Final Report on FMD workshop held as part of the FMD 2010 International Symposium
Table of Contents

Aim of the Workshop ........................................... 2
Format of the Workshop ....................................... 2
The Scenario ..................................................... 2
Group Questions ................................................ 4
Workshop Programme ......................................... 6
Outcome of Group Discussions ............................. 6
Summary of Outcomes ........................................ 9
Appendix .......................................................... 11
1. **Aim of the Workshop**

To allow all participants to discuss a number of management issues in the control of a major FMD outbreak and make relevant recommendations for response and control plans, including identifying research needs.

2. **Format of the Workshop**

The workshop took the form of a series of discussion groups based around a simulated FMD outbreak in the Goulburn Valley dairy region, a high density livestock production area in Victoria, Australia.

The simulated outbreak was presented to the symposium in plenary before participants broke up into five discussion groups.

The discussion groups were as follows:

- Vaccination (two groups)
- Proof of Freedom
- Diagnostic Technologies
- Management of FMD in endemic situations

Following discussions lasting the morning and part of the afternoon, the groups were recalled to present their findings to the plenary. A brief panel discussion followed.

3. **The Scenario**

The scenario/exercise considered that FMD had been diagnosed in Australia and that it had entered Victoria’s Goulburn Valley from an infected property in New South Wales. While the situation in New South Wales was under control, the extent of disease in Victoria was giving cause for concern.

The Victorian Chief Veterinary Officer (CVO) declared four shires (Greater Shepparton, Campaspe, Moira and Strathbogie) which cover most of the Goulburn Valley region, as a Restricted Area.

The Goulburn Valley, with its irrigated dairy farms and high livestock population density, strongly favours the spread of highly infectious diseases such as FMD. By the end of the second week of the simulated emergency response, 30 infected premises (IPs) had been detected and the disease had spread to two other dairy areas of the State hundreds of kilometres from the initial area of infection in Victoria.

During the initial response phase, 15 properties were depopulated, but a backlog was rapidly building.
Over 170 properties were linked to existing IPs through high-risk animal, people or vehicle movements and were classified as Dangerous Contact Premises.

Carcass disposal on-farm through incineration proved problematic due to a lack of fuel wood. The Victoria Department of Primary Industries (DPI) had identified landfill sites where carcasses could be buried.

Victoria DPI staff were at this point stretched to the limit, and alternative human resources were being sought. Epidemiologists predicted that the number of IPs would rapidly grow to 50 within the next 6 weeks.

Although OIE rules around vaccination favour eradication through stamping out, it is recognised that, regardless of eradication strategy followed, Australia would be excluded from the livestock and livestock products trade for a year or more. With this in mind, the Victorian CVO was considering vaccination as an eradication option.
Modelling showed that vaccination in a 5km radius of IPs would have the effect of shortening the epidemic.

The participants in this workshop were asked to recommend the next course of action for the scenario.

4. Group Questions

The participants were divided into groups to discuss the following:

**Group 1 – The decision to vaccinate**

**(NB This group was subdivided into two due to high participant numbers).**

**Background**
The logistics associated with the outbreak make it virtually impossible to slaughter all infected animals and bury or incinerate the carcasses in a timely fashion.

Public opinion is strongly against mass culling, especially for the uninfected but susceptible animals within FMD-affected areas.

500,000 doses of FMD vaccine have arrived in the country and are available immediately; and a further 500,000 will be available within another month at a cost of AUD$1.20 per dose.

**Questions**

i. Would you vaccinate or use an alternative control strategy?

ii. If vaccination is used, design an appropriate vaccination strategy – noting whether vaccination resulting in slaughter or allowing vaccinates to live is the better option.

**Group 2 – Surveillance for proof of freedom**

**Background**
To control the outbreak a ‘vaccinate to live’ strategy was followed. All domestic ruminants within 5km of known IPs are to be vaccinated: this involves 350,000 cattle and 5,000 sheep on affected Goulburn Valley properties and a further 100,000 cattle and 50,000 sheep elsewhere in Victoria.

Pigs on known IPs and in backyard situations will be slaughtered; uninfected pigs in biosecure piggeries will be placed under surveillance.

A 3ABC ELISA is available which works in cattle and sheep and goats with Sp=99% and Se=99.5% in all three species. It is to be assumed that these figures apply for within-herd testing as well as at the herd level.

This test is less reliable in pigs.

A real time PCR is available to detect viral RNA.

**Questions**

i. Design a surveillance strategy, taking OIE guidelines into account, including targeted and random surveillance based on an appropriate prevalence level determined by the group.
ii. Describe implementation of the sampling procedures on the ground, including some ideas as to resource requirements.

iii. What are the implications for control costs, trade, and national standards and guidelines?

**Group 3 – Diagnostic methods used during the control exercise**

**Background**

Advice is needed by the CVO to inform various stages of the diagnostic work, from initial diagnosis through to proving disease freedom.

The initial samples were epithelium and probangs from cattle where the lesions were aged at about 2-5 days. The serotype was unknown at that time.

The outbreak virus had to be characterised (sequenced) and the r-values determined against the vaccine strains in Australia’s vaccine bank.

Forensic tracing of the outbreaks between the different farms was necessary during the outbreak investigations.

Sero-surveys to measure the vaccine efficacy were performed.

DIVA testing was needed to determine disease freedom.

**Questions**

i. Consider the laboratory tests needed for the different stages of the outbreak outlined above.

ii. Discuss the advantages and disadvantages of the methods currently in use for these types of work, including their sensitivity and specificity. What would you advise the CVO to use?

iii. Discuss the possible use of penside tests in an outbreak such as this.

iv. Make recommendations as to research needed to improve diagnostic assays.

**Group 4 – The endemic setting (what to do if the disease becomes uncontrollable)**

**Background**

The outbreak has spread beyond control within the outbreak focus and is deemed endemic.

The CVO requests advice on whether a submission should be made to the OIE about Australia’s new FMD status, whereby Victoria is an endemic [containment] zone and the rest of the country is recognised as FMD free.

There is an unknown number of deer, goats and pigs in the area and their movements are impossible to control.

**Questions**

i. At what point would you abandon eradication and establish an endemic zone?

ii. What would be your holding / transitional strategy while preparing to declare / implement an endemic zone?
iii. What strategies would remain in place in an endemic zone? Around the endemic zone? In the rest of Australia?

iv. How long would you accept endemicity before possibly revisiting the idea of eradication?

v. What wildlife surveillance would you perform and how would you ensure no infected wildlife move out of the endemic zone?

5. Workshop Programme

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:00 – 09:15</td>
<td>Introduction</td>
</tr>
<tr>
<td>09:15 – 10:00</td>
<td>Technical background and scene setting</td>
</tr>
<tr>
<td>10:00 – 13:00</td>
<td>Breakaway groups</td>
</tr>
<tr>
<td>13:00 – 14:00</td>
<td>Lunch</td>
</tr>
<tr>
<td>14:00 – 15:00</td>
<td>Breakaway groups</td>
</tr>
<tr>
<td>15:00 – 15:30</td>
<td>Report back in plenary</td>
</tr>
<tr>
<td>15:30 – 16:00</td>
<td>Tea</td>
</tr>
<tr>
<td>16:00 – 17:00</td>
<td>Report back to plenary, further discussion and debating</td>
</tr>
<tr>
<td>17:00 – 17:30</td>
<td>Final summary</td>
</tr>
</tbody>
</table>

6. Outcome of Group Discussions

Surveillance/Proof of Freedom

The group identified three different sub-populations of animals requiring surveillance, based on their vaccination status and area classification: vaccinated animals in the restricted area (RA), non-vaccinated animals in the RA, and animals (all unvaccinated) in the control area (CA).

Vaccinated herds/flocks: Sero-surveillance (using NSP ELISA) would be based on detecting 10% sero-prevalence with 95% confidence in these herds/flocks – meaning sampling 30 animals in each group.

Unvaccinated properties in the RA: clinical surveillance mixed with sero-surveillance to detect a herd prevalence of 1% with 95% confidence. The group identified that a farm-level bulk milk ELISA would be useful to augment this. Clinical and abattoir surveillance of pigs would also be carried out.
Surveillance in the CA: based on reports of suspicions, surveillance at aggregation points and stratified sampling of randomly selected herds/flocks.

The surveillance of vaccinated herds in the RA under the given scenario would involve 2,250 cattle properties and 65 sheep properties – with samples from a total of 68,000 cattle and 2,000 sheep. With 30 teams of two people each managing four farms per day, it was hoped to complete a task of this size in about three weeks. Follow-up of an estimated 700 false positives would have to be catered for.

Identification and traceability of vaccinated animals was considered vital.

Risk perceptions with respect to products from vaccinated animals (on the part of both local consumers and trading partners) would need addressing, and it would be sensible to have advance agreements with abattoirs for processing vaccinated animals.

More work was required to AUSVETPLAN to “mainstream” a vaccinate-to-live approach, and to reconsider the testing of all vaccinated animals for presence of antibodies to the non-structural proteins to ensure proof of freedom from infection.

**Endemic situation**

The decision to abandon immediate eradication and allow a period of endemic FMD would be influenced by a number of factors – for example, the exhaustion of resources needed for eradication, the need to maintain business continuity in some form within Victoria while allowing other jurisdictions to declare FMD freedom and resume trade, and the need to confine FMD to limited areas of Victoria. The discussion group failed to agree on whether Victoria would be declared an endemic zone, with the topic generating robust discussion. The group did, however, agree on the principles for maintaining an infected zone, controlling the outbreak, managing wildlife and returning to a ‘free’ state, if such a decision were imposed.

State borders and natural barriers such as national parks and rivers would be considered in setting up zone boundaries, and appropriate movement controls put in place. The use of buffer or stock-free zones would be considered. A concerted public relations effort would be needed to ensure awareness and compliance with movement restrictions.

Vaccination and surveillance within the infected zone would need to continue, with animal/product processing taking place within the zone largely for domestic use.

Surveillance of wildlife would be necessary to determine if feral animals were involved in FMD virus transmission. Such surveillance may involve trapping or culling and testing. Barriers (such as stock free areas on farms and between farms and wildlife reservoirs) might be necessary to separate livestock from feral animals as much as possible.

In returning to a FMD-free status, the infected areas within the state would be reduced in size by concentrated control efforts including vaccination. Surveillance for proof
of freedom would start 28 days after the last reported clinical case. OIE guidelines would be followed, and a recovery plan for the State would be required.

**Diagnostic methods**

The group divided the tests required into three functional groups:

**First case detection** [samples=lesions material and blood] [Ag Elisa, PCR, VI and EM & sequencing, vaccine matching]

**Operational tests** required during the outbreak response [samples=lesion material and blood] [High throughput RT-PCR with Ag Elisa, sequencing, vaccine matching for a proportion of samples and SP and NSP Elisa for Vaccine efficacy and for screening]

**Recovery and proof of freedom** [samples= blood] [NSP Elisa and backup tests]

It was envisaged that PCR testing would be the main method used for diagnosing new cases. The outbreak would be monitored periodically using gene sequencing to check the strain involved. NSP ELISA was the test of choice for proof of freedom surveillance – VNT would be used to follow up positives.

There was much discussion on the use of lateral flow devices (LFD – “penside testing”) – it was seen as an adjunct to clinical surveillance to be used where lesions were suggestive of FMD. It may also be of use in sheep flocks where lesions were not always clear, and in useful in follow-up traces. The group did not reach a clear consensus on the use of penside tests.

A number of research needs were identified, including:

**Agent detection**
- Bulk milk test to screen tankers for infected herds
- Microarray for serotyping devices
- Microarray for detecting serotyping subtypes
- Improve the speed of Ab and Ag ELISA through conjugation
- Real time LAMP test
- Test for carrier status

**Serology**
- One step Ab assay
- NS ELISA – improved sensitivity
- ABC ELISA validation in pigs and sheep

**LFD**
- LFD or PCR to give serotype
- Rapid and reliable POC – minutes not hours
- LFD with Ag and Ab capability
- Increased sensitivity for LFD

**Other**
- More cost effective reagents
- New cell lines for all species
- Simple, Rapid high throughput sample processing
• Thermal imaging
• Need tests stable at higher temperatures
• Development of Luminex technology for nucleic acid detection

Recommendations to CVO

• Provide field vets with good tools and instructions to ensure the optimal samples arrive at the lab / used for LFDs
• Early in the outbreak: Focus on rapid detection – do vaccine matching quickly
• Operational- keep it simple: RT-PCR high throughput, VI and Ag ELISA
• Recovery + Proof of freedom: serology based, robust high throughput NSP testing with confirmatory NSP tests backup. Ignore detection of persistence – not worth it.

The decision to vaccinate

The number of participants subscribed to this group was very large, necessitating the splitting of the group into two. These two groups were further subdivided to ease discussions. It was thus difficult to achieve consensus across all of the issues.

While it was realised that under the scenario supplied, vaccination (and probably vaccination to live) was the best option to follow, there was much discussion on the role of carriers, the sensitivity/specificity of the NSP ELISA in exposed populations, and the actual size of vaccinated buffers around IPs.

There was a strong feeling that apart from buffer vaccination (with buffer radii from 1 to 3 to 5 and even 10km being considered), vaccination of dangerous contact properties (DCPs) should also be undertaken. One group also discussed the value of vaccination of the known IPs, which couldn’t be de-populated, as a means of reducing the risks to other properties.

There is a need to decide upon, and communicate very early, whether vaccinated animals would be removed or allowed to live out their commercial lives. It was also made clear that a swift return to FMD-free without vaccination was the ultimate aim of the disease control effort.

The participants felt that under a vaccinate-to-live scenario, only cattle would be vaccinated, pigs and sheep could possibly be subject to sero-monitoring. All animals on infected premises would be destroyed in any case.

There was perhaps a role for point of care testing during vaccination – such tests might be helpful in determining the status of a property prior to vaccinating.

Effective identification and tracing of vaccinates was seen as essential.

Major recommendations from these groups were:
• Post-outbreak serological monitoring – DIVA testing and remove only +ve animals to slaughter (if testing strategy can be applied on an individual animal basis via serial testing or new/better DIVA assays)
• Review the terminology – ‘vaccinate to live’ and ‘vaccinate to die’. Vaccination to die can be either slaughter and recover of protein (post withdrawal period) or kill and dispose (burn, render, compost, bury, landfill)
• Review decision tree – doesn’t allow for vaccination as initial option
• Early PoF arrangements to support zoning/compartments
• Negotiate arrangements with OIE/trading partners. Prior agreement on vaccination strategies and possible zones would remove a lot of uncertainty from the issue and avoid the need to negotiate “in the heat of battle”.
• Pre-prepare a communication strategy
• Pre-emptive RFID policy
• More research needed on reliability of DIVA testing
• Human resource planning
• Refine decision processes and exit strategies for ‘vaccinate to live’ and ‘vaccinate to die’

7. Summary of Outcomes

There was general agreement from participants that the scenario workshop was an interesting and valuable exercise. While the symposium served to place the issue of vaccination (with a bias towards vaccination to live) firmly on the agenda, it also highlighted the need for more research, discussion and communication on the use of vaccination in the eradication process.

• The role of point-of-care testing (e.g. LFD) requires further clarification.
• The true role of carrier cattle needs to be spelled out more clearly, especially to producers who still have concerns in this area.
• Further clarification regarding specificity and sensitivity of DIVA testing is needed; particularly where potentially exposed animals may be vaccinated.
• Clear planning of responses involving vaccination is needed. AUSVETPLAN and the ‘decision tree’ need revision, and more planning around communication is needed. Consumer acceptance of ‘vaccinated’ products is one issue amongst many that will need management.
• Given the novelty of vaccination usage, it would be advisable to initiate discussions with trading partners on this prior to an emergency.
• The introduction of a subunit vaccine would enable the manufacture of vaccine in uninfected countries such as Australia; it may also add to the reliability of current DIVA tests.
• There is a need for further research on testing for agent presence, a more sensitive NSP ELISA and better reagents, amongst others.
Appendix

FMD 2010 workshop discussion – brief overview of questions and answers

Diagnostics

Audience member: asked if any diagnostic techniques have been validated for wildlife species

David Brake: asked which specific devices the diagnostics group was recommending for the scenario as their presentation did not seem to be an overview and not based on the scenario.

Hugh Millar: asked what follow-up should be done for positive DIVA tests and if there is a straightforward diagnostic pathway for reactors.

Chris Morrissy: probang, PCR, VI. Resampling. Different ELISAs. Should be aware that more than one positive would be expected if the herd was actually positive.

Vaccine 1

Ross Cutler: Lot of debate about vaccinate to live vs vaccinate to die – comments?

Kris De Clercq: How long would it take to kill 500 000 animals?

Lori Miller: suggested with information provided 1 property would take 3 days.

Graeme Garner: reminded the audience that vaccinated animals would not necessarily need to be slaughtered on farm. Animals can be sent to an abattoir for slaughter. Vaccination provides flexibility regarding the time of slaughter so it can be done in a more orderly fashion.

Peter Milne: talked about the 2007 UK FMD outbreak where supermarkets would not stock vaccinated (thus animals could not enter the normal supply chain).

Aldo Dekker: discussed the 2001 Netherlands outbreak – slaughter of vaccinates began 2 weeks after vaccination. It took two to three weeks to slaughter 200 000 animals and estimated one and a half months to slaughter the 500 000 animals in the scenario, providing approximately a one month advantage over a vaccinate-to-live policy.

Vaccine 2

Martyn Jeggo: asked the group why pigs had been left unvaccinated?

Audience member: Analysis of Dutch outbreak in 2001 showed that pigs were not as important in transmission as previously believed. Pigs can be slaughtered if required.

Luis Rodriguez: asked about use of blanket vaccination in the area.
Hugh Millar: discussed used of vaccine for DCPs and IPs should resources not be available for immediate slaughter.

Simone Tolson: asked about the risk benefit of vaccination of high risk/infected properties as the team undertaking the vaccination would be most likely contaminated and unlikely to be able to continue vaccination.

Lori Miller: commented that vaccination can buy time if there are excessive numbers of animals to be slaughtered.

Luis Rodriguez: commented that four days after vaccination there is up to a 99% drop in viral shedding, even if vaccine isn’t fully effective.

Proof of freedom
The group asked the room what is the likely within-herd sero-prevalence in vaccinated exposed herds. The group also highlighted that surveillance in wildlife would be opportunistic and of lower priority than surveillance in livestock populations.

Aldo Dekker: highlighted that the group was looking for herds without clinical disease and would expect that a 10% prevalence would be in the upper level of what would be expected (in other words, many more animals would need to be sampled).

Audience member: commented that the sensitivity in the scenario was 99% but the actual test would have a sensitivity of 90% resulting in many more false negatives.

Keith Sumption: suggested that with the high volume of samples, national laboratories in the area could be set up in an affected area to get thorough testing.

Lori Miller: asked how long until animals would be antibody negative to FMD after vaccination.

Aldo Dekker: commented that he found a seropositive animal 13 years later.

Endemic
The group could not reach consensus on establishment of an infected zone. They asked the plenary session whether it was possible for the rest of Australia (or individual states) to declare freedom or resume unrestricted trade without declaring Victoria an endemic or infected zone.

Dorothy Geale: said that it was possible to have a containment zone for countries that are free.

Questions were asked about vaccination strategy for the area. Ross stopped discussion to begin panel discussion.
Panel discussion

Members of the panel: Graeme Garner, Keith Sumption, Jef Hammond, Victor Saraiva

Ross Cutler asked Sam McCullough to think about vaccination strategy in an endemic zone.

Wilna Vosloo: asked about disease in wildlife. Commented that if disease in domestic animals was controlled then wildlife tend to be ok?

Audience member: commented that high densities and certain circumstances are required for transmission between wildlife and domestic animals (Israel – gazelles)

Victor Saraiva: commented that they have never had any proof that disease has been caused by deer or wild pig. In fact, domestic populations tend to infect wild populations. Sero-conversion seen tends to be artificial, requiring close contact between species.

Graeme Garner: suggested disease surveillance efforts should be concentrated on domestic livestock.

Jef Hammond: suggested that perhaps different serotypes have different abilities to infect wildlife – perhaps Cathay-O may potentially cause infection in wild pig populations.

Sam McCullough: suggest blanket vaccination was appropriate in an endemic zone.

Simone Warner: commented that one paper has been published about potential transmission by marsupials (Jef Hammond: Bill Snowden)

Keith Sumption: commented that deer were a dead end host in the UK outbreak. Serology was not included to prove freedom.

Jef Hammond: commented that farmed deer sero-converted but no viral antigen could be isolated

Luis Rodriguez: commented that research on Plum Island showed deer were excellent transmitters of disease to cattle.

Aldo Dekker: commented that subsequent research around the world has not implicated deer. That data from Plum Island was old at which Luis commented that they did their work in different species of deer.

Victor Saraiva: highlighted the need it develop statistical models to sample areas. On average 8-10% of animals were positive. Vaccine needs to be NSP free to reduce false positives:

1. Need to wait 100 days before sampling.
2. Sample young animals not over 2 years old.
3. Test in clusters – if animal is positive resample and test using the same test, then further sampling if still positive.

**Keith Sumption**: commented that vaccinated animals in Europe are tagged for life. There is a sequence of tests available with a clear decision trees.

**Keith Walker**: commented that we need to work on the notion that there are limited resources. He argued that the mindset should be that resources will/must be found to respond adequately.

**Victor Saraiva**: commented that resources really are limited. Decisions must be made swiftly as it is important for an exporting country such as Australia to keep export markets open.

**John Stewart**: commented that under the EADRA, the default cap for the response cost is 2% of the industry’s GVP, and 20% of that would be recovered from industry.. In the case of the beef cattle sector and a GVP of $16 billion, the default cap is $320 million. Of that $64 million would be recovered from industry and repaid from levies over an agreed period.

**Martyn Jeggo**: whole point of EADRA is to stop worrying about money.

**Graeme Garner**: if using vaccination it must be used early in the outbreak response. Resourcing issues require more exploration.

**Jantien Backer**: commented on her modelling of FMD vaccination in the Netherlands. Up to governments to decide – predetermined strategy for each region.

**Aldo Dekker**: commented that recommendations were to have vaccination applied immediately in densely populated areas but have a ‘wait to decide’ strategy in sparsely populated areas. This was not acceptable with their government.

**Audience member**: Should stocks of vaccine be held in Australia?

**Hugh Millar**: Stakeholders have invested in an emergency supply arrangement. Antigen is required for vaccine development – held in vaccine banks by company specialised in the area. Interested in possibility of storing and manufacturing the adenovirus vaccine.

**Rupert Woods**: commented that there is a fact sheet on risk of FMD in wildlife on the Australian Wildlife Health Network website.

**Aldo Dekker**: suggested that Australia should look at dividing the country into separate compartments and outline these in contingencies plans (as in the Netherlands). The Netherlands initially had a problem with milk companies who had tankers moving between compartments but found that this was resolved as milk tankers plan their routes in compartments now.
Keith Sumption: suggested that the meeting of the European commission for the control of FMD in September (in Vienna) would be a place where FMD could be discussed on a global scale.

**Table of Acronyms and Abbreviations**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag ELISA</td>
<td>Antigen ELISA</td>
</tr>
<tr>
<td>AUSVETPLAN</td>
<td>Australian Veterinary Emergency Plan</td>
</tr>
<tr>
<td>CA</td>
<td>Control Area</td>
</tr>
<tr>
<td>CVO</td>
<td>Chief Veterinary Officer</td>
</tr>
<tr>
<td>DCP</td>
<td>Dangerous Contact Premises</td>
</tr>
<tr>
<td>DIVA</td>
<td>Differentiate Infected from Vaccinated Animals</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
</tr>
<tr>
<td>EM</td>
<td>Electron Microscopy</td>
</tr>
<tr>
<td>FMD</td>
<td>Foot and mouth disease</td>
</tr>
<tr>
<td>IP</td>
<td>Infected Premises</td>
</tr>
<tr>
<td>LFD</td>
<td>Lateral Flow Device</td>
</tr>
<tr>
<td>NSP</td>
<td>Non-Structural Protein</td>
</tr>
<tr>
<td>OIE</td>
<td>Office International des Epizooties (the World Organisation for Animal Health)</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RA</td>
<td>Restricted Area</td>
</tr>
<tr>
<td>RFID</td>
<td>Radio Frequency Identification</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real-time PCR</td>
</tr>
<tr>
<td>VI</td>
<td>Virus isolation</td>
</tr>
</tbody>
</table>