The 2007 Equine Influenza Outbreak in Australia – Lessons for EAD response planning.

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Overview

- An overview of the outbreak;
- The laboratory role and response;
- Lessons from this outbreak;

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Background

- A country free of equine influenza – never occurred before;
- A naive population but some vaccinated horses (had travelled overseas or were imported);
- 24 August 2007: disease suspected in large equestrian centre (200 horses);
- Over next 2 days, multi-focal outbreak identified spanning 8 locations over 700km range and into southern Qld
Initial spread of the virus

Maitland horse event
25-26/8

Narrabri horse event
25-26/8

Centennial Park Equestrian Centre Clinical signs 22/8

Warwick 3-day horse event 24-26/8
Extent of the outbreak

- 11pm 24 August 2007 – EI confirmed at Centennial Park
- 2am 25 August – horse standstill declared across whole State
- 21 September – zoning defines regions to allow control in infected areas (Amber, Red and Purple) and freedom of movement in free areas (Green)
- 22 September - Peak of infected properties – 5895
- 24 September - Peak number of new cases (farms) on 1 day - 225
- 29 September – Vaccination begins
- 22 December 2007 – last Infected Property reported in NSW
- 50,000 infected horses on 8500 farms
The Disease

- Typical clinical signs in most horses – nasal discharge and cough highly suggestive
- Disease more severe in large intensively managed populations compared to horses on pasture
- Short incubation period (48-72h, sometimes 24hrs)
- Very high morbidity; mortalities uncommon
- Incidence approached 100% except for some very isolated dispersed groups.
Epidemiology

- Direct or close contact between horses
- Rapid spread between horses – All horses in intensively managed situations infected within 2-5 days
- Transmission by fomites – movement of people, equipment extremely important
- Aerosol and windborne spread – at least 2km
- Very high levels of virus excretion for 7-10 days
- Role of other species – birds, dogs
Control Strategies

Quarantine of infected properties;
Control Strategies

Cleaning and decontamination of vehicles, equipment
Control Strategies

Cleaning and decontamination of stables & enclosed buildings
Control Strategies

- Canary pox vectored subunit vaccine selected – efficacy and DIVA potential
- Vaccination around perimeter;
- Accelerate infection in heavily infected zones (encourage movement!);
- Late in outbreak, vaccinate uninfected horses in purple zone
- Surveillance in free areas (eg buffer zones during vaccination);
- Clearance “Proof of freedom” testing of quarantined properties to reduce zones
Role of the state laboratory

- Confirmation of EI infection in clinical cases;
- Investigation of ‘dangerous contacts’;
- Surveillance in free areas (eg buffer zones during vaccination);
- ‘Assurance of freedom’ testing – eg prior to races;
- Movement testing – for movement into free areas;
- ‘Clearance testing’ – release of properties from quarantine
- “Proof of freedom” testing
Specimens and laboratory assays for EI

Detection of agent

- Nasal swabs in viral transport medium (PBGS);
- Influenza A qRT-PCR (matrix gene);
- At the Reference Lab:
  - Virus isolation, Nested RT-PCR, Antigen ELISA/PoC

Detection of antibody

- Blocking ELISA – ‘Flu A group assay;
- HI test – H3 specific
Requirements of a high throughput system

- Maximise number of samples tested per day;
- Maintain rapid turn around;
- Accuracy of results not affected;
- Able to respond quickly to changing needs;
- Cost effective;
- Does not compromise biosecurity.
Critical aspects to achieve high throughput

THE GOAL: TO MINIMISE HANDLING OR REDUCE STEPS AT ANY STAGE

- Equipment:
  - integration from ‘start to finish’ – compatible formats, minimising handling
  eg RNA extraction through to PCR; serum for dilution, testing and storage

- Assays:
  - appropriate type for HT (qRT-PCR vs gel, ELISA vs HI)
  - format: 8 x 12 matrix;
  - can be automated;

- Reagents: can be supplied/prepared in bulk or large quantities;

- Require a consistent specimen type and packaging
Systems for a high throughput – qRT- PCR

- From RNA extraction through to PCR;
Handling of serum for ELISA: dilution, testing and storage
A high throughput system - achievements

- **Maximised number of samples tested per day:**

  During the EI outbreak
  
  - 21,000 accessions: a 10-15 fold increase in accessions and samples (peak 550/day)
  
  - qRT-PCR – 71,000 assays completed (>30,000 in 4 week period)
  
    (2,300 in 2 shifts/day, 300 per 2 hr period), plus “business continuity” (total 3,000)

  - bELISA – 66,000 sera tested

- **Maintained rapid turn around:**

  - Samples for qRT-PCR tested ‘same day’ – diagnostic sample turn-around ave 3 hrs;

  - ELISA results ‘next day’
A high throughput system - achievements

- Accuracy has not been affected;
- Able to respond quickly to changing needs;
- Cost effective;
- Does not compromise biosecurity.
qRT-PCR for EI testing

- Very high sensitivity and specificity.
- Easy technology to transfer and train new staff
- Robust, highly reproducible, relatively low cost
- Detects RNA before onset of clinical signs (24 hrs post exposure)
- Detects very low levels of residual RNA in recovering horses (some implications)
- Need to exercise EXTREME care with inactivated vaccines or products containing target nucleic acid sequence
- Capacity to multiplex assays for diagnosis in ‘peacetime’ – both DNA & RNA (eg EI, EHV).
Large scale testing – the challenges

- Specimen receipt:
  - No of accessions: disinfection, processing, data entry
- Sample type: swabs, blood samples (impact of wrong type);
- Sample labelling and documentation;
Large scale testing – the challenges

- Reliable supplies of consumables – operation can be very delicately balanced and dependent on unexpected shortages
- LIMS data entry and reporting of results;
- Changes of field staff, sampling strategies, reporting requirements;
- Media scrutiny;
- Sustained effort;
- Maintaining “normal business”.

www.dpi.nsw.gov.au
Responding to questions that arise during an EAD response

- Can people become infected? (Species=‘P’)
- Is the virus being spread by wild birds – eg pigeons in feed troughs
- Is there any evidence of infection in dogs?
- Is there any evidence of ‘dog to dog’ transmission?
- Some lessons regarding the role of humans
- Expect the ‘unexpected’ – difficult to plan for – a need for flexibility
Keys to a successful laboratory response

- Suitable sample format for assays to be used;
- Appropriate assays – suitable for needs, adequate performance data for adaptation to a new situation, high specificity to minimise retests/confirmatory testing;
- Suitable equipment and facilities with adequate backup;
- Reliable suppliers of consumables and for equipment maintenance (‘trivial’ components can be critical – 5mL vials, swabs, mastermix)
Keys to a successful laboratory response

- Appropriately trained, skilled and committed staff who are dedicated to a prolonged response
In the field:

- Logistical issues vary markedly with species affected:
  - horses (the most complex species?): highly mobile, ratio of animals to owners very low & large number of ‘affected’ owners and associated workers;

- Property and animal identification a challenge;

- A need for access by field staff to a permanent property/owner database;

- Immediate availability of preferred sample vials and transport medium;

- Systems to streamline specimen identification and data entry must be considered (eg Pre-labelled specimen tubes and permanent property ID)

- Data entry close to point of collection should be considered;
At the laboratory:

- Require systems/strategies to minimise delays during specimen receiveal, disinfection, cataloguing and data entry;
- Availability of basic consumables can be critical (swabs, tubes);
- Clearly defined, unambiguous lines of communication from field to laboratory for supplies (cannot afford duplication);
- Regular, reliable communication between control centres (local and state) and laboratory to convey short and medium term plans and needs;
- Clear lines of communication for provision of relief staff with appropriate skills, in an appropriate timeframe;
- A need for flexibility to respond to unexpected demands or changes.
Keys lessons from the EI outbreak - 3

Overall – what really made the difference:

- Early detection and rapid diagnosis critical;
- Rapid implementation of standstill;
- Industry and owner co-operation essential;
- Availability of suitable assays, reagents and equipment;
- Vaccine must be available or delivered at short notice
- DIVA strategy very successful
- Without real time PCR, EI would be endemic in Australia
The EMAI team

- Regional Veterinary Laboratory
  - specimen receipt;
  - reporting

- Virology Laboratory:
  - specimen receipt & processing
  - qRT-PCR, bELISA, HI
  - reporting, specimen storage and disposal
Australia free of EI

- NSW provisionally free – 15 March 2008
- Australia officially free of EI – 30 June 2008
- Imported horses:
  - vaccinated;
  - Negative PCR within 3 days of departure;
  - PCR within 24 hours of arrival;
  - PCR with 72 hours of release from quarantine