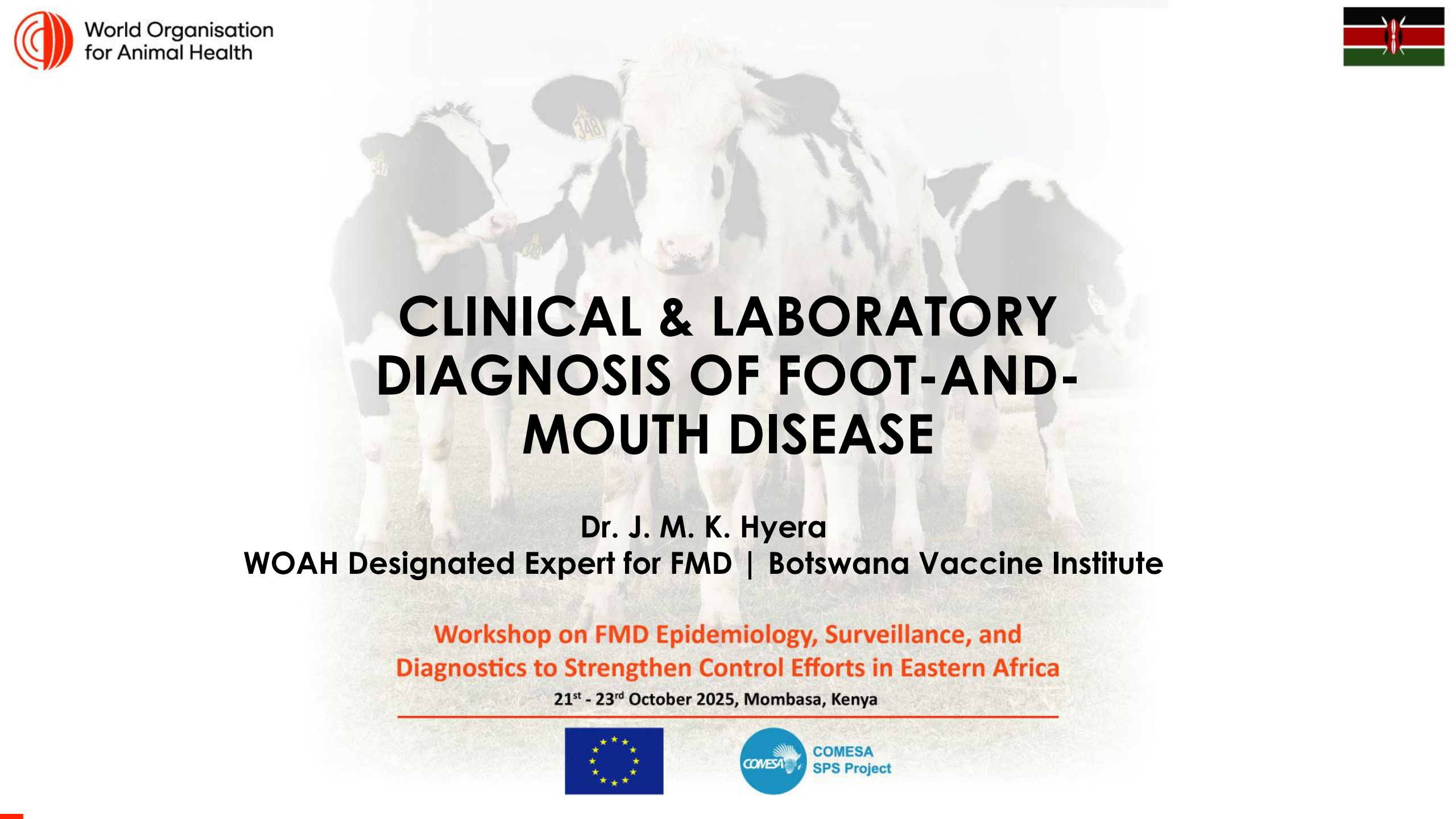
World Organisation for Animal Health



CLINICAL & LABORATORY DIAGNOSIS OF FOOT-AND-MOUTH DISEASE

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Workshop on FMD Epidemiology, Surveillance, and Diagnostics to Strengthen Control Efforts in Eastern Africa

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COMESA SPS Project

OUTLINE

1.Background

2.Clinical Diagnosis of FMD

- 2.1 Clinical signs (Cattle, sheep and goats, pigs)
- 2.2 Differential Diagnosis
- 2.3 Epidemiological information

3. Laboratory diagnosis

- 3.1 Sample collection
- 3.2 Sample packaging and shipment
- 3.3 Laboratory diagnosis
  - 3.3.1 Virological diagnosis
  - 3.3.2 Serological diagnosis
- 3.4 Interpretation of serological test results

1. BACKGROUND

Foot-and-Mouth Disease (FMD)

- Highly contagious viral disease of domestic and wild animals: Domestic ruminants and pigs most susceptible
- Not a public health threat but has great potential of causing economic losses in farmed animals (cattle, sheep, goats, pigs & water buffalos)

Aetiology of FMD

- RNA virus, non-enveloped, single-stranded, 25-30 nm; icosahedral symmetry
- Genus – Apthovirus
- Family – Picornavirdae

HOSTS OF FMDV

- All domestic cloven-hoofed animals are susceptible, including cattle, pigs, sheep, goats, and water buffalo *(Bubalus bubalis)*
- All wild cloven-hoofed animals are also susceptible, including African buffalo (*Syncerus caffer*), deer, antelope, wild pigs, elephant, giraffe, and camelids.
- Strains of FMD virus that infect cattle have been isolated from wild pigs and deer
- African buffalos are the only wildlife species to play a significant role in the epidemiology of FMD in Africa

TRANSMISSION OF FMD

- Direct contact between infected and susceptible animals
- Direct contact of susceptible animals with contaminated inanimate objects (hands, footwear, clothing, vehicles, etc.)
- Consumption (primarily by pigs) of untreated contaminated meat products (swill feeding).
- Ingestion of contaminated milk (by calves)
- Artificial insemination with contaminated semen
- Inhalation of infectious aerosols
- Airborne, especially temperate zones (up to 60 km overland and 300 km by sea)

TRANSMISSION OF FMD CONT.

- can harbour FMDV in their respiratory tract for 24–48 hours, leading to the common practice of 3-5 days of personal quarantine for personnel exposed in research facilities
- During an active outbreak, this may be reduced to an overnight period of time after thorough shower and shampoo, change of clothing, and expectoration

SOURCES OF FMD VIRUS

- Incubating and clinically affected animals
- Breath, saliva, faeces, and urine; milk and semen (up to 4 days before clinical signs)
- Meat and by-products in which pH has remained above 6.0
- Carriers: recovered or vaccinated and exposed animals in which FMDV persists in the oropharynx for more than 28 days
- The rates of carriers in cattle vary from 15–50%
- The carrier state in cattle usually does not persist for more than 6 months, although in a small proportion it may last up to 3 years

SOURCES OF FMD VIRUS CONT.

- Domestic buffalo or water buffalo *Bubalus bubalis*), sheep and goats do not usually carry FMD viruses for more than a few months; African buffalo (*Syncerus caffer*) are the major maintenance host of SAT serotypes, and may harbour the virus for at least 5 years

- Circumstantial field evidence indicates that on rare occasions carriers may transmit infection to susceptible animals of close contact: the mechanism involved is unknown

2. CLINICAL DIAGNOSIS OF FMD

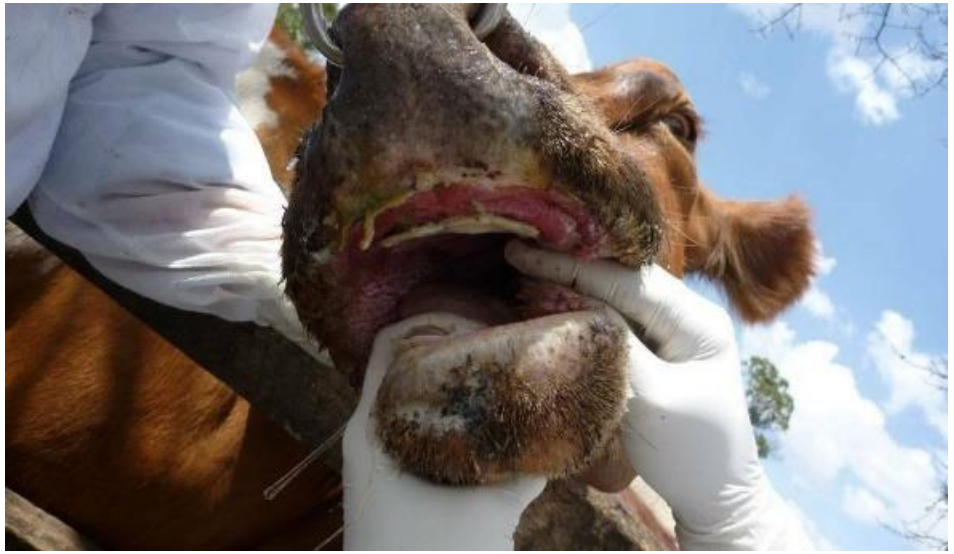
2.1 CLINICAL SIGNS

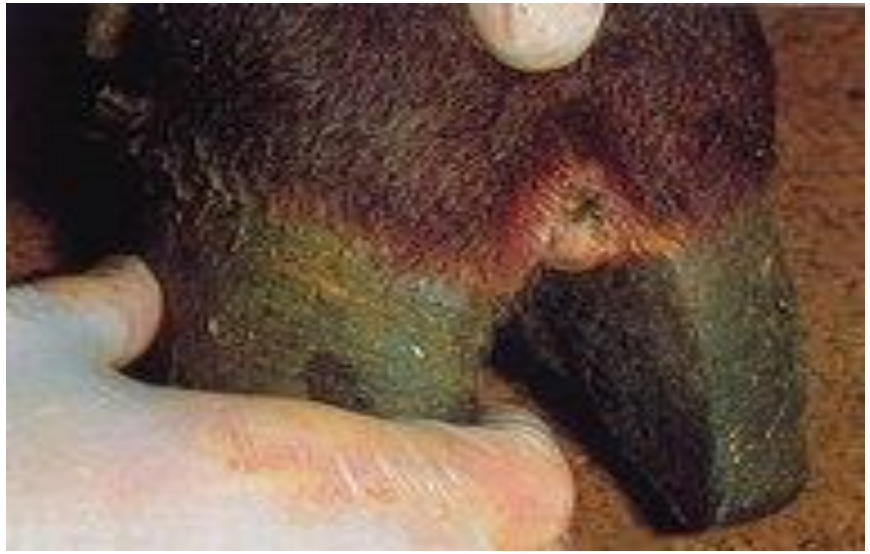
Cattle

- Pyrexia, anorexia, shivering, reduction in milk production for 2–3 days, then;
  - smacking of the lips, grinding of the teeth, profuse salivation (drooling), lameness, stamping or kicking of the feet: caused by vesicles (aphthae) on buccal and nasal mucous membranes and/or between the claws and coronary band
  - after 24 hours: rupture of vesicles leaving erosions
  - vesicles can also occur on the mammary glands

CONTINUATION

- Recovery generally occurs within 8–15 days
- Complications: tongue erosions, superinfection of lesions, hoof deformation, mastitis and permanent impairment of milk production, myocarditis, abortion, permanent loss of weight (severe emaciation)
- Death of young animals from myocarditis





SHEEP

- Pyrexia. Lameness and oral lesions are often mild
- Foot lesions along the coronary band or interdigital spaces may go unrecognised, as may lesions on the dental pad
- Agalactia in milking sheep and goats is a feature. Death of young stock may occur without clinical signs

PIGS

- Pyrexia
- May develop severe foot lesions and lameness with detachment of the claw horn, particularly when housed on concrete
- Vesicles often occur at pressure points on the limbs, especially along the carpus ('knuckling')
- Vesicular lesions on the snout and dry lesions on the tongue may occur. High mortality in in piglets is a frequent occurrence.

2.2 DIFFERENTIAL DIAGNOSIS

- **Clinically indistinguishable diseases**
  - Vesicular stomatitis in cattle
  - Swine vesicular disease
  - Vesicula exanthema of swine
- **Other differential diagnosis (oral lesions – salivation):**
  - Rinderpest
  - Bovine viral diarrhoea and Mucosal disease
  - Infectious bovine rhinotracheitis (IBR)
  - Bluetongue

CONTINUATION

- Epizootic haemorrhagic disease
- Bovine mammillitis
- Bovine papular stomatitis; Contagious ecthyma
- Malignant catarrhal fever

OTHER DIFFERENTIAL DIAGNOSIS (HOOF LESIONS - LAMENESS):

- Infectious diseases
  - Foot rot
  - Digital dermatitis
  - Interdigital dermatitis
- Non-infectious diseases
  - Traumatic injury
  - Sole haemorrhages
  - Sole ulcers

2.3 EPIDEMIOLOGICAL INFORMATION

- Description of the spread of infection in the herd or flock.
- Number of animals on the premise by species, the number of animals dead, the number of animals showing clinical signs, and their age, sex and breed.
- Type and standard of husbandry, including biosecurity measures and other relevant factors potentially associated with the occurrence of cases.
- History of foreign travel by owner or of introduction of animals from other countries or regions.

CONTINUATION

- Any medication given to the animals, and when given.
- Vaccination history describing the type of vaccines used and dates of application.
- Other observations about the disease, husbandry practices and other disease conditions present.

3. LABORATORY DIAGNOSIS

3.1 Sample collection

- **Who should collect the samples**
  - Veterinarians
  - Veterinary paraprofessionals
- **Why is sampling important?**
  - Collecting appropriate samples (epithelial tissue, blood, etc.) is critical for foot and mouth disease (FMD) diagnosis of suspected cases.
  - Laboratory diagnosis helps in the detection of either the FMD virus itself or antibodies to the FMD virus.
  - Timely detection plays a crucial role in managing and possibly stopping FMD outbreaks.

➢ Stages for FMD diagnosis sampling

Step 1: Sample Collection

- Collect samples from animals showing clinical signs.
- Vesicular fluid and epithelium are the preferred samples for agent detection since they are rich sources of the virus. Vesicular fluid is collected in sterile, screw-capped glass or plastic test tubes. Epithelial tissues are contained in similar tubes; the tubes are provided with virus transport medium (VTM) as a preservative. Blood should be collected in vacutainer tubes without preservative for harvesting serum for FMD antibody detection.
- Collect duplicated samples if you need to submit them to different laboratories for diagnosis.

Step 2: Sample Labelling & Handling

- Each sample should be suitably and legibly labelled on the container with a water proof marker.
- The sample should be accompanied by animal description and details (age, sex, breed, vaccination status and ID) and their owner details (name and address). A duly completed laboratory specimen submission form must also accompany the samples.

Step 3: Storage & Transportation

- Maintenance of the cold chain is important for sample quality.
- Ensure the samples are kept at 4°Celsius for short shipments (1 to 2 days).Store samples in a cool box containing ice packs.

- **3.2. Sample Packaging and Shipment**

➢Triple packaging is used to ensure safe shipment of FMD samples:

- ➢ Primary container
- ➢ Secondary container (leak-proof; e.g. resealable plastic bag)

CONTINUATION

- Robust container with absorbent material (cotton wool or paper towel)& ice packs
- Outer container –strong cardboard box; seal box and label
- **Hazard label**: The outer box must be labelled as UN 2900 "Infectious substance, affecting animals" and include a warning such as **"CAUTION: FOOT-AND-MOUTH DISEASE. CAN ONLY BE OPENED BY THE RECEIVING LABORATORY".**

- **Hazard label**: The outer box must be labelled as UN 2900 "Infectious substance, affecting animals" and include a warning such as **"CAUTION: FOOT-AND-MOUTH DISEASE. CAN ONLY BE OPENED BY THE RECEIVING LABORATORY".**

3.3 Transport method:

By air freight following International Air Transport association (IATA) regulations

3.3.1 Virological diagnosis

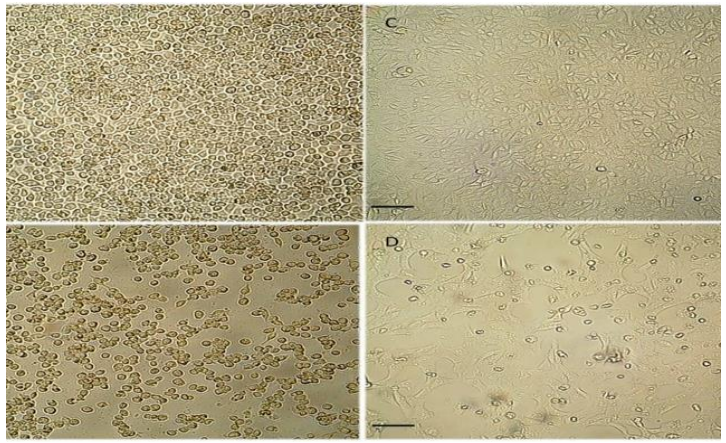
Virus isolation	Confirmation of clinical cases	Contribute to disease eradication policies
Antigen detection by ELISA	Confirmation of clinical cases	Contribute to disease eradication policies
Agarose-based RT-PCR	Confirmation of clinical cases	Contribute to disease eradication policies
Real time RT-PCR	Confirmation of clinical cases	Contribute to disease eradication policies

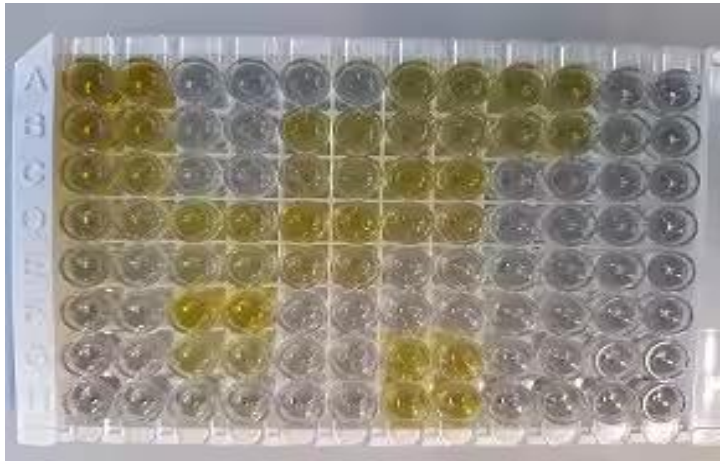
[1]Based on detection and identification of virus agent. It is essential to confirm the presence of FMDV following virus isolation by an antigen or nucleic acid detection test. [2]Recommended for this purpose by WOAH

3.3 LABORATORY DIAGNOSIS

3.3.1 Virological diagnosis

- Virus and viral antigen detection & identification
  - Virus isolation in cell culture (primary & permanent cells)
  - Virus infection in cells expressed as cell degenerative changes (CPE)
  - Virus serotype identified by antigen ELISA (Ag. ELISA) using monospecific, serotype specific antiserum.

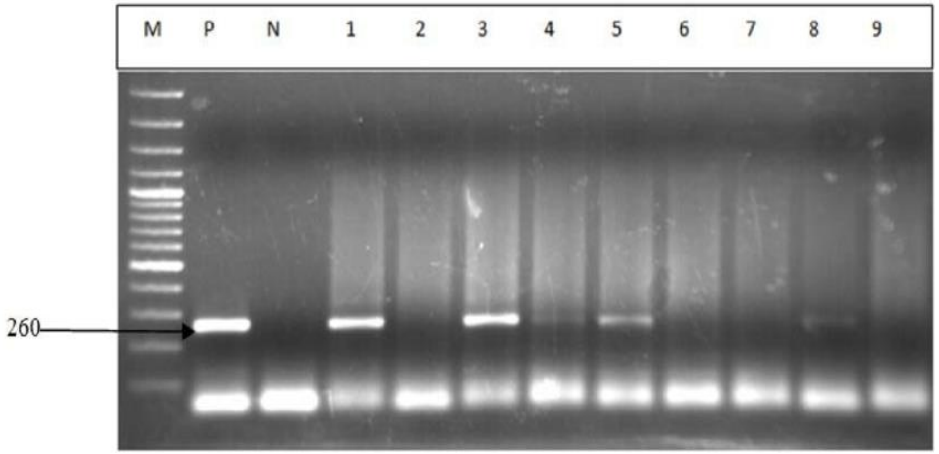




3.3 LABORATORY DIAGNOSIS

➢ 3.3.1 VIROLOGICAL DIAGNOSIS

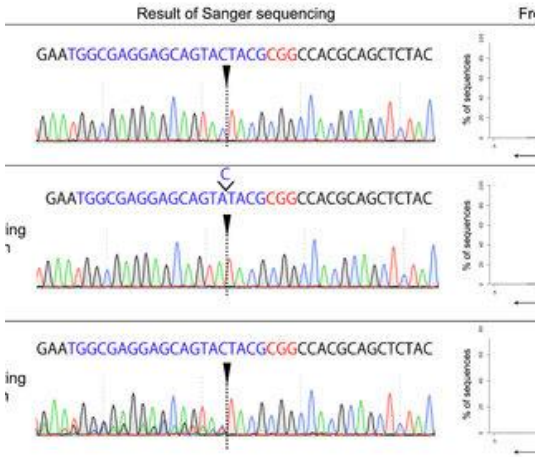
- ➢ Virus nucleic acid (RNA) detection
  - Total RNA extraction from sample
  - Synthesis of complimentary DNA by reverse transcriptase polymerase chain reaction (RT-PCR)
  - Sequencing PCR products (VP1

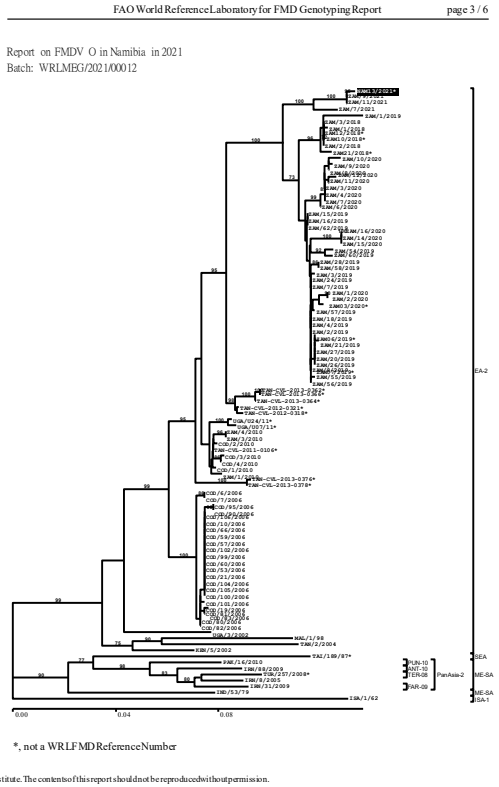


3.3 LABORATORY DIAGNOSIS

3.3.1 VIROLOGICAL DIAGNOSIS

- Virus nucleic acid (RNA) detection
  - VP1 Sequencing and phylogenetic analysis





3.3.2 SEROLOGICAL DIAGNOSIS

FMD Serological test methods and purposes of the test methods[1]

Test method	Purpose of test method					
	Population freedom from infection	Contribute to disease eradication policies	Confirmation of clinical cases	Prevalence of infection-surveillance	Individual animal freedom from infection prior to movement	Immune response in individual animals or populations post vaccination
NSP-Ab-ELISA	+++	+++	+++	+++	++	-
SP-Ab-ELISA	++	+++	+++	++	++	+++
VNT	++	++	+++	++	++	+++

3.3.2 SEROLOGICAL DIAGNOSIS

- Antibody detection against NSPs
  - PrioCHECK NS Antibody ELISA (Applied biosystems, Thermo Fisher Scientific: Commercial kit)
- Antibody detection against SPs
  - Liquid phase blocking ELISA (LPBE)
  - Solid phase blocking ELISA (SPBE)
  - Virus neutralization test (VNT)

3.4 Interpretation of serological test results

3.4.1 SP (Liquid phase blocking ELISA)

Antibody titres are expressed as the 50% end-point titre, i.e. the dilution at which the reaction of the test sera results in an optical density equal to 50% inhibition of the median optical density (OD) of the reaction (antigen) control wells. Antibody titres are expressed as reciprocal of highest serum dilution with OD values of ≥50%.

3.4 Interpretation of serological test results

3.4.1 SP (Liquid phase blocking ELISA)

- In general sera with titres ≥90 are considered to be positive. A titre of less 40 is considered to be negative.
- For certification of individual animals for the purposes of international trade, titres of >40 but <90 are considered to be doubtful (grey zone), and further serum samples may be requested for testing; results are considered to be positive if the second sample has a titre of ≥40.
- For the purposes of herd-based serosurveillance as part of a statistically valid serological survey, a cut-off of ≥90 may be appropriate.

3.4 INTERPRETATION OF SEROLOGICAL TEST RESULTS

3.4.1 SP (Liquid phase blocking ELISA)

- Cut-off titres for evaluating immunological protection afforded by vaccination have to be established from experience of potency test results with the relevant vaccine and target species. In cattle a titre of ≥100 (2 log10) is generally accepted as being protective.

3.4.2 VNT (Virus neutralization test)

- A titre of ≥45 or of the final serum dilution in the serum/virus mixture is regarded as positive.
- A titre of less than <16 is considered to be negative

- For certification of individual animals for the purposes of international trade, titres of 16 to 32 are considered to be doubtful, and further serum samples may be requested for testing; results are considered to be positive if the second sample has a titre of ≥16
- For the purposes of herd-based serosurveillance as part of a statistically valid serological survey, a cut-off titre of 45 may be appropriate.

3.4.3 NSP (PrioCHECK NSP Antibody ELISA)

- Test results are expressed as percentage inhibition (PI). The latter is calculated according to the following formula:
- PI = 100 – [OD (test serum at 450 nm) – OD (serum control at 450nm)] x100

3.4.4 Interpretation of the PI values

PI score	Test Results	Remarks
PI = <50%	Negative	Antibodies against NSP of FMDV are absent in test sample
PI = ≥50%	Positive	Antibodies against NSP of FMDV are present in test sample

Adjust the cut-off at 95% confidence interval according to your laboratory working conditions. At the WOAH lab in BVI, the measurement uncertainty (MU) of the NSP test at 95% confidence interval has been estimated at 50±3.9% (46 – 54%). Any positive result (PI >50%) that is equal to or higher than 54% is positive with 95% confidence but any positive result that is less than 54% is not positive with 95% confidence. Similarly, a negative result (PI <50%) that is less than 46% is negative with 95% confidence but a negative result that is equal to or higher than 46% is not negative with 95% confidence. The zone of low confidence (PI ≥46 to <54%) correlates with the "grey zone" or "inconclusive/suspect/doubtful zone"; animals exhibiting these results are resampled for retesting.

THANK YOU FOR YOUR ATTENTION