



NSW DEPARTMENT OF
PRIMARY INDUSTRIES



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Equine Influenza in Australia –
the importance of new technologies in the laboratory

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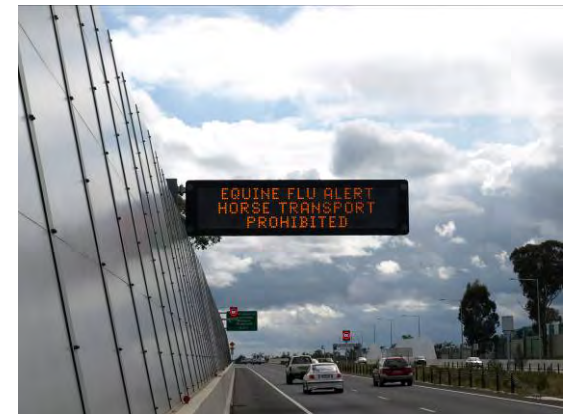
Is there a need for laboratory tests?

- Clinical signs of equine influenza are considered to be highly suggestive but not specific to EI;
- Laboratory tests necessary to prove conclusively that a horse has been infected;
- Diagnostic testing can involve:
 - detection of the virus in first 7-10 days;
 - detection of antibodies after 10-14 days



Is rapid diagnosis important?

- 24 August 2007: disease suspected in large equestrian centre;
- Samples arrive 7.50 PM
- Advice to CVO 23.05
- Standstill imposed early AM 25 August 2007
- Samples reach AAHL mid morning 25 August;
- Subsequently, urgent cases could be confirmed at EMAI within 2 hours of arrival at laboratory

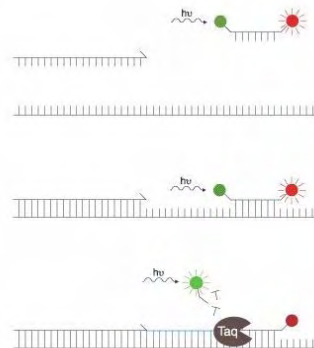
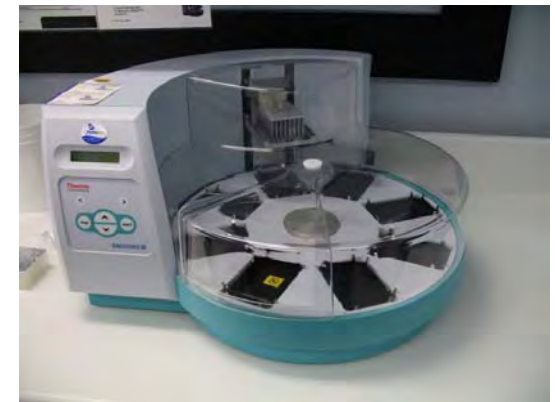




New laboratory tests – the EI outbreak

- **Detection of virus:**

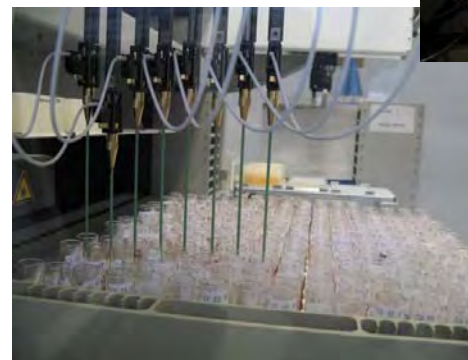
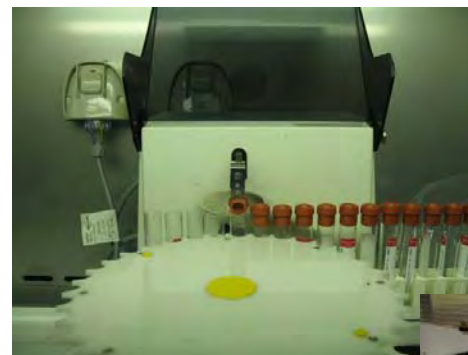
- Virus isolation (culture) – many weeks;
- Antigen ELISA – rapid but lacks sensitivity;
- ‘Conventional’ polymerase chain reaction (PCR) labour intensive, prone to cross contamination;
- “Real time” PCR – rapid, efficient, can be automated, permits high throughput capability



New laboratory tests – the EI outbreak

Detection of antibodies:

- Haemagglutination inhibition (HI) test
 - relatively labour intensive;
- Blocking (b) ELISA
 - rapid, specific, detects antibodies to specific virus ‘subunits’ and proteins
 - detects antibodies to virus core;
 - canary-pox vectored vaccine only induces antibody to ‘spike’ protein (H3);
 - allows discrimination between infected and vaccinated horses



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





Benefits of 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
- A key tool in proving freedom from infection (antibody tests of no value in infected zones – all infected horses remain positive)

Questions that needed to be answered during an EAD response – the impact of new technology

- How long do horses continue to excrete virus?

NSW Police horses – monitoring of EI virus ‘shedding’ by qRT-PCR

Name	Onset	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16	Day 17	Day 18	Day 19	Day 20
		Angus	29/08/2007			20.65	23.18	21.61	28.51	30.89	32.59	31.65	31.59	29.76	35.34	31.73	36.19	42.16	40.48	33.87	38.08	34.95
Anzac	27/08/2007	19.80				21.14	25.54	35.00	32.22	26.22	28.34	34.15	33.07	37.55	38.41	39.32	39.47	undet	36.22	undet	undet	undet
Beau	28/08/2007																					
Beaufort	27/08/2007	31.01				21.63																
Bill	28/08/2007			20.21	26.12	23.86	28.96	30.09	29.26	32.07	30.89	undet	35.69	undet	36.81	undet	undet	undet	undet	undet	undet	undet
Bodie	29/08/2007		22.45	21.75	27.25	26.36	23.52	27.50	27.57	29.55	32.18	30.69	29.58	32.92	30.92	33.53	undet	undet	undet	undet	undet	38.15
Bruce	28/08/2007			24.14	22.75	27.87	26.64	31.45	30.71	33.54	35.49	undet										
Captain	27/08/2007	22.24				27.93	32.94	27.18	28.75	31.27	33.23	32.87	36.26	33.47	undet	34.00	undet	undet	undet	undet	undet	undet
Cedar	27/08/2007	19.66				25.13	29.07	29.25	26.13	26.82	32.45	33.00	31.96	32.01	undet	35.65	undet	undet	undet	undet	undet	undet
Dandy	29/08/2007			21.70	25.89	26.01	25.10	28.17	32.10	24.44	30.78	31.79	31.28	32.11	32.07	40.04	undet	undet	39.31	undet	undet	35.62
Darcy	28/08/2007																					
Doctor	27/08/2007	28.71				27.26	24.84	28.86	26.55	30.89	23.65	33.28	31.12	40.13	undet	35.16	39.42	41.69	undet	undet	undet	undet
Duke	31/08/2007		21.01	19.16	23.45	28.89	35.00	33.93	35.54	33.51	35.81	undet	33.87	undet	undet	undet	undet	undet	undet	undet	undet	undet
Echo	29/08/2007			22.08	23.17	27.31	25.44	25.78	29.42	31.31	35.65	36.58	39.62	30.05	34.48	33.70	44.05	undet	undet	undet	undet	39.72
Fred	28/08/2007				22.11	25.12	32.06	26.91	28.40	32.09	39.15	33.10	undet	undet	41.57	undet	undet	undet	undet	undet	undet	undet
Gallant	28/08/2007				24.18	27.55	26.33	27.14	28.44	29.73	27.29	27.96	26.20	28.41	27.44	30.47	33.48	32.89	undet	undet	undet	undet
General	30/08/2007		16.36	23.27	25.61	24.90	24.20	28.83	31.85	29.36	29.92	34.94	37.79	33.76	undet	43.04	undet	undet		undet	undet	undet
Hannah	26/08/2007	24.52					25.68	29.30	24.21	28.85	31.46	34.18	35.60	35.71	35.00	undet	undet	40.51	37.21	undet	undet	undet



bELISA for EI testing

- Rapid assay – can be semi-automated (strategic combination of robotic liquid handling of specimens and technicians)
- High sensitivity (>99.5%) and acceptable specificity (97%)
- Can detect antibody in most horses from about day 5 of illness
- Long lasting antibody response (>6 months in absence of re-exposure)
- Will detect antibodies induced by inactivated and subunit vaccines
- Distinguishes horses that are naturally infected (or vaccinated and exposed) vs vaccines that induce a H3-specific response (DIVA)

- Another question: How soon can antibodies be detected in bELISA?

NSW Police Horses – what was the first day on which all test positive

Name	Onset	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Angus	29/08/2007			17	16	13	40	68	83	77	89	82	89	87	91	92
Anzac	27/08/2007					61	70	78	81	88	88	86	82	89	88	90
Beau	28/08/2007															
Beaufort	27/08/2007					17										
Bill	28/08/2007				18	14	36	66	80	88	92	81	92	91	90	92
Bodie	29/08/2007			15	27	50	73	86	86	89	87	84	88	86	89	91
Bruce	28/08/2007				21	26	27	53	80	89	89	92	89	84		92
Captain	27/08/2007					32	66	80	84	88	84	87	88	70	81	84
Cedar	27/08/2007					21	48	77	75	84	82	85	88	84	81	87
Dandy	29/08/2007			10	9	32	56	74	86	87	89	83		89	92	79
Darcy	28/08/2007															91
Doctor	27/08/2007					21	36	66	82	89	90	83	91	89	92	91
Duke	31/08/2007	6		8	10	19	59	77	87	85	89	89	91	93	91	93
Echo	29/08/2007			13	16	31	53	78	83	93	90	88	89	90	90	93
Fred	28/08/2007				15	83	91	93	92	90	80	92	90	90	91	88
Gallant	28/08/2007				19	29	21	46	67	59	80	83	79	87	88	92
General	30/08/2007	14		17	20	32	67	73	87	89	78	88	91	85	88	88
Hannah	26/08/2007					43	61	76	82	89	79	88	87	91	83	87
Hero	30/08/2007	13		13	24	38	65	68	80	83	80	70	78	70	89	87
Isaac	29/08/2007			9	19	51	60	80	74	80	88	86	88	85	91	92
Joker	29/08/2007			16	19	34	50	64	60	60	64	62	64	64	91	73
Major	30/08/2007	30		34	46	51	80	70	90	91	91	91	87	92	92	89
Monarch	30/08/2007	11		12	15	30	62	56	87	88	89	90	82	88	93	91
Monty	28/08/2007				18	30	30	56	75	82	89	89	87	89	80	94
Patezza	29/08/2007			18	19	50	70	88	90	91	91	84	90	73	93	92
Prince	29/08/2007			12												
Reveille	28/08/2007				77	92	88	92	92	90	91	88	93	91	78	92
Rommel	29/08/2007			16	20	29	37	67	79	87	88	89	90	87	93	
Rory	29/08/2007			10	18	23	58	76	85	81	89	89	92	89	91	92
Souvla B	29/08/2007			22	61	72	86	86	88	80	92	91	89	90	93	94
Toby	28/08/2007				18	32	45	73	82	84	91	90	89	87	89	90
Tommy	28/08/2007				16	25	73	91	91	82	92	90	92	90	89	86
Trump	30/08/2007	16		14	3	19	48	76	89	89	84	90	87	88	91	93
Wahoo	28/08/2007				23	31	63	78	86	87		82				92
Warringa	28/08/2007				10	7	34	62	83	85	89	77	84	91	79	86
Woody	29/08/2007			14	8	-1	25	70	77	81	77	88	87	83	88	89



A high throughput system - achievements

- Maximised number of samples tested per day:
 - 21,000 accessions: a 10-15 fold increase in accessions and samples
 - qRT-PCR – 71,000 assays completed (>30,000 in 4 week period)
(2,300 in one day plus “business continuity”)
 - bELISA – 66,000 sera tested
- Maintained rapid turn around:
 - Samples for qRT-PCR tested ‘same day’ – diagnostic sample turn-around 3 hrs;
 - ELISA results ‘next day’
- These achievements dependent on use of new technologies – never used before for EI diagnosis and support of control measures in an outbreak

Keys to a successful laboratory response

- Appropriately trained, skilled and committed staff who are dedicated to a prolonged response





The EMAI team

- **Regional Veterinary Laboratory**

- specimen receipt (SR & D);
- reporting (Duty veterinarians and CSU)



- **Virology Laboratory**

- specimen receipt & processing
- qPCR, bELISA, HI
- reporting, specimen storage and disposal



- **Visitors**

- Interstate & NZ

