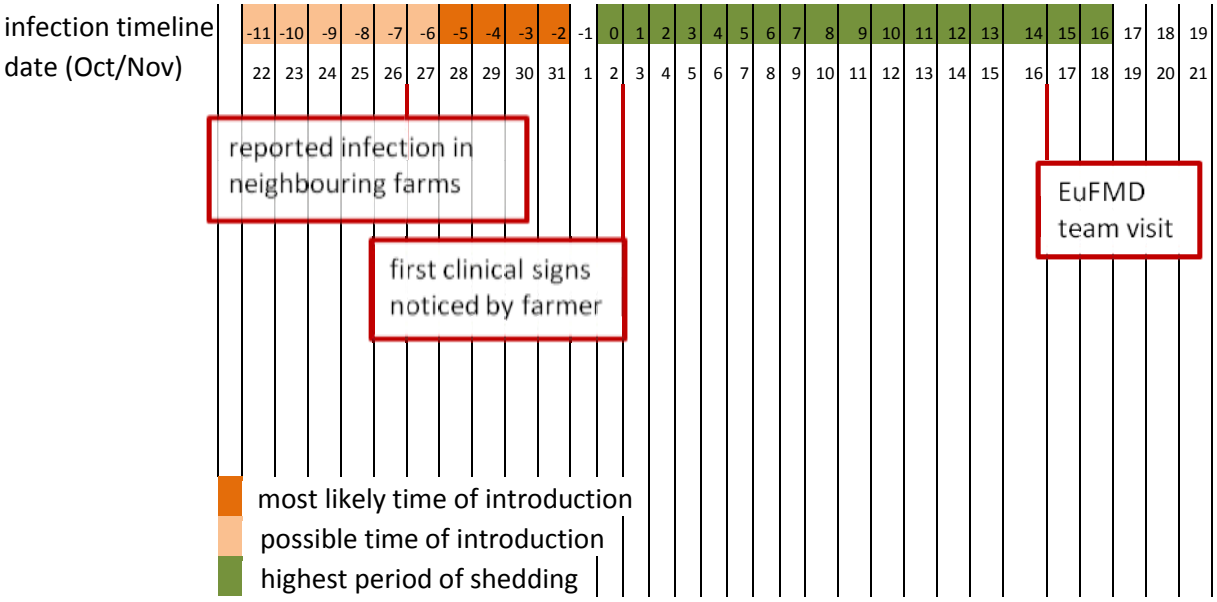
Human traffic between neighbouring farms is extensive.

Zebras are seen on the farm approximately once a month. No other wildlife is present on the farm.

The farm shares a spray pump for tick control with the neighbours with animals sprayed weekly.

The milk truck that visits the farm also visits a number of other farms.

Timeline



Farm summary

Laboratory results indicate FMD serotype O. Animals were vaccinated against this serotype approximately six weeks prior to the outbreak, antibodies, therefore, should be present. Vaccine failure could have possibly occurred resulting in these cows becoming infected.

We are assuming more than one transmission cycle occurred within the herd as fresh lesions were observed by the team despite the farmer reporting first clinical signs two weeks ago.

Most likely source of introduction

The most likely source of introduction to this farm was neighbouring farms, which showed signs of disease approximately a week before this farm. Introduction of FMD into the area was likely to be from the livestock market as outbreaks have been reported in nearby districts/ locations/divisions. Further information on the serotypes and exact locations of these outbreaks would be valuable.

Estimated risk of spread:

The District veterinarian reported that there was usually less spread of FMD during the rainy season (probably due to reduced animal movements) however this was not the case in the recent outbreak which had continued spreading through the rainy season.

Recommendations

Direct and indirect spread of the disease to neighbouring farms is highly likely due to shared water points and equipment. However, the administrative unit had been quarantined and milk from the farm was no longer being sold so extensive further spread is not likely. It is recommended that quarantine of the administrative unit as a whole is maintained until there are no further cases in the unit.

It is recommended that future vaccination is specific for the serotype and strain circulating in the area at the time of the outbreak.

Additional information that could be useful in the epidemiological investigation of this case includes more information about movements of animals and vehicles to stock markets as well as information on how animals that remain unsold at stock markets are treated- (i.e. are they reintroduced to the herd on their return?)

It would be of interest to perform active surveillance at stock markets to determine if clinically affected animals are present there. Sero-prevalence of circulating strains could be evaluated. Related to this point, it would be helpful to assess the knowledge of the market organisers to FMD spread and biosecurity. A sero-prevalence study and examination would also be useful within the herd examined and would create a more complete clinical picture of disease course This information would address the areas of uncertainty around the vaccination status of the herd and may be able to provide clarity on why infection with type O is present after vaccination for that serotype.

Antibodies should have been sufficient to protect against disease for this herd by around the 25th of September. The animals were reported to have been vaccinated previously 18 months before. Given this information, protective levels of- immunity should have been reached by the time of the disease challenge Information on the reason for vaccination failure is of value both at a district and at a herd level. Obtaining additional information from the manufacturer about the batch of vaccine used on this herd would be helpful. Other herds in the area vaccinated with the same batch could be prioritised for investigation to look for clinical disease and also for serology if necessary. For the sake of completeness, preservation of the cold chain and vaccination technique could be checked. The manufacturer may also wish to conduct testing to ensure efficacy of the vaccine. Another point raised in by the Kenyan veterinarians conveyed that infection with Type O seldom results in such severe mouth lesions as those seen on this farm. The question of the efficacy of the use of sodium

bicarbonate as a topical treatment was also raised. Some felt that application of sodium bicarbonate may have led to more severe mouth lesions.

Case farm 3 – Eden Farms

Farm history

Location

Kampimoto

Production system

This is a dairy operation with 44 bovines on the property. The cattle run as one herd. Currently the bull is kept in a separate camp. Two heifers and two steers are also separated.

There are 11 Dorper-cross sheep on the property. The sheep and cattle co-graze and share a water source.

Dairy meal is bought in weekly from Nakuru for the calves. Other feed for the animals is produced on the farm.

Dogs and poultry are present on the property.

Farm workers are resident on the premises.

Farm biosecurity

The farm is well contained with an electric fence and a hedge. There is only one entrance to the property on a well travelled road. The farm workers live on the premises, therefore, human movements are limited compared with some production units.

The neighbouring farm boundary is approximately 600m away and animal contact with the neighbouring property is unlikely. The neighbouring farm has in excess of 100 bovines and has experienced a severe acute FMD outbreak with reported mortality within the last 3 weeks.

There is no contact with wildlife.

Clinical examination and sampling

Seven animals were examined; five cattle and three sheep. Clinical signs observed were salivation, nasal discharge and limping. Lesions were observed on four cattle and one sheep. The age of lesions on the cattle ranged from three days to more than seven days. The lesions observed on the sheep were approximately ten days old. Serum was collected from all sheep and four of the cattle. Epithelium was collected from three cattle and penside tests were conducted. Two tests were positive for foot and mouth disease virus antigen while one was negative. A probang sample of orophangeal fluid was collected from one cow. All animals sampled were treated with multivitamin and antibiotics. Further details are provided in Appendix A: Clinical examination form – Farm 3.

Preliminary laboratory results yielded positive antigen ELISA results from the epithelial tissue from Animal 2 and Animal 3 as well as positive cell culture from the epithelial tissue from Animal 2 and Animal 3. The virus was typed as serotype O. NSP test results yielded a strong positive from Animal 1 and Animal 3, weak positives for Animal 4, Animal 5 and Animal 6 (sheep). NSP test was negative for Animal 2, Animal 7 (sheep) and Animal 8 (sheep). Since these lesions were aged between day 3 to greater than 10 days, the positives could possibly be due to previous vaccination with vaccine containing NSPs, previous exposure to any serotype of FMD or improperly aging of the oldest lesions.

Top Left: Animal 2 (bull) salivating.
Top Right: Note blanching of epithelium on the tongue representing an unruptured vesicle (bull).
Bottom Left: Animal 3 with lesions along the dental pad aged at 5 days old (cow).
Bottom Right: Animal 7 (ewe) with lesions on the tongue aged to >10 days.

The animals kept separate from the main herd, included the bull, two heifers and two steers also displayed clinical signs of FMD despite of being kept separate from the herd.

Epidemiological findings

FMD & vaccination history

Bovines on this farm were vaccinated by the district veterinarian as a routine measure last year. The vaccination took place more than 6 months ago. The vaccination was performed with a trivalent vaccination containing O, A and SAT1 serotypes.

Good biosecurity measures have prevented any previous outbreaks on this property.

Current outbreak

The farmer reported the first clinical signs appeared on the farm on the 15th of November (2 days before the EuFMD team visit). The clinical signs noted were salivation, anorexia and depression. Only two days after the appearance of the first clinical signs, 37 of the 44 bovines present on the property were affected.

The sheep on the property were reported to be limping.

Animal movements and other contacts

Movements on and off the property were well documented by the farmer.

As previously noted, farm workers reside on the property. Some visitors to the farm workers may have gone unrecorded.

Milk is collected daily by Brookside, a local dairy purchaser.

Hay is purchased by neighbouring farmers on the farm. This is a daily occurrence.

Hay was harvested starting on the 29th of October and the harvesting is ongoing at this time. Harvesting is performed by a contractor who also performs this service on neighbouring farms. Cattle were moved onto the field where hay was harvested on the 13th of November.

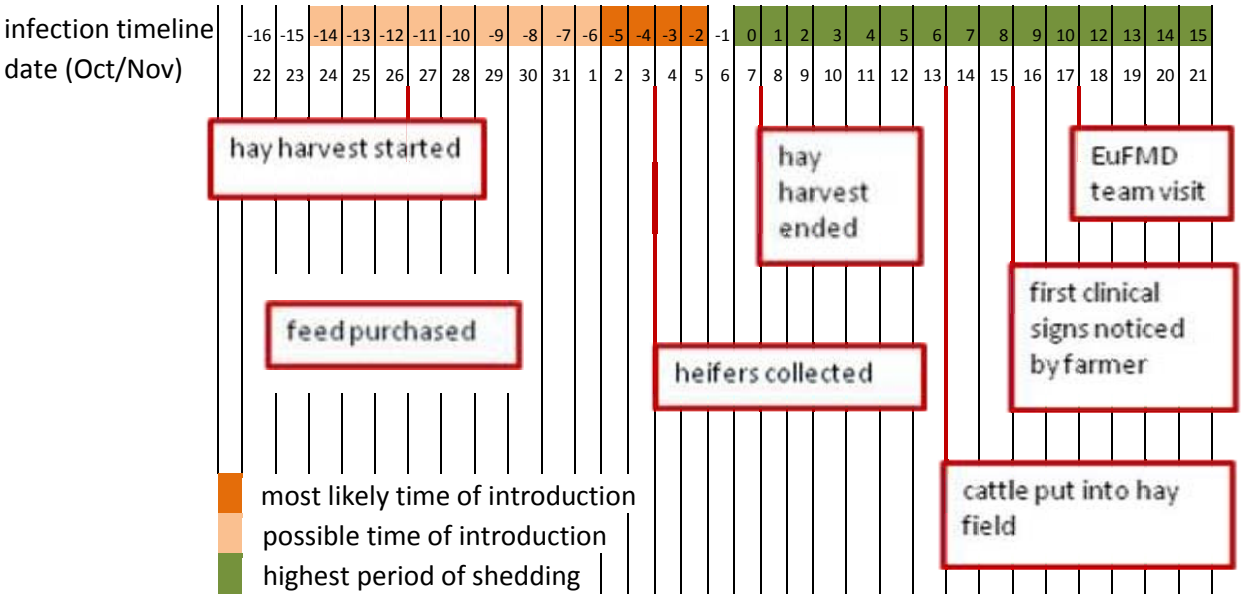
Weather conditions two weeks ago were cool and humid.

A veterinary practitioner visits this farm every fortnight. No artificial insemination services have been used on this property in the last 9 months. The bull which was examined was brought onto the property in place of the artificial insemination between 9 months and one year ago.

Heifers were sold off the property on the 3rd of November and collected by a livestock transport company. These animals were taken to the Karichu district.

There is no record of any new animals being brought onto the farm in the last month.

Timeline



Farm summary

Potential sources of introduction are listed in order of likelihood.

The livestock transport truck is considered to be high risk both because of the temporal location of the visit and because of the high levels of exposure to large numbers of animals. From the age of the lesions examined and the history given by the farmer, it seems that the sheep were infected first. However it is possible that older and less severe lesions have gone unnoticed in the cattle herd.

The vehicles used to purchase hay from the property are also possible in the disease pathway. Introduction by manure/ infectious material on the wheels of the vehicles and the shoes of people coming onto the farm is considered possible.

The contractor who harvested the hay from the property is also a possible point of infection. Equipment used for cutting and baling hay may have been a mechanical vector for infectious material.

The milk collection vehicle is a possible vector of disease though less likely.

Human movements onto the property are not logged and are a possible entry pathway.

The neighbouring farm is reported to be involved in an acute, severe outbreak with some cows having died. This property should be regarded as a possible source of aerosol transmission. In the preceding 3 weeks, the weather has been humid and rainy. These conditions could be consistent with aerosol transmission whilst the likelihood of infection to this farm by aerosol transmission is low

Recommendations:

More information is required on farms in the locality. It would be beneficial to know the strain present on the neighbouring farm. Further tracing is required to clarify the disease state of the heifers transported off the property and the other animals present on the farm that they were taken to.

It is recommended that the farm remain under quarantine until the cases are resolved. Contact with any outside animals should be eliminated and animal-human contact limited as much as possible. Ideally sales of hay and milk should be halted although this may not be feasible. The sale of hay from the farm is a high risk pathway of spread to further properties.

To prevent future outbreaks the farmer could implement further biosecurity measures such as a vehicle disinfection station on the single access road. Additionally, routine vaccination of all bovines at 6 month intervals with a quadri-valent vaccination containing O, A, SAT1 and SAT2 would provide a further level of protection. However, tri-valent vaccines are much more common and less expensive in Kenya.

It is also recommended that feed retailers take measures to minimise spread of disease by avoiding purchase of feed from known infected areas. Farmers purchasing feed should also be aware and purchase feed locally or from areas not experiencing outbreaks of FMD.

Additional information that would be of benefit in this case includes accurate information on the locations that the heifers that were moved off the farm were relocated to. Heifers were sold to Kerichu and they should be traced to investigate if the outbreak spread from Farm 3 to other farms. The truck which picked the heifers up on Farm 3 may have introduced FMD on Farm 3. This truck came from Kipkelion, a city close to the area Kerichu which has been experiencing a type O FMD outbreak. Sequencing the isolates obtained on Farm 3 and from the outbreak in the Kerichu area would provide evidence of a link between the outbreaks.

Estimated risk of spread:

The timelines show that farm 3 was infected before farm 2. Although the two farms were infected with serotype O it is not likely that farm 3 infected farm 2. Further tracing information would be needed to try to link these farms, if possible.

Appendix A:

Clinical examination form– Farm 1

CLINICAL EXAMINATION FORM

Name of Owner/ Household: Tere Estate

Date: 16 November 2010

Animal ID:	Species	Age of animal	Vaccination Status of Animal	Clinical Signs	Temp	Vesicular Lesions on Mouth and/or Feet	Age of Lesions	Samples Taken	Penside	NSP Results	Epithelial sample results:	Comments
A	Bovine cross breed cow	4	Not vaccinated	BCS 2, lameness, not lactating	37.9	Y(M&F)	8d old	Tissue from the lesions on the foot and serum sample	Negative- sample too old	-ve (14%)	Ag ELISA: negative Cell culture: negative	Treat with multivitamin and antibiotics Lesions are old and healing in the mouth. Foot lesions appear to be secondarily infected. There may be some scars on the teats- but they are not in the same time line as the mouth & foot lesions &there is no evidence to support that they are caused by FMD.

B	Bovine cross breed bull	2	Not vaccinated	BCS 3.5	38.1	Y (M)	8d Plus	Serum sample	Not done	+ (79%)		Small lesions filling up already and healing
C	Bovine cross breed calf	18mo	Not vaccinated	BCS 3 Lame and salivating	38.9	Y(M&F)	7d mouth 7 d foot	Serum sample, probang ++	Not done	+ (74%)		2 notable lesions on the tongue, one small on the tip and one large on the dorsum of the tongue, concurrent joint effusion hind limb at the level of the hock
D	Bovine cross breed calf	1yoa	Not vaccinated	BCS 2	39.4	Y (F&M)	10d plus	Serum sample	Not done	+ (79%)		Healing lesions in the mouth, this animal has a history of eating plastic packets and appears to have an impacted rumen.

Clinical examination form – Farm 2

CLINICAL EXAMINATION FORM

Name of Owner/ Household: Solai Arus

Date: 16 November 2010

Animal ID:	Species	Age of animal	Vaccination Status of Animal	Clinical Signs	Temp	Vesicular Lesions on Mouth and/or Feet	Age of Lesions	Samples Taken	Penside	NSP Results	Epithelial tissue results	Comments
E (Pembe)	Bovine cross breed cow	3 years	serotypes O, SAT1 and SAT2 on the 11th September 2010	BCS 2.5 Salivation, nasal discharge, painful, protruding tongue	41.5	Y(M)	D1	Tissue from the lesions in the mouth serum sample	Positive result	+ (75%)	AgELISA: positive Cell culture: positive	On exam, the tongue has a blister ruptured leaving the most distal part of the tongue without epidermis. The owner reports that this animal is gravid.
F	Bovine cross	4 years	serotypes O, SAT1	BCS 3.5, salivating,	40.5	Y (M)	D1	Serum sample Probang	Not done	+ (79%)	AgELISA: Positive	Lesions present on

(Chetakampuni)	breed bull		and SAT2 on the 11th September 2010	lame				sample of oropharyngeal fluid Tissue sample from the mouth			Cell culture: positive	the dental pad Fluid aspirated from the vesicle and tissue samples collected from the the region.

Clinical examination form – Farm 3

CLINICAL EXAMINATION FORM

Name of Owner/ Household: Eden Farms

Date: 17 November /11/2010

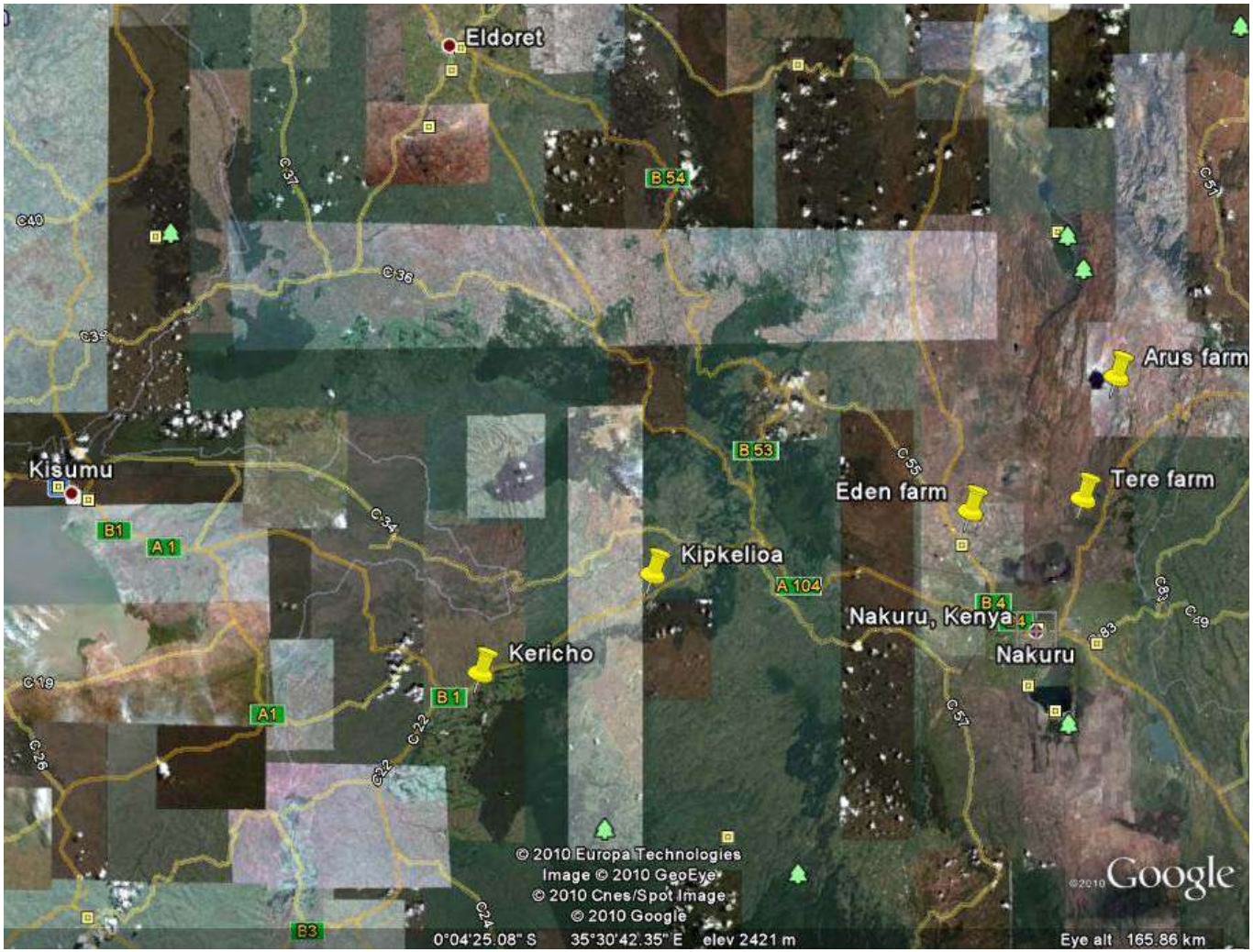
Animal ID:	Species	Age of animal	Vaccination Status of Animal	Clinical Signs	Temp	Vesicular Lesions on Mouth and/or Feet	Age of Lesions	Samples Taken	Penside	NSP Results	Epithelium Test Results	Comments
1	Bovine, Holstein cross Cow	Older than 5 years old	Serotypes O, A and SAT1 about 1 year ago	No lesions	40.94	No lesions		Serum	Not done	++ (83%)		
2	Bovine Holstein cross bull	3 yoa	Unknown	BCS 3 Salivation, nasal discharge	40.39	Y (M)	D3	Serum sample Epithelial sample	positive	-ve (44%)	AgELISA: positive Cell Culture: positive	Mouth treated with sodium bicarbonate yesterday – lateral flow device penside test still positive. Lesions on the tongue are about 1 day old and those on the dental pad about 3d old

3	Bovine Holstein cross breed cow		Serotypes O, A and SAT1 about 1 year ago	BCS 2 Nasal discharge	39.36	Y(M)	D5	Epithelium	negative	++ (87%)	AgELISA: positive Cell culture: positive	Lesion present on the dental pad granulating lesion with fibrin still present
4	Bovine Holstein cross cow		Serotypes O, A and SAT1 about 1 year ago		39.1	Y(M)	D7+	Serum Orophangeal fluid collected with probang	Not done	+ (64%)		It is reported by the farmer that this animal was the first affected and started with clinical signs on the 15th of November
5	Bovine Holstein cross calf	8 months	Unknown	Salivating, limping	40.3	Y(M&F)	D5	Serum, Epithelial tissue from the mouth lesions (used for Penside only)	positive	+ (59%)		Lesions on the upper lip, the dental pad and the tip of the tongue, fibrin filled and starting to granulate. Granulating foot lesions between the cleats.
6	Dorper cross ovine, ram		Not vaccinated	Nasal discharge, limping	NT	No lesions		Serum	Not done	+ (59%)		Abscess on the foot, no mouth lesions
7	Dorper cross ovine, ewe		Not vaccinated	Nasal discharge	NT	Y (M)	D10+	Serum	Not done	-ve (37%)		No foot lesions noted. The mouth lesions are present on

												the dorsum of the tongue, with no lesions on the dental pad (expected location for sheep)
8	Dorper cross ovine ewe		Not vaccinated	Nasal discharge	NT	No lesions		Serum	Not done	-ve (42%)		No lesions on the feet or in the mouth

Appendix B:

Overview map of farms visited (Arus Farm, Tere Farm and Eden Farm). Kipkelion is where the truck which obtained heifers from Farm 3 originated. The heifers were delivered in Kerichu. Outbreaks have been confirmed as serotype O in Kerichu many weeks prior to the outbreak on Farm 3.



Appendix C:

People to interview

District official veterinarian
farmer of outbreak farm
animal technician (animal health assistance) of this village – reported outbreak
private livestock officer

History

FMD (last year and last outbreak) what serotypes?
Levels: Province, District, village, Farm

Vaccination (incl. what serotypes?)

Routine vaccination:
Outbreak vaccination:
Levels: District, village, Farm

Animal movements

- In and out

a) Levels: District(in the last 3 months), village (in the last month), Farm(in the last 3 weeks, depending on age of lesions)

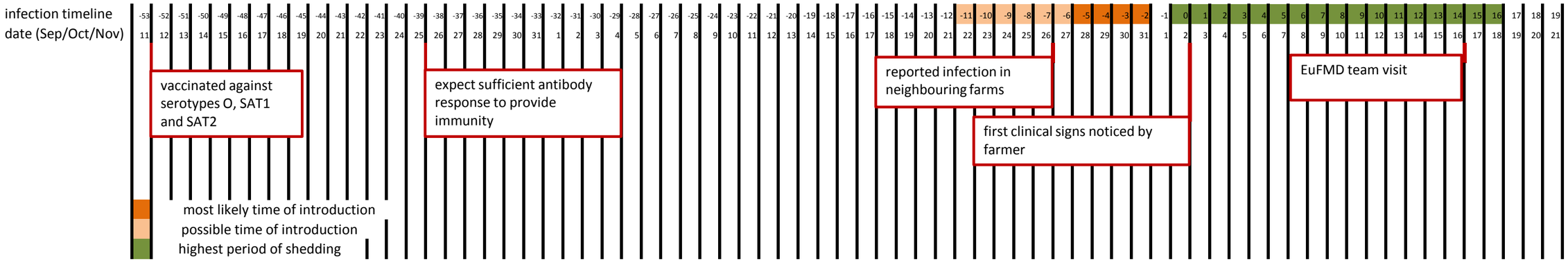
Map

number of farms incl. number of susceptible species in the 1km zone

A) number of farms incl. number of susceptible species in the 3km zone
B) number of farms incl. number of susceptible species in the 10km zone
C) markets, coming water places, common grazing, common dippings
D) wildlife (national parks)?

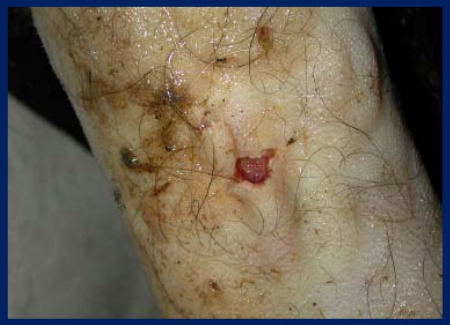
Case history:

Report from clinical team
How many animals (species, age distribution) on farm,
date of first clinical signs (what kind)
Source of feed (for the 1 week before day of most likely introduction)
Other farmers with ill animals, when?
Shared equipment with other farmers
Animal products traded
Biosecurity measures on farm (in general and after outbreak, especially vermins)
Other animals on farm and are they moving? Where?
Contact to wildlife (how often, what species)?
Personnel movements (especially those who have direct contact to animals) – 2 weeks before first clinical sign
- In and out
Animal products (milk, meat, by-products (incl. dead animals)) – 2 weeks before first clinical signs
- In and out


infection timeline
date (Sep/Oct/Nov)
vaccinated against serotypes O, SAT1 and SAT2
expect sufficient antibody response to provide immunity
reported infection in neighbouring farms
first clinical signs noticed by farmer
EuFMD team visit
most likely time of introduction
possible time of introduction
highest period of shedding

ESTIMATING AGE OF LESIONS

Cattle - Day 1

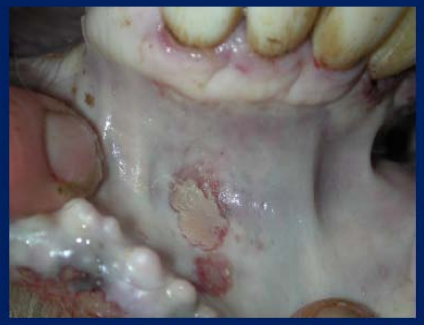


One day old vesicle, ruptured when tongue drawn from mouth

Cattle - Day 3

Lesions start to lose their sharp demarcation, fibrin deposition starts to occur on edge of lesions

Cattle - Day 5



Note progressive loss of lesion margination and extensive fibrin infilling

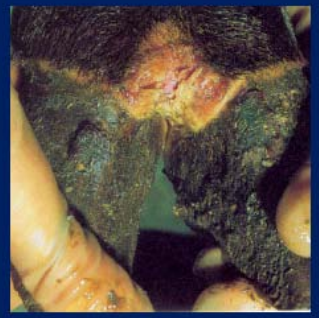
Cattle - Day 7

Field example. Extensive scar tissue formation and healing has occurred. Some fibrin deposition is usually still present

Cattle - Day 3

Note friable epithelium, deposition of fibrin starting to occur

Cattle - Day 7



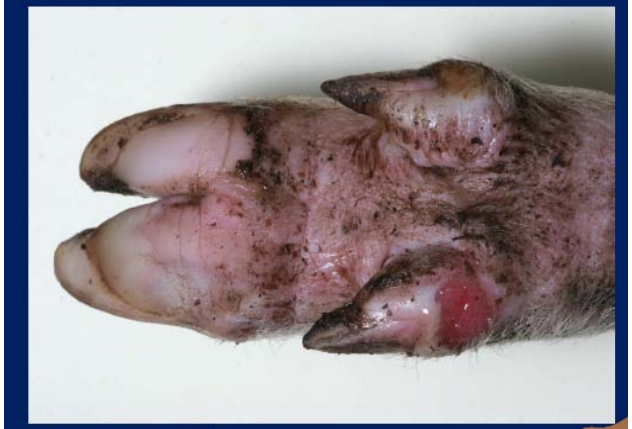
Healing progressing under the necrotic epithelium

eofmd
european commission for the control of foot-and-mouth disease

© Nick Juleff, IAH

Sheep - Day 1

Pigs – Day 3



Supernumerary digits

Pigs

- Most information obtained from foot lesions
- If lesion is at coronary band < 1 week old
- Thereafter horn grows at 1mm per week

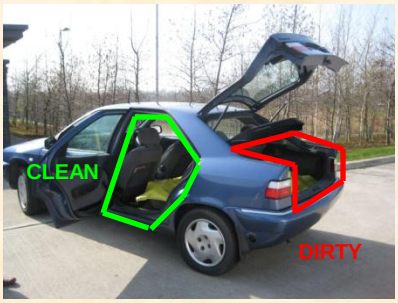
Estimating the age of lesions

Day of Clinical Disease	Appearance of lesion
Day 1	Blanching of epithelium followed by formation of fluid filled vesicle
Day 2	Freshly ruptured vesicles characterised by raw epithelium, a clear edge to the lesion and no deposition of fibrin
Day 3	Lesions start to lose their sharp demarcation and bright red colour. Deposition of fibrin starts to occur.
Day 4	Considerable fibrin deposition has occurred and regrowth of epithelium is evident at the periphery of the lesion.
Day 7	Extensive scar tissue formation and healing has occurred. Some fibrin deposition is usually still present.

PROTOCOL FOR PROTECTIVE CLOTHING

PROTOCOL FOR PUTTING ON PROTECTIVE CLOTHING (FMD)

1. Prepare equipment & PPE (protective clothing) before travelling



2. Prepare “clean” & “dirty” areas in car

3. Remove watch & jewellery before leaving car

4. Place mobile phone in zip-lock bag

5. Carry equipment to site in plastic bag

6. Identify suitable location for clean/dirty separation

7. Prepare clean & dirty disinfection points at hygiene barrier

8. Put on disposable suit

9. Put on waterproof suit

10. Put on boots – putting legs of disposable suit inside boots

11. Pull down legs of waterproof suit outside boots

12. Put on boot covers

13. Put on two pairs of disposable gloves

14. Pull sleeve of disposable suit down between the two pairs of gloves

15. Tape outer glove to sleeve of disposable suit

16. Pull down sleeves of waterproof suit outside gloves

17. Carry equipment onto site

18. When on site, do not remove mobile phone from zip-lock bag

PROTOCOL FOR TAKING OFF PROTECTIVE CLOTHING (FMD)

1. Disinfect equipment & outside of sample boxes/bags

2. Place equipment & samples in equipment bag on clean side

3. Remove boot covers & place in waste bag for disposal

4. Disinfect legs of waterproof suit

5. Clean soles of boots thoroughly & disinfect

6. Disinfect remainder of waterproof suit

7. Remove waterproof suit

8. Place suit in PPE bag on “clean” side

9. Remove first outer glove – touching only the outer surface

10. Remove second outer glove by hooking thumb under cuff - place in waste bag

11. Step onto clean side & disinfect boots

12. Remove disposable suit & place in waste bag on “dirty” side

13. Remove first inner glove – touching only the outer surface

14. Remove second inner glove by hooking thumb under cuff - place in waste bag

15. Double bag & seal the equipment & PPE bags

16. Disinfect outside of bucket

17. Disinfect hands using 6-step procedure

18. Wipe face with wet-wipes

19. Pour disinfect over plastic mat & place mat in waste bag

20. Place equipment & PPE bags in “dirty” area of car

CLINICAL EXAMINATION FORM

Clinical Examination Form (can be adapted)

Name of the Owner/Household: ..... Farm ID: …… Epidemiological unit:

Date:

Animal ID	Species	Type	L/S/M/PD/A	Temp.	Vesicular Lesions on Mouth and Feet Y/N	Age of Lesions	Samples **T**issue/**B**lood/**S**erum	Number of Photos	Comments
if no eartag, give order of animals examined..No. 1, 2 etc	bov.	calf	-	n.m.	Y (M + F)	> 7d		PA 130073, PA 130077	

L: … Lameness, S: … Salivation, M: … Drop of Milk Yield, PD: … Perinatal Death, A: ……Abortion

Estimated age of oldest lesion seen:

…My Notes…