Senate Community Affairs Committee

ANSWERS TO ESTIMATES QUESTIONS ON NOTICE

HEALTH PORTFOLIO

Supplementary Budget Estimates 2013-14, 20 November 2013

Question: E13-195

OUTCOME: 1 - Population Health

Topic: Zoonotic Diseases in Kangaroo Meat

Type of Question: Written Question on Notice

Senator: Rhiannon

Question:

On Food Standards website http://www.foodstandards.gov.au/publications/Documents/Toxoplasma%20gondii.doc there is a document titled Toxoplasma Gondii. Its Table 1 on page 7 lists 13 fatalities during 1993 in Australia associated with toxoplasmosis (T.gondii), caused by consumption of undercooked kangaroo meat.

a) May I please have details of those cases?

b) Are there any other recorded cases of T.gondii in Australia caused by human consumption of undercooked kangaroo meat? i. What warnings were and are given to the public by either government or by the industry regarding this potentially fatal health risk?
ii. What was the health or mortality outcome of those cases?
iii. What is the process for reporting and collating this information, and where can that information be publically accessed?

Answer:

a) During the outbreak of toxoplasmosis in 1994 (incorrectly listed as 1993 in the Food Standards Australia New Zealand (FSANZ) report) there were 13 cases of infection. There were no deaths. Symptoms included myalgia, headache, lethargy, fever, chills lymphadenopathy. Four of the 13 cases had no symptoms. The cases are detailed in the attached report (Attachment A) that was published in the Department of Health's journal Communicable Diseases Intelligence.

While the question the Honorable Senator asked stated that there were 13 deaths, as mentioned above, there were no deaths. The table below taken from the report in question listed the total no. of cases involved in each outbreak, and the number of associated fatalities would be given in brackets "Total no. cases (fatalities)" and "No. congenital cases (fatalities)", which could fairly easily be misinterpreted. If there had been any fatalities, they would have been listed as "176(3)" for example. FSANZ has since updated the document to remove the possibly of misinterpretation, and the updated version can be found at http://www.foodstandards.gov.au/publications/Documents/Toxoplasma%20gondii%20-%20dec%202013.pdf

Table 1: Selected major foodborne outbreaks associated with *T. gondii* (≥5 cases and/or ≥1 fatality), from report *Toxoplasma gondii*, Food Standards Australia New Zealand.

Year	Total no. cases (fatalities)	No. congenital cases (fatalities)	Food	Country	Comments	Reference
2001-2002	176	0	Water and ice cream	Brazil	Kittens lived on top of the water reservoir tank. Rainfall may have carried oocysts into water reservoir. Ice cream prepared from contaminated water	(de Moura et al. 2006)
1995	5	0	Pork liver	Korea	Consumption of raw pork offal from a domestic pig	(Choi et al. 1997)
1993	13	1	Kangaroo meat	Australia	Consumption of undercooked meat	(Robson et al. 1995)
1993	17	0	Mutton	Brazil	Consumption of raw mutton	(Bonametti et al. 1997)

b) The outbreak in 1994 in Australia is the only outbreak associated with the consumption of kangaroo meat.[1] Despite a widespread prevalence of toxoplasmosis among humans and other vertebrates, overt outbreaks are rarely reported. A 2002 report prepared for the New Zealand Food Safety Authority summarized knowledge of foodborne outbreaks of toxoplasmosis worldwide. Only 15 food or water-borne outbreaks were published up to that time. There has only been one other outbreak reported in Australia due to any food (an outbreak due to suspected raw lamb in 1979).

i. Public Health messaging on toxoplasmosis is available from State and Territory health departments. These mention toxoplasmosis as a potentially foodborne disease and offer precautions to prevent infection such as not eating rare or medium-rare meat, thoroughly washing vegetables and washing hands and utensils after contact with raw meat, for example: http://www.betterhealth.vic.gov.au/bhcv2/bhcarticles.nsf/pages/Toxoplasmosis_reducing_the_risks An example of information contained within broader advice for pregnant women published by New South Wales is available from: http://www.foodauthority.nsw.gov.au/_Documents/consumer_pdf/pregnancy-brochure.pdf

National evidence-based antenatal care guidelines for medical practitioners are currently being reviewed by the Australian Government in collaboration with state and territory governments, and will include a section on toxoplasmosis. Prevention messages in the section will include the need to avoid the consumption of raw and undercooked meat. Public release of the guidelines is expected in late 2014.

[1] Risk Profile: *Toxoplasma gondii* in red meat and meat products, Dr Rob Lake, Dr Andrew Hudson, Peter Cressey, August 2002

ii. There were no fatalities in the outbreak with 13 cases (see details of symptoms above and in the attached report), and no other known outbreaks.

iii. Toxoplasmosis is not nationally notifiable in Australia, and is not notifiable in any Australian state or territory. Therefore, there is no collation of information on individual cases. OzFoodNet, Australia's enhanced foodborne disease surveillance network investigates outbreaks of foodborne disease. OzFoodNet has maintained a register of foodborne disease outbreaks since 2000. There have been no recorded outbreaks of foodborne toxoplasmosis in Australia since surveillance began 2000. OzFoodNet reports can be found on the Department's website (http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-pubs-cdi-cdiintro.htm)

period 1991 to 1994 (Figure). Cases have, however, occurred throughout the year, probably reflecting different seasonal peaks in cases due to *L. pneumophila* (in summer and autumn) and *L. longbeachae* (for which spring peaks have been reported). The NNDSS will distinguish between cases caused by *L. pneumophila*, cases caused by *L. longbeachae* and cases due to other species, beginning in the near future.

There were 179 notifications of legionellosis in Australia in 1994, with a peak in onset in March-April. So far this year there have been 144, about the same as by this time last year, and equivalent to 193 for the whole year. One hundred and seven have been for males and 36 for females (the sex of one was not reported). Fifty-seven reports (40%) have been for persons over the age of 65 years and a further 44 cases (31%) have been reported for persons between 50 and 64 years.

A PROBABLE FOODBORNE OUTBREAK OF TOXOPLASMOSIS

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Introduction

Toxoplasma gondii is a ubiquitous intracellular protozoan parasite. Cats are the only known definitive hosts, but a wide range of animals and man are susceptible to infection as intermediate hosts[1,2].

Three life forms of *T. gondii* occur: the oocyst (which contains sporozoites and is the product of the sexual cycle in the small intestine of cats), the tachyzoite (the asexual invasive form), and the tissue cyst (which contains bradyzoites capable of persisting in tissues during the chronic or latent phase of the infection). Cats acquire infection by hunting wild mice and birds, from being fed raw meat or by the faecal-oral route from other cats. In other hosts, ingestion of oocyts results in initiation of an asexual cycle with the formation of tissue cysts. Ingestion of these tissue cysts by a wide range of mammals and birds leads to release of motile bodies (bradyzoites) by the action of digestive enzymes; thus initiating fresh asexual cycles and acute infection. Herbivores, including livestock, become infected by ingestion of grass and other foodstuffs that have been contaminated by cat faeces. Their tissue may remain infectious for life.

The two major routes of transmission to humans are oral and congenital. Ingestion of environmentally resistant oocysts shed from infected cats may contaminate hands, food or water and result in infection. Eating raw (uncooked or undercooked and unfrozen meat) which contains tissue cysts is the other common source of exposure. The incubation period was said to be 10 to 23 days in one outbreak associated with undercooked meat and 5 to 20 days in an outbreak associated with cats[3]. Person to person transmission is known only from mother to child *in utero*. Tachyzoites cause infection in the fetus during a primary maternal infection. These rapidly proliferating forms may also result in infection from contaminated blood transfusions.

It is not known what proportion of human *T. gondii* infection is due to eating raw or undercooked meat containing tissue cysts and what is due to oocysts on unwashed hands or vegetables. Estimates of the rate of infection of meat vary widely depending on the animal species and locality. The changes in food preparation and eating habits that have taken place throughout the world in the past few decades have increased the risk of human infection through consumption of undercooked meat. The impact of new game meats such as kangaroo is unknown.

T. gondii infection in children and adults is often asymptomatic or characterised by a mild influenza-like illness or lymphadenopathy, while congenital infection may lead to severe defects or death *in utero*. Infection is serious in immunocompromised hosts, such as transplant recipients and AIDS patients, in whom reactivation of latent infection may also occur.

This report details a probable foodborne outbreak of toxoplasmosis which involved 12 acute infections in adults and a case of congenital toxoplasmosis.

Index cases

The index case was a 31 year old woman diagnosed with acute toxoplasmosis after her baby was found to have chorioretinitis. At 34 weeks gestation an amniocentesis has been performed to investigate possible Rhesus incompatibility. One week later (in January 1995) she went into premature labour and delivered a live female infant who was jaundiced because of rhesus disease. Phototherapy was required for 10 days. No transfusions were administered.

At three months of age, delayed visual maturation was found but thought to be unrelated to an incidental finding of bilateral mature looking retinal scars clinically suggestive of congenital toxoplasmosis. Serological testing of the baby in May 1995 at 16.5 weeks revealed a positive IgG (index 8.902) and IgM (index 2.224) by EIA. The positive IgM was confirmed by an indirect immunofluoresence antibody technique (IFA) at a titre of 1:160. Serology on the mother at the same time was consistent with acute toxoplasmosis; both IgG (index 7.720) and IgM (index 2.136) were positive. IFA testing demonstrated a IgM titre of 1:160. Antenatal serum collected at 21 weeks was tested in parallel and showed no evidence of previous exposure to toxoplasmosis (IgG negative, index 0.075; IgM negative 0.779; IFA<20), confirming primary toxoplasmosis

Antenatal serum collected at 21 weeks was tested in parallel and showed no evidence of previous exposure to toxoplasmosis (IgG negative, index 0.075; IgM negative 0.779; IFA<20), confirming primary toxoplasmosis in the intervening period. The child was commenced on pyrimethamine and sulphamethoxazole together with twice weekly injections of folinic acid in June 1995 when she was five months of age. Treatment is anticipated to continue for one year.

Possible exposures were explored. The mother recalled eating undercooked kangaroo meat on 25 November 1994 when she was 28 weeks pregnant during a Christmas function at a suburban Brisbane restaurant. She was aware that another person who attended the restaurant had also been diagnosed with toxoplasmosis, in December 1994. In retrospect, she recalled lethargy and myalgias three weeks after the party. The index case worked as a preschool teacher but stopped work at 36 weeks gestation. The family did not own a cat but had a sandpit at home. In September 1994 they holidayed on an outback Queensland property but did not consume rare meat and drank boiled tank water. There were many feral cats about the property but direct contact was avoided.

The second case, of whom the first case had been aware, was a male aged 34 years who presented to his general practitioner nine days after the party with fever, nausea, myalgias and arthralgias. Liver function revealed a mild hepatic picture. Serological testing of paired sera on days 13 and 63 demonstrated seroconversion to toxoplasmosis. His wife who also attended the party was five weeks pregnant. Testing in a previous pregnancy had shown evidence of past exposure to toxoplasmosis.

Hypothesis

Related foodborne acquisition of toxoplasmosis for the two index cases was suspected. Its relative rareness suggested that even two cases acquired in the same time frame may have a possible epidemiological link. Hot foods served at the dinner party prior to their illness were spicy Thai chicken wantons, lamb satays, merguez sausage and marissa rolls, marinated eggplant, capsicum and fetta pizzas. Cold foods served were roasted eggplant dip with crudites and house made bread, smoked salmon and dill quiches, rare kangaroo medallions on crispy rissoto cakes and duck liver pate with naan bread.

The most likely 'risk food' was thought to have been the rare kangaroo medallions. Kangaroos are known to be an intermediate host for toxoplasmosis. Other meats (chicken, lamb and sausage) were thought to be less likely potential sources because they had been more thoroughly cooked. Whilst vegetables contaminated with oocysts may also have been a source, an environmental health inspection suggested hygiene practices in the restaurant were appropriate.

Methods

Approximately 60 people attended the function. Seven months afterwards a questionnaire was mailed to 46 attending persons asking about foods eaten and requesting a serum sample for *Toxoplasma* testing. Information was entered and collated using Epi Info v6.0. *Toxoplasma* IgG was tested using an in-house indirect EIA. IgM was tested using an indirect EIA (Gull Laboratories, Inc, Salt Lake City, Utah, USA). Various neighbours of the index case were also tested for serological evidence of acute toxoplasmosis.

Case definition

Evidence of acute infection was defined as:

a. Presence of *Toxoplasma* IgG and IgM on a single serum specimen, or

b. Evidence of seroconversion from negative to positive specific IgG for *Toxoplasma* on a retrieved and current serum specimen. Due to the timing of the blood collections in relation to the exposure time (up to seven months), the IgM may still be positive or have become negative. (Specific *Toxoplasma* IgM may remain detectable for many months after infection.)

Seroconversion provides the most reliable evidence for acute toxoplasmosis as there is the possibility of false positive IgM results with single specimens.

Those classified as not fulfilling the case definition included those with no previous exposure to *Toxoplasma* (*Toxoplasma* IgG and IgM negative) as well as those persons defined as having evidence of past exposure (*Toxoplasma* IgG positive without the presence of IgM, unless a stored serum specimen was available prior to the suspected exposure to enable seroconversion to be demonstrated).

This last group of patients may have been subject to misclassification because at the time of the study, those who experienced acute infection in December 1994 may have lost their IgM response. Alternatively, they may have acquired toxoplasmosis independently from alternative sources in the past. On a population basis one would expect approximately 30% of the cohort to be seropositive. A seroprevalence higher than this could have suggested that some patients have been misclassified as past rather than acute cases.

Results

Thirty-eight questionnaires were returned, a response rate of 83%, and all respondents also supplied a serum sample for testing. The mean age of the repondents was 38 years (range 24 to 61years). Seventeen (45%) were female and 21 (55%) males.

Twelve adults in addition to the infant of the index case showed evidence of acute toxoplasmosis (Table 1). For six of these cases, acute seroconversion was demonstrated. (Retrospective sera were available for some subjects.) Of those that did not fulfill the case definition, 20 had no evidence of exposure. As a group,

Table 1. Serologically confirmed cases of acute toxoplasmosis

Case	Sex	Age (years)	Serology	Symptoms	Attended LMO[1]	Onset	Duration
1	M	35	Seroconversion	Asymptomatic	-	-	-
2	F	35	IgG+ IgM+	Asymptomatic	-	-	-
3	F	30	Seroconversion	Muscle pain, headache, lethargy, lymphadenopathy	-	16.12.94	3-4 weeks
3a[2]	F	0	IgG+ IgM+	Chorioretinitis			
4	M	26	IgG+ IgM+	Myalgia, headache, lethargy, sore throat, cervical lymphadenopathy, night sweats, fever	3.12.94 6.12.94	30.11.94	1 week
5	F	45	IgG+ IgM+	Myalgias, headache, tiredness, sore throat, cervical lymphadenopathy, fever, chills	-	3.12.94	4 weeks
6	M	52	Seroconversion	Myalgias, headache, lethargy, fever, chills, altered liver function	6.12.94	3.12.94	4 weeks
7	F	35	IgG+ IgM+	Asymptomatic	-	-	-
8	M	39	Seroconversion	Myalgias, lethargy, cervical and axillary lymphadenopathy, fever, night sweats	10.12.94	10.12.94	4 weeks
9	M	34	Seroconversion	Myalgias, cervical and axillary lymphadenopathy, fever, night sweats, altered liver function, atypical lymphocytes	4.12.94	4.12.94	2 weeks
10	M	25	IgG+ IgM+	Cervical lymphadenopathy	-	20.12.94	6-8 weeks
11	F	33	IgG+ IgM+	Asymptomatic	-	-	-
12	M	55	Seroconversion	Myalgias, headache, tiredness, rash, fever, night sweats, cough, altered liver function, atypical lymphocytes	1.12.94	28.11.94	2 weeks

1. LMO Local Medical Officer.
2. This patient was the congenitally affected five month old infant.

Table 2. Symptoms and signs documented for cases

	Cases	
	Number (n=12)	%
Fever	6	50
Night sweats	4	33
Myalgias	7	58
Headache	6	50
Lethargy	7	58
Sore throat	3	25
Rash	1	8
Lymphademopathy		
cervical	6	50
axillary	1	8
inguinal	1	8
Weight loss	1	8

therefore, at least 32 of 38 (84%) were susceptible to primary *Toxoplasma* infection prior to the party. The remaining six (16%) had detectable IgG only, suggesting past exposure. As at least seven months had elapsed prior to testing, those with evidence of past infection may also have acquired their infection in December 1994.

The incubation period was as short as three days with a mean of 11 days and range from three to 25 days.

Of the 12 cases that had evidence of acute toxoplasmosis, eight had associated symptoms and four had been asymptomatic. Five of the symptomatic patients had sought medical advice. The pregnant index case did not attend a doctor at the time, nor did the wife of one of the patients who sought medical attention who suffered symptoms very similiar to her husband. The remaining case noted persistent cervical lymphadenopathy without accompanying symptoms and was reassured by a medical colleague. The symptoms of the cases were varied and are summarised in Table 2.

Table 3. Attack rates for foods consumed at the cocktail party

	Persons who *ate* specified food				Persons who did *not* eat at specified food					Persons who were *uncertain* if they ate specified food		
Food item	Case	Non-case	Total	Attack rate (%)	Case	Non-case	Total	Attack rate (%)	*p* Value[1]	Case	Non-case	Total
Spicy chicken	10	17	27	37	1	0	1	100	0.39	1	9	10
Lamb satay	11	18	29	38	0	3	3	0	0.53	1	5	6
Sausage rolls	5	11	16	31	3	1	4	75	0.25	4	14	18
Eggplant pizza	9	19	28	32	1	0	1	100	0.34	2	7	9
Eggplant dip	5	10	15	33	2	5	7	29	1.00	5	11	16
Smoked salmon	9	16	25	36	1	4	5	20	0.64	2	6	8
Rare kangaroo	10	16	26	38	0	7	7	0	0.07	2	3	5
Duck liver	8	11	19	42	3	6	9	33	1.00	1	9	10

1. Fisher's exact test; 2-tailed.

Three patients had liver function tests performed at the time of acute illness. Alanine transaminase (ALT) and to a lesser extent, aspartate transaminase (AST) and lactate dehydrogenase (LDH) were elevated. Atypical lymphocytes (10–11%) were present in two patients. Duration of symptoms ranged from one to eight weeks.

Testing the other child of the index case as well as several neighbours revealed no previous exposure to *Toxoplasma*.

The attack rates for cases versus non-cases for the eight items on the menu are presented in Table 3. No statistically significant association could be demonstrated between the aquisition of toxoplasmosis and any of the foods ingested using the 2-tail Fisher's exact test.

Only one non-case described an illness with onset of symptoms three days after eating at the restaurant. He suffered fever, night sweats, myalgias, headache, tiredness and diarrhoea. The symptoms lasted two days and no further investigations were performed. Serological testing showed no exposure to *Toxoplasma*.

Confounding as a result of previous blood transfusions, ingestion of unpasteurized milk or raw eggs was not found. Three of the 12 cases (25%) were cat owners (no kittens) who fed their cat raw, canned and cooked meat. Seven of the 26 non-cases (27%) were cat owners. Nine of the cases were gardeners, five of whom wore gloves while gardening. None were vegetarian and all had consumed pork, lamb and beef in the previous 12 months. One or possibly two had consumed kangaroo in the preceding 12 months. Seven enjoyed their meat moderately to very well cooked, and five usually ate meat medium rare to very rare. All the cases, except one, usually washed their raw fruit and vegetables prior to ingestion and separated raw meat from other foods while cooking.

The majority of those that acquired toxoplasmosis did not usually mix socially or eat at common venues.

In addition to the index case and the woman who was five weeks pregnant, a third woman who was approximately 28 weeks pregnant attended the function and she also developed acute toxoplasmosis. Her infant was tested in May and July at two and 4.5 months and on both occasions had no demonstrable *Toxoplasma* IgG and IgM detected.

Discussion

The most important implication from this apparent outbreak was the risk posed to non-immune pregnant women and their unborn children. In a study from the Royal Women's Hospital (Melbourne) the seroprevalence of *Toxoplasma* antibodies in pregnant women was 45% on initial screening and the primary infection rate was 4 per 1000 births in the group studied[4]. Other data have shown that 60% of fetuses of infected mothers become infected[5]. The rate of congenital toxoplasmosis in the Melbourne population would therefore be 2-2.5 per 1000 births. If this is extrapolated to the whole of Australia (260,000 births per annum), there may be 520 to 650 infants born with congenital toxoplasmosis each year. Other studies have demonstrated lower rates of infection. In a recent Western Australian survey of pregnant women, 35% were seropositive on screening[6]. The rate of maternal infection in susceptible pregnancies was 1.6 per 1000 with a birth prevalence of congenital infection of 0.23 per 1000 births to non immune mothers. Studies in South Australia and Queensland have shown seroprevalences of 23 to 26% and in the Queensland study a congenital infection rate of 0.44 per 1000 non-immune pregnancies[7,8].

In Australia, neither serological screening nor patient education about *Toxoplasma* infectivity is routinely or systematically undertaken. The incidence of fetal infection relates to the stage of gestation at which a pregnant woman acquires the infection. Without treatment the incidence of congenital infection is approximately 10 to 15% for acquisition during the first trimester, 30% for the second trimester and 60% for the third trimester. The earlier in gestation transmission occurs, the greater the severity of infection in the fetus and newborn. Early maternal infection may result in death of the fetus *in utero* and spontaneous abortion. Almost all infected newborns of mothers who acquired the infection dur-

ing the third trimester are born without obvious signs of infection. Approximately 85% of infants with congenital infection appear normal at birth[5]. Without some form of screening, very few cases of congenital toxoplasmosis are recognized but, according to French studies, 85% will have long term sequelae including chorioretinitis or neurological damage[9].

In a recent study of 42 mothers of infants with congenital toxoplasmosis, 52% identified specific exposures to cat excrement; 52% had eaten raw or undercooked meat, and 16% had consumed raw eggs or unpasteurized milk. These data suggest that education can prevent or reduce the frequency of infection[5], however one study from Brussels reported a non-significant reduction of the rate of seroconversion in pregnant women routinely given a written list of recommendations[10].

Pregnant women should be advised on the modes of acquisition so they can take appropriate preventative measures. Ingestion of infected meat appears to be the main form of transmission in Australia. Rothe et al found that none of 115 stray cats examined shedded oocysts, agreeing with other studies that found a low prevalence of oocyst shedding in domestic cats[11]. These authors also reported one of 30 pork and none of 30 lamb chops contained viable cysts when the sensitivity of the detection method was one cyst per 100g of tissue[11]. As most pigs for domestic consumption in Australia are raised under clean conditions and without soil contact, infection should be unlikely. That none of the lamb chops showed detectable levels of cysts could have been due to the small sample size or to the fact that the samples were from animals probably less than nine months old which had not yet acquired infection. A survey on the prevalence of toxoplasmosis in Australian (Tasmanian) meat animals in 1975 showed the highest prevalence was in lambs (16.9%) and other sheep (61.7%), while the prevalence in vealers was 2.3% and in other cattle 0%. Pigs had an intermediate seroprevalence; cracker pigs 23.3% and other pigs 7.2%[12]. The prevalence of antibody to *Toxoplasma* in a more recent study of a sheep population of South Australia was 8%[13].

Whilst this study was unable to confirm the source of infection, the kangaroo meat would on theoretical grounds be the most likely source. A number of repondents paticularly recalled the kangaroo meat as being extremely rare and 'oozing with blood'.

Marsupials are highly susceptible to *T. gondii* infection because they evolved in the absence of this parasite until European settlement in Australia introduced the domestic cat, only 200 years ago[14]. Very severe clinical toxoplasmosis has been reported in wallabies and kangaroos[15], so many may die from the infection and leave only a small proportion of those surveyed as seropositive. In a study of 151 Bennett's wallabies (*Macropus rufogriseus rufogriseus*) and 85 Tasmanian pademelons (*Thylogayle billardierii*) which were tested to determine the prevalence of acute toxoplasmosis of macropods in the wild, 4% of the wallabies and 1.2% of the pademelons possessed *T. gondii* specific IgM in their sera[16]. The same workers demonstrated an overall seroprevalence of IgG to *Toxoplasma* in wild macropods of 8.5%[17]. There are probably significant differences in cat densities in various parts of the country, leading to differences in pasture contamination and exposure to infective oocysts.

Kangaroo meat served in restaurants, which is from field shot game, probably contains no more infective tissue cysts than other meats. The difference is that it is often presented for consumption very rare with the possibility that any tissue cysts may remain viable.

Whilst the disease is usually asymptomatic and without consequence in the immunocompetent adult, pregnant women should be alert to the risk to their unborn fetus if they ingest undercooked meat of any kind. Infected meat is rendered safe by adequate cooking (61°C for four minutes)[17]. Smoking and brine curing destroys bradyzoites in infected pork. Freezing at -21°C for 24 hours followed by thawing is reported to be effective; however conflicting results cast doubts on the reliability of this method. Other preventative measures should also be emphasized. Cutting boards and knives used for preparing meat and vegetables for human consumption should be thoroughly washed in hot water and detergent. Fruit and vegetables to be eaten raw should be thoroughly washed before consumption. Meats should not be eaten or tasted raw. Pet cats should not be fed uncooked meat scraps, especially pork. Cats should be fed on dry, canned or boiled food and discouraged from hunting (collar and bell) or scavenging; their faeces and litter should be disposed of daily before the development of infective sporozoites from oocysts, which takes up to five days depending on environmental conditions. Faeces should be disposed of carefully (burying or burning); litter pans should be disinfected daily by scalding with boiling water and gloves worn when handling faecal material. Outside sandpits should be covered when not in use. Hand washing is important after handling raw meat and possible contamination.

Acknowledgment

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RELATIONSHIP OF A DENGUE 2 ISOLATE FROM TOWNSVILLE, 1993, TO INTERNATIONAL ISOLATES

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Introduction

Between March 1992 and August 1993 there were over 700 cases of dengue fever notified from Townsville and the Charters Towers region of North Queensland[1,2]. Analysis of the epidemic pointed towards the suburb of South Townsville as the likely origin[3]. The next most recent epidemic occurred in 1981 and was caused by dengue 1[4], and there and have been small numbers of cases of this serotype reported as recently as 1991[5]. The 1992-93 epidemic was therefore most likely the result of the importation of a dengue 2 strain from overseas; it is of interest that a number of backpacker hostels are located in South Townsville. Importation and subsequent local transmission of dengue 2 has recently been demonstrated in Cairns[6] and highlights the risk of future epidemics from this source.

The molecular characterisation of dengue viral isolates provides a means to track more precisely the origins of epidemics and may provide insights that better enable control. Previous studies have compared sequences from 32[7] and 40[8] dengue 2 isolates creating an opportunity to relate dengue isolates to one another. We therefore undertook to sequence a dengue 2 isolate from the 1992-93 epidemic. The preliminary results are presented.

Methods

A dengue 2 viral isolate (TSV01) was obtained in April 1993 from a patient whose clinical illness was typical of dengue. The viral RNA genome was isolated and DNA encoding the 1485 base pair envelope (E) gene was obtained by reverse transcription and polymerase chain reaction (RT-PCR) using specific oligonucleotide primers. The PCR amplicon was cloned into a plasmid vector and the nucleotide sequence determined by the dideoxy chain termination method. Nucleotide sequences of two independent clones were obtained to identify any errors introduced by the PCR reaction.

The TSV01 sequence was compared with the sequence information on 35 other dengue 2 isolates from different geographical locations for which the complete E gene sequence is available and an additional 33 sequences where the last 111 bases of the E gene is known[7,8]. These data were obtained from the Division of Vectorborne Diseases at the Centers for Disease Control and Prevention in Atlanta, United States.

Results

The TSV01 gene sequence (data not shown) and the amino acid sequence deduced from it were compared with the nucleotide or aligned deduced amino acid sequences of the complete 35 E glycoprotein sequences.