

ANIMAL HEALTH UPDATES

Animal Health Group

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Jan-Mar'09

In this issue :

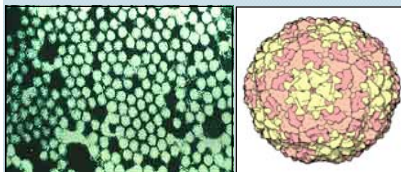
- **Foot and Mouth Disease (FMD) and its control in farms.**
- **Significant animal diseases reported to OIE during January to March 2009.**
- **New test for detecting fake organic milk.**
- **New bacteria found in upper atmosphere by Indian scientists.**

Disease - Foot and Mouth Disease (FMD) and its Control in farms

Foot and mouth disease (FMD) causes severe economic losses and is **the most contagious disease** of cloven footed animals. A short incubation period, release of virus prior to appearance of clinical signs, massive quantities of virus released, extended survival of virus in the environment, multitude of routes of virus transmission, minimal size of the infective dose, aerosol transmission over long distances are some of the important factors that qualifies it as the most contagious disease.

Etiology

FMD is caused by a virus of the genus *Aphthovirus*, family *Picornaviridae*. There are seven serotypes of FMD virus, namely Oise (O), Allemagne (A), C, South African Territories (SAT 1), SAT 2, SAT 3, and Asia 1. Infection with any one serotype does not confer immunity against another. Within serotypes, several strains have been identified. There are several variants within a serotype and the antigenic variation seems to be greatest for serotype A.



Electronmicrograph of FMD virus (left) and graphical representation of a virion (right)
Source : vetmed.illinois.edu (lt); 1.bp.blogspot (rt)

Host Range

All cloven footed domestic and wild animals are susceptible. Dromedarian (one humped) camels are naturally resistant to FMD.

Epidemiology

The key factors in epidemiology of FMD are as follows:

- The disease is highly contagious, spreading by aerosols and with movements of infected or contaminated animals, products, fomites, objects and people.

- Large amounts of virus are excreted by infected animals, with pigs excreting approximately 3000 times more virus than cattle in aerosols. Pigs are therefore the main amplifying hosts, and are extremely important in disease spread.

- Cattle are mainly infected by inhalation of infected aerosols and are considered the best indicator species of the disease.

- Infected sheep and goats may show mild or inapparent signs and therefore they may be important in the maintenance and spread of disease.

- Winds carrying infected aerosols can spread the disease over considerable distances if climatic and environmental conditions are favourable.

- Carriers may emerge following recovery, after infection.

A. Incubation

Incubation period depends on the strain of FMD virus, dosage, and the route of entry. It can vary from as short as 2-3 days when animals are in close contact to as long as 10-14 days from windborne infection. Experimentally, incubation period is 18-24 hours. For regulatory purposes, a 14 day incubation period is considered.

B. Persistence of the virus

FMD virus may remain infective in the environment for several weeks and longer in the presence of organic matter or on chemically inert



Profuse viscous salivation and vesicles on feet due to FMD
Source : cvm.tamu.edu

materials. Important characteristics of the virus are as follows:

- Inactivation is very rapid below pH 5.0 or above pH 11.
- Exposure to 56°C for 30 minutes is sufficient to destroy most strains although there is some variation between strains.
- Sunlight has little or no direct effect on infectivity; any loss is due to secondary drying and temperature.
- Pasteurization (72°C for 15 seconds) inactivates FMD virus in milk.
- The pH of normal bovine urine inactivates FMD virus.
- The survival of airborne virus is mainly influenced by relative humidity (RH) with good survival above 60% RH and rapid inactivation below 60% RH.

Virus in tissue fluids or blood allowed to dry on different materials and kept indoors at room temperature may remain infective for periods as shown in table 1.

Table 1: Infectivity of virus on different materials			
wool	Up to 2 weeks	Rubber boots	13 weeks
Cow hair	4 weeks	Hay	15 weeks
Boot leather	11 weeks	Bran	20 weeks

C. Excretion of virus

FMD virus can replicate and get excreted from the respiratory tract of animals. Airborne excretion of virus occurs during the acute phase of infection. FMD viruses may occur in all the secretions and excretions of acutely infected animals, including expired air. Excretion in semen and milk can occur for up to 4 days before the clinical phase and sheep excrete virus in their breath for around 24 hours before signs are apparent. Virus has been recovered from bovine semen stored at -50°C for at least 320 days. Most excretion of virus ceases within six days of the appearance of vesicles. FMD virus has been detected in the milk and semen of experimentally infected cattle for 23 and 56 days respectively. The virus is reported to survive for various periods (table 2) in the environment.

Table 2: Survival periods of virus

Item	Period	Item	Period
Water	50 days	Pasture at $8 - 18^{\circ}\text{C}$	74 days
Soil, sacking, hay or straw, depending on storage or climatic conditions	26-200 days	Wood contaminated with fat	398 days
Cardboard, wood or metal contaminated with serum, blood or issue of infected animal	35 days	Animal rooms	48 hours
Dry manure	14 days	Moist manure	8 days
Liquid manure	34-42 days ($12-22^{\circ}\text{C}$)	Water from shed washings	21 days ($17-21^{\circ}\text{C}$)

D. Carrier State

Following recovery from the acute stage of infection, infectious virus disappears from all secretions and excretions with the exception of oesophageal – pharyngeal (OP) fluids from some ruminants, where live virus may persist. Animals in which the virus persists in the OP for more than 28 days after infection are referred to as ‘carriers’. Pigs do not become carriers. 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. Domestic buffalo, sheep and goats do not usually carry FMD viruses for more than a few months.

D. Transmission

Transmission is generally effected by di-

rect contact between infected and susceptible animals or, more rarely, exposure of susceptible animals to the excretions and secretions of acutely infected animals. Experimental transmission of FMD by insemination of infected semen has been demonstrated. FMD virus has been found in bull semen 4 days before, during, and up to at least 37 days after the disappearance of clinical signs. The virus enters semen as a result of viraemia or lesions around the preputial orifice. Spread in cattle by embryo transfer should not occur, provided the embryos have been properly washed according to the International Embryo Transfer Society (IETS) protocols, and people and equipment are free of contamination.



Hoof evulsion and udder damage may result from FMD
Source : cvm.tamu.edu

Once liberated into the atmosphere, infected aerosols can form a ‘plume’ which is dispersed horizontally and vertically. For sufficiently high quantities of virus to be maintained near the ground, vertical dispersion must be limited. However, under certain atmospheric conditions, bodies of infected air may rise vertically, travel large distances, then descend to ground level with little dilution of virus having occurred.

Cattle are highly susceptible to FMD because of their higher respiratory tidal volume. A lower Tissue Culture Infectious Dose₅₀ (TCID₅₀) also contributes to their higher susceptibility. (Table 3)

Table 3: TCID₅₀ in various species

Species	Respiratory route (TCID ₅₀)	Oral route (TCID ₅₀)
Cattle	12	1×10^6
Pigs	20	8×10^3
Sheep	10	

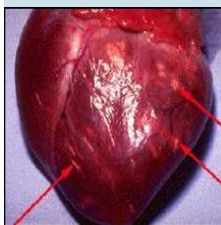
People examining the head area of clinically affected pigs can harbour FMD virus in their nasal passages and throat for a period up to 36 hours. During this time the virus is expelled during coughing, sneezing, talking, breathing and in the saliva.

The virus can be spread mechanically both by animate and inanimate objects.

Clinical signs

Infection of susceptible animals with FMD virus leads to the appearance of vesicles in and around the oral cavity, on the feet, and on the mammary glands of females. Coronary band lesions may give rise to growth arrest lines whose progress down the side of the hoof can be used to indicate the time since infection occurred. In severe infections of the feet, hooves may be shed. Mastitis is a common sequel of FMD in dairy cattle.

Vesicles can also occur at other sites, such as inside the nostrils and at pressure points on the limbs – especially in pigs. The severity of clinical signs varies with the strain of



Tiger heart due to FMD
Source : cvm.tamu.edu

virus, the exposure dose, the age, breed of animal, the host species and its degree of immunity. The signs can range from a mild or inapparent infection to one that is severe. Death may result in some cases.

Mortality from a multifocal myocarditis (tiger heart) is most commonly seen in young animals: myositis may also occur in other sites.

Appearance of lesions on tongue of cattle may provide a guide to how long the infection has been present in a herd. (Table 4)

Table 4: Progression of tongue lesions from the onset of clinical signs (0 day) of FMD

Unruptured vesicles	0-2 days
Newly ruptured vesicles with epithelial remnants at margins	1-3 days
Ruptured vesicles without epithelium or fibrous healing	3-10 days
Open lesions with marked fibrous tissue at margins	Above 7 days

Specimens that can be collected

A. For virus isolation

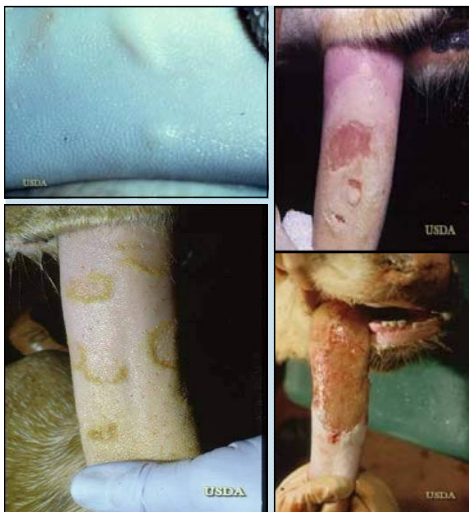
Live animals - Vesicular fluid, vesicular lesion, epithelial coverings or flaps, OP fluid & whole blood.

Dead animals - Tissue samples include lymph nodes, thyroid, adrenal, kidney, spleen and heart.

B. For Serology - Serum

On-farm control

On-farm control of FMD would involve all the biosecurity measures that need to be put in place to obviate the persistence of virus in the environment and its transmission, isolation of any focus of infection, providing an immune umbrella against the prevalent strains by vaccination and, assessing



Clock wise from top left : (i) Vesicle at day 1 (ii) Lesion at day 4 (iii) Lesion at day 6 (iv) Lesion at day 8 to 14.
Source : cvm.tamu.edu

the immune status of the animal from time to time.

A. Biosecurity measures

The general biosecurity measures adopted for reducing the chances of virus entering, spreading or leaving the premises would hold in good stead for control of FMD (also refer Oct-Dec'08 issue).

B. Vaccination

I. Farm herd

Primary vaccination against FMD can be carried out at 4-5 months of age. Revaccination may be conducted for all the animals during March / April and again during September / October each year. Synchronise the routine so that all animals are vaccinated at the same time.

II. Susceptible population of surrounding/project villages

Routine vaccination of the susceptible population (including sheep, goat and pig) in a radius of 5 km of the farm and, in the villages from where frequent purchases are planned (eg. villages in progeny testing programme) to the farm is recommended.

III. Newly introduced animals

Purchased animals are to be vaccinated at the earliest or 21 days after, if vaccinated before purchase, while in quarantine.

C. Vaccine storage and administration

Vaccine is to be stored between 2 & 8° C and protected from light at all times up to the point of administration to animals. The integrity of the cold chain for vaccine storage is important to maintain the potency of the vaccine.

Two sterile needles are required for each vaccine vial – one for withdrawal of vaccine (21G x 1/2 inch) and the other to

allow air into the vial to avoid creation of a vacuum (23G x 2 inch). Both needles remain in the vaccine vial plug and are not to be used for injections.

Recommended needles for vaccinating the susceptible species is given in table 5.

Table 5: Recommended needles for vaccination	
Large ruminants	18 or 16G x 1 1/2 inch
Small ruminants	21 or 19G x 1 1/2 inch
Adult pigs	18 or 19G x 1 1/2 inch
Piglets	21G x 1/2 inch

Only enough vaccine for about half hour's work should be taken from refrigeration.

Intra-muscular injection site (for oil-based vaccine)

Cattle : Deep in the muscle of the neck, in front of the shoulder, about one-third down from the top ridge of the neck and two-thirds up from the lower edge of the neck.

Goat : Similar to cattle but about half way up the neck.

In both cases the needle should be inserted at 45° angle to the skin to avoid leak-back.

Pigs : Deep in the muscle of the neck, behind the ear. The needle should be inserted at 45° angle to the skin to avoid leak-back, and backwards down the neck to avoid the ear canal.

D. Seromonitoring

The efficacy of vaccination can be ascertained by assessing the antibody response to vaccination. For this, serum samples need to be collected randomly from the vaccinated population both at 0 day and 30 day post vaccination (DPV). More samples (90 & 180 DPV) also may be collected if continuous monitoring is preferred.

E. FMD outbreak management in farm

In the event of an FMD outbreak in the farm, the following measures need to be undertaken:

I. Animals

- Isolate infected animal (s) immediately till recovery, do not cull.
- Isolate in-contact animals for a period of 14 days and observe for any symptoms.
- Re-vaccinate all healthy and in-contact animals irrespective of vaccination status and avoid vaccinating in-contact animals.
- Care should be taken to use individual needles for each animal while administering the vaccine.

II. Bulls and semen

- Stop semen collection from all (both infected and healthy) bulls during the period of outbreak.
- Destroy all the semen collected one month preceding the onset of outbreak.

iii. Give 90 days rest to infected bulls.

iv. Semen collection may be resumed from affected bulls 3 months after the last case of FMD in the farm recovers and after 1 month from healthy unaffected bulls after the last case recovers.

v. Each batch of semen meant for export may be tested by virus isolation and PCR techniques.

III. Premises, feed & fomites

i. Clean and disinfect the premises and all fomites in contact with the infected animal(s).

ii. Destroy all the feed and fodder in contact with the affected animal(s).

IV. Personnel

i. Personnel who have been working with FMD animals must stay away from healthy animals for at least 3 days.

F. Cleaning and Disinfection

Some disinfectants effective against FMD virus are shown (table 6). They are to be applied on clean surfaces for best results.

Table 6 : Disinfectants effective against FMD virus		
Product	Dilution	Notes
Sodium hypochlorite (bleach 5.25%)	3%	Not effective on dirty surfaces.
Acetic acid	4-5%	Undiluted vinegar (4% acetic acid) may be used.
Sodium hydroxide (lye)	2%	Highly caustic, always add lye to water.
Sodium carbonate (soda ash)	4%	Mildly caustic

G. Monitoring field strains of FMD virus

It is important for the vaccine manufacturer to keep a continuous tab on the field strains of FMD virus to maintain maximum homogeneity with the strains used in the vaccine, which in turn would help provide maximum protection to the vaccinated animals, thereby reducing the outbreaks. To this end, it would be appropriate to intimate Indian Immunologicals Limited (IIL), Hyderabad in the event of any outbreak in the field so that they could arrange to collect samples for virus strain identification.

Diagnosis

I. Virus detection

A. Virus isolation - A range of sample types including epithelium, OP samples and serum may be examined by virus isolation by inoculating the processed sample onto cell cultures.

B. Enzyme Linked Immunosorbent Assay (ELISA) - It is a preferred procedure for the detection of FMD viral antigen and identification of viral serotype. This is an

(Continued on page 4)

OIE - Significant animal diseases reported to OIE during Jan – Mar'09

Sl.No	Disease Outbreak	Countries reporting
1	Foot and Mouth Disease (Strain in parenthesis)	China (Asia 1 & A), Taipei (O), Israel (O) , Lebanon
2	Blue Tongue	Italy, Norway, Australia, Greece
3	Lumpy Skin Disease	Mali
4	Highly Pathogenic Avian Influenza	Nepal, China, Hongkong, Germany, Laos
5	Low Pathogenic Avian Influenza	South Korea, Canada, France, Romania, Japan, Czech Republic
6	Peste des petits ruminants (PPR)	Tanzania
7	Trichinellosis	Vietnam
8	Epizootic Haemorrhagic Disease	Reunion Island (France)
9	Classical Swine Fever	Israel, Brazil
10	Rift Valley Fever	South Africa
11	New Castle Disease	Netherlands Source :www.oie.int

New test for detecting fake organic milk

Scientists in Germany have developed a new method to determine whether milk marketed as "organic" is genuine or just ordinary milk mislabeled to hoodwink consumers. The test is based on analysis of milk fat for the ratio of stable isotopes of carbon which is used to identify milk samples from cows raised on feed containing a higher ration of maize, a feeding regimen that is typical of conventional milk production. Organically raised cows are fed less maize but more pasture feed. In addition, the team identified a significant difference in the alpha-linolenic acid content of milk fat between organic and conventional milk samples. Organic milk typically has a higher alpha-linolenic acid content than conventional milk. This report appeared in April'09 edition of *Journal of Agricultural and Food Chemistry*.

Source : www.engormix.com

Indian scientists find new bacteria in upper atmosphere

Three new species of bacteria have been identified by Indian Space Research Organisation (ISRO) in the upper atmosphere. One of the three, identified as a member of the genus Janibacter, has been named Janibacter hoylei, the second, Bacillus isronensis, and the third, Bacillus aryabhata. All the three are not found elsewhere on earth and are highly resistant to ultra-violet radiation. The samples were collected using a 26.7 million cubic feet balloon carrying a scientific payload from heights ranging from 20 to 41 km.

These samples were analysed independently by scientists at the Centre for Cellular and Molecular Biology, Hyderabad as well as the National Centre for Cell Science (NCCS), Pune. The experiment detected 12 bacterial and six fungal colonies, nine bacterial and all fungal colonies showed greater than 98 percent similarity with known species on Earth.

This was the second such experiment conducted by ISRO, the first one being in 2001. Even though the first experiment had yielded positive results, it was decided to repeat the experiment by exercising extra care to ensure that it was totally free from any terrestrial contamination.

Source :<http://in.news.yahoo.com>

(Continued from page 3)

indirect sandwich test in which different rows in multiwell plates are coated with rabbit antisera to each of the seven FMD serotypes. ELISA is suited to the examination of epithelial suspensions, vesicular fluids or cell culture supernatants .

C. Nucleic acid recognition methods - This is used to amplify genome fragments of FMD virus in diagnostic materials including epithelium, milk, serum and OP samples. Tests are: (i) Reverse transcription polymerase chain reaction (RT-PCR) assay and (ii) Real-time RT-PCR assay.

II. Serological tests

Serological tests for FMD are performed in support of four main purposes, namely to: (i) Certify individual animals prior to import or export (i.e. for trade) (ii) Confirm suspected cases of FMD (iii) Substantiate absence of infection and, (iv) Demonstrate the efficacy of vaccination. Serological tests for FMD are of two types; those that detect antibodies to viral structural proteins (SP) and, those that detect antibodies to viral nonstructural proteins (NSPs).

A. Serological tests to detect SP

The SP tests are serotype-specific and detect antibodies elicited by vaccination and infection. These tests are serotype-specific and are highly sensitive, providing that the virus or antigen used in the test is closely matched to the strain circulating in the field. **They are the tests prescribed for trade by Office International des Epizooties (OIE)** and are appropriate for confirming previous or ongoing infection in non-vaccinated animals as well as for monitoring the immunity conferred by vaccination in the field. The tests are: (a) Virus neutralization test (b) Solid phase competition enzyme-linked immunosorbent assay (ELISA) (c) Liquid phase blocking ELISA

B. Serological tests to detect NSP

The detection of antibody to the NSPs of FMD virus can be used to identify past or present infection with any of the seven serotypes of the virus, whether or not the animal has also been vaccinated. Therefore the tests can be used to confirm suspected cases of FMD and to detect viral activity or to substantiate freedom from infection on a population basis. For certifying animals for trade, the tests have the advantage over SP methods that the serotype of virus does not have to be known. However, there is experimental evidence that some cattle, vaccinated and subsequently challenged with live virus and confirmed persistently infected, may not be detected in some anti-NSP tests, causing false-negative results. The tests are: (a) Indirect ELISA (b) Enzyme-linked immunoelectrotransfer blot assay (EITB)

There are five OIE reference laboratories for FMD which are situated in UK, Botswana, Brazil, Russia and South Africa respectively.

III, Hyderabad, is well equipped to conduct most of the viral detection and serological tests for FMD virus.

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