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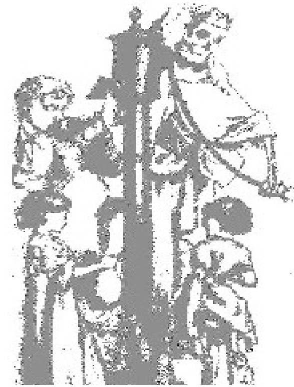
icpmr Institute of Clinical Pathology and Medical Research



FEATURE ARTICLE – LINDA HUESTON

Serological Super Sleuthing

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The arbovirus laboratory diagnoses arboviral infections, confirms arboviral diagnoses for other laboratories, determines immune status, maps arboviral antibody prevalence and investigates outbreaks or unusual cases.

A variety of techniques are used to achieve these goals. Some are well known and include virus isolation and various serologic techniques such as haemagglutination inhibition, complement fixation, immunofluorescence, ELISA and neutralisation. Equally important but lesser known techniques include interviewing and information gathering regarding geography, ecology, weather, disease trends nationally and internationally. Individual patient information is also gathered regarding clinical presentation, onset dates, duration of symptoms, travel histories, family history, vaccination history and occupations.

A review of the laboratory and non-laboratory data leads to a final diagnosis. Some diagnoses are straightforward but others take us on unusual and unpredictable journeys. Below are two cases to illustrate these points.

Case 1: How do you get a case of locally acquired dengue in metropolitan Sydney?

The story: A resident of Manly (a beach suburb in Sydney) presented to his GP with a short history of bi-phasic fever, with rash and joint pain. No travel history was given but the request specified dengue serology.

The results: The acute sample was negative for flavivirus IgG antibody but was positive for flavivirus IgM antibody suggesting this was an early primary flavivirus infection. Additional dengue specific serology suggested the infection was due to dengue fever. The clinician was contacted to obtain a travel history. The clinician confirmed there was no travel outside Sydney.

We assumed a false positive IgM and set up neutralisation tests (the "gold standard") and requested a second sample. The results demonstrated a rise in IgM and an IgG seroconversion to dengue fever. The neutralisation tests confirmed this and indicated the infection was a primary dengue 3 infection.

The dengue vector *Aedes aegypti* was eradicated from NSW following WW2. As NSW doesn't have a vector for dengue the only cases we see are acquired overseas or are from north Qld following re-introduction of the virus to that state by viraemic travellers. For this patient to have acquired dengue in Sydney the vector *Aedes aegypti* would need to have been introduced, established and become infected with dengue 3 before this transmission could have occurred OR was there more to the story? Both the patient and GP were interviewed.

We discovered the patient was a biomedical engineer, working on equipment used to produce commercial dengue fever kits. We attended an inspection of the production premises where we determined that the kits use live, high titred virus as the antigen. The production process involved significant aerosol generation, use of mucosal irritants, no safety gear was used (no gloves or breathing protection) and staff reported paper cuts from virus-impregnated membranes.

The outcome: We determined the patient acquired his dengue infection from his workplace either by direct inoculation through paper cuts and/or inhalation of lyophilised virus. Production was halted; safety warnings were issued with the kits to advise users of the need for appropriate biosafety measures. Arboviral infections are not thought to be acquired by aerosol; while this is true in nature it is a reminder that this route can transmit organisms if there is enough organism and significant aerosol generation. This incident highlighted the need for better laboratory and production safety requirements at a national level.

Case 2 How do you prove a dengue infection was not acquired in Australia?

The story: In 2000 an air force officer was deployed to East Timor. During his deployment he developed an illness consistent with dengue and was returned home.

The results: Original testing (not done at CIDMLS) in 2000 showed low level dengue IgM with no IgG. A diagnosis of dengue was issued and then later rescinded when a convalescent sample showed no IgG. This was assumed to be a false positive IgM, the possibility of a false negative IgG had not been considered and neutralisation tests were not performed.

In 2002 routine testing at CIDMLS suggested a past dengue infection. Where had he acquired dengue?

Samples from 2002 and 2004 were located and tested for dengue. Only dengue 4 antibodies were detected and the titres had remained static over a 2-year period. Travel history (including military deployments) from 1999 to 2004 was researched. Travel and deployment included East Timor, Darwin, Richmond (NSW) and Amberley (Qld).

The outcome: We determined that he did not acquire dengue during his time in NSW or the Northern Territory– as these areas did not have a vector capable of transmitting dengue. Dengue could have been acquired in tropical north Qld but this would exclude Amberley.

All four serotypes of dengue circulate in East Timor. However, dengue 4 had not been locally transmitted in North Queensland until 2003.

Continued overleaf

This patient's antibody predated the introduction of dengue 4 into Australia. We concluded the patient had most likely acquired dengue 4 during his deployment to East Timor.

These two cases illustrate that serology is more than generating antibody titres or positive and negative results. – it's about detection and deduction. To quote Sherlock Holmes *"- it's elementary my dear Watson".*

GLENDA ARNOLD TALKS ABOUT FOOD MICROBIOLOGY

The ICPMR's Division of Analytical Laboratories provides a range of scientific services essential to the State's public health system. The laboratories are widely acknowledged as the State's leading authority in food analysis and food safety, having been responsible for monitoring the microbiological and chemical quality of foods, and investigating food – associated problems since the late 1880's. The laboratories were pivotal in the analysis of foods during the lead up to the 2000 Olympic Games in Sydney. The laboratories are currently negotiating a service level agreement with the newly created NSW Food Authority, for the provision of analytical services required by the Authority in fulfilling its responsibilities in food regulation, food safety and the investigation of foodborne disease outbreaks in NSW.

The Food Microbiology Laboratory is one of the laboratories within the Public Health Sciences Branch, conducting microbiological examination of foods submitted from all over NSW. A wide variety of foods are routinely tested for compliance with regulatory standards stipulated in the Australian Food Standards Code. Specific surveillance programs are also undertaken to assess and identify public health risks and to provide data to aid in the formulation of new microbiological standards. In addition, the laboratory has extensive experience in the isolation and identification of microorganisms of public health significance, as it plays a major role in the investigation and control of incidents and outbreaks of foodborne illness in NSW.

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In the first 6 months of 2004, the number of cases of gastroenteritis almost doubled, compared to the same period in 2003. In particular more than 1000 cases of Salmonella infection were reported in NSW during this period necessitating a large increase in the analysis of suspect foods for *Salmonella*, prompting NSW Health and the NSW Food Authority to issue a health alert to the community warning of the importance of properly preparing and cooking meat
and chicken products.

The Food Microbiology Laboratory utilities leading-edge diagnostic technologies, including molecular microbiology methods, to allow rapid and reliable detection of foodborne pathogens which may include *Salmonella, Shigella, Bacillus cereus, Clostridium perfingens, Campylobacter jejuni/coli,* coagulase positive staphylococci, *Listeria monocytogenes, Vibrio parahaemolyticus, Vibrio cholerae, Vibrio vulnificus* and verocytotoxic *Escherichia coli.* Furthermore the Molecular Microbiology Laboratory of this Division has developed molecular typing techniques to compare clinical isolates with pathogens isolated from foods implicated or suspected of being implicated in human illness – hence assisting epidemiological investigations.

Food surveys undertaken by the Food Microbiology Laboratory over the last few years have included investigations into the microbiological quality of:

- hospital and cook-chill foods;
- shell eggs in NSW, particularly for the presence of *Salmonella*;
- smorgasbord foods;
- smallgoods in general;
- food safety survey of hospitals and aged care institutions;
- *Salmonella* in Poultry.

CIDM PUBLIC HEALTH – EDUCATION PROGRAM

- **19 August 2005: Parasitology Symposium – Are we winning the parasitology war?**

The recent TV series *'The Body Snatchers'* has highlighted some of the measures employed by parasites in a bid to take us hostage. How is it that parasites have an army of mechanisms with which to invade us - whilst our bodies are equipped with only very limited defence? Why have some attempts to treat parasitic infections been successful whilst others have failed? At this one-day symposium some of Australia's leading parasitologists will uncover a four-decade search for the truth behind parasitic infections and bring you up-to-date with parasitology in Australia today.

- **04 November 2005: The Salmonella Journey – From diarrhoea to database.**

To receive further information & join our e-list, please email judithh@icpmr.wsahs.nsw.gov.au

Congratulations!

Congratulations go to Sasha Elliott in the Hepatitis Lab at CIDMLS. She has just topped her year at TAFE for the Diploma in Laboratory Technology (Pathology Testing). Sasha, who is now a permanent Technical Officer, says that she appreciates the support of other staff during her studies. She received an award which is sponsored annually by Mayne Laverty Pathology.

Congratulations also to Dr Vitali Sintchenko (Public Health Microbiologist & Health Informatician employed by CIDM Public Health) who has just been awarded a PhD entitled "Decision by design - Decision support for antibiotic prescribing in critical care" (University of New South Wales)