Karen Smith Founder: Lyme Australia Recognition & Awareness Website: http://www.lymeaustralia.com/

Attention: Dr Gary Lum Department of Health Canberra

Re: Submission of Response to Scoping Study to Develop and Research Project(s) to Investigate the Presence or Absence of Lyme Disease in Australia

Thank you for the opportunity to submit responses to the scoping study on Lyme disease. I write on behalf of myself, my children, and indeed all Lyme patients and their families looking for recognition of Lyme in Australia. I was one of the 125 people in the consultative group mentioned by the Lyme Disease Association of Australia (LDDA), in which the LDAA Scoping Study response was discussed. Whilst I fully support LDAA's patient submission, as a researcher who has spent thousands of hours researching his topic (especially the transmission and maintenance of Lyme in the environment) I also feel compelled to submit an additional individual response.

As numerous other Australian Lyme Patients have done, I had plans to submit a "patient impact statement" to accompany this response. However, I have had a health setback, and been quite unwell for the last month and unable to do a great deal of anything, least of all focusing the brain and writing cohesively. I mention this, not only to introduce a short patient history, but also to explain that with the comments, scoping study response, a lot of the research/ responses are copied from my research articles in order to fit in with the requested – page and paragraph response, however on one occasion this was not strictly possible.

A short patient impact statement for me would be:

Pre- Lyme: I worked, raised three children and completed a four year university degree: Bachelor of Psychology with Honours. I graduated from this degree at the top of my class, with a final score of 94%, and was Awarded the Australia Psychological Association prize, and when i went on to enrol for a PhD, was awarded an Australian Postgraduate Awards (APA) scholarship, which as you may know are awarded to students of "exceptional research potential". The monetary value of this scholarship, the loss of 5 years, has nothing on what it feels like for me – to no longer be able to concentrate for longer than 20 minute spans, or indeed to be unable to spell properly, or punctuate correctly. This loss of function I am determined to regain – though do fight the anger/frustration in knowing the damage could more than likely have been prevented if I had of received treatment on my initial test results and brain scans which read; "The pattern of widespread inhomogeneous cortical hypoperfusion with involvement of the basal ganglia, but sparing of the frontal lobes is non specific, but can be due to the encephalopathy of Lyme Disease".

Whilst I would dearly love to get back to full time work, research – there are still days where my brain just cannot "function". To reduce my own sense of loss of identity, self-worth and self-esteem – since/when I have been able to be out of bed - I have endeavoured to help others and raising awareness of Lyme in Australia through : Research ; Starting my website – Lyme Australia Recognition & Awareness; Starting a facebook support group - Lyme Australia & Friends ; Advocating for patients ; Undertaking National awareness events (Attended the first Australian protest in Sydney organised by Danielle Ryan and Dayna Parkinson ; Co-organised the protest outside QLD health with Rachel Robins), as well as International Awareness events - Signing Australia up for the Inaugural Worldwide Lyme Protest in 2013, and co-coordinating the National (with Janice Foster & Sharon Whiteman) and International events; Establishing International Red Shoe Day in memory of Theda Myint and all those lost to invisible illness.

With all the above/awareness work throughout the last few years one glaring/outstanding factor that I can attest to is – Lyme Patients numbers are increasing at a rapid rate. (When i first joined facebook to raise awareness – Lyme patient numbers equalled less than a dozen – they are now well over 1000). The pain and suffering of hundreds of people is something I would never have thought possible in Australia. I have "seen" the death, of a number of Australian Lyme patients, and dealt with the sadness, anger, frustration and feelings of helplessness that this brings about. Primarily for me – the fact that my children also have Lyme, and to read of the number of children suffering, and the way they suffer, is why I will never give up this fight. My children deserved better – they deserved a healthy "teenage years", and they didn't deserve to "lose" their active/outgoing Mum. All our children and future generations are entitled to the best healthcare possible. They deserve for acknowledgement and awareness – with this brings the benefit of early treatment of Lyme, and hopefully will result in far less needless suffering.

Below/ Attached is:

Cover Letter and patient story / Reason for Interest in Lyme Research	1
Scoping Study Response/Comments	Pages 3-21
References	Pages 22- 42

Attachments:

Attachment 1: A little More on Patient Story. I originally wrote my "story" in 2011. This is attached. As is my brain scans, positive blood results. Attached also is a picture of an EM on daughters face – She also has a positive PCR blood result. She has never left Australia. And nor had I before I went to the UK for Lyme treatment in 2012. My story – including interview with Today Tonight: http://www.lymeaustralia.com/karen-founder-lara.html

Attachment 2: "Lyme Disease: A Counter Argument of the Australian Government's Denial". This can also be seen on my website – as can a video I prepared –to detail the problems in audio format. <u>http://www.lymeaustralia.com/k-smith-lara-research.html</u>

The second research paper: : "Lyme Disease / Borreliosis: An Overview of Lyme and Direction for further Research required in Australia". Will be available in pdf as soon as health permits, though the majority of information has been up on my website since 2012: <u>http://www.lymeaustralia.com/lyme-borreliosis.html</u>

Attachment 3: Seabird Areas around Australia Coastline – I prepared this a couple of years ago in order to show how often/where seabirds interact with human population. This information has also been available on the website since 2012; and can be seen at: <u>http://www.lymeaustralia.com/migratory-marine-bird-areas-around-aust-coastline.html</u>

Scoping Study Response/Comments

Comment 1 : Page 5, Para 1: "It is now recognised to be the most frequent cause of tick-borne disease with an estimated 65,000+ cases in Europe and a further 20,000+ cases in the United States, but this may be a significant underestimate with many cases unreported, and compounded by the small number of countries in Europe to make Lyme disease notifiable, and the actual total may be closer to 255,000 cases annually (Rudenko et al 2011; Radolf et al 2012)".

In August 2013 (Before the release of the Scoping study), the Centre for Disease Control (CDC), in) the United States of America (USA), released figures of around 300,000 new cases of Lyme disease each year in America alone.

Comment 2 : Page 6, Para 2: New genospecies in the Lyme *Borrelia* complex are being recognised almost every year (Stanek and Reiter 2011) and more would be undoubtedly found if a concerted effort was made in collecting and processing ticks, especially in new areas. Examples of this have been demonstrated in Canada (Scott et al 2010; Ogden et al 2011), and in Uruguay (Barbieri et al 2013). The latter report is the first isolation of indigenous *B. burgdorferi* s.l. in the Southern Hemisphere, and also demonstrates that novel *Borrelia* genospecies in the *B. burgdorferi* s.l. complex may occur in new geographic areas.

The acknowledgement that "New genospecies in the Lyme *Borrelia* complex are being recognised almost every year", makes it all the more incomprehensible to understand why there has been no government research with regards to the possibility of Lyme in Australia since the early 90's.

Not knowing the writers intent, I am making the assumption that the mention of the 2013 research with regards to the "first isolation of indigenous *B. burgdorferi* s.l. in the Southern Hemisphere, is perhaps to suggest that Lyme in the Southern Hemisphere is a new possibility that is just being investigated. Whilst it may be the first isolation of a "novel species" belonging to the B. burgdorferi sl complex in ticks, it is certainly not the first isolation of Borrelia DNA from ticks in the Southern Hemisphere. Since the 80's, there has also been numerous reported cases of seropositivity or suspected Lyme disease in the Southern Hemisphere, including Australia.

In 1993 study looking at the role of seabirds in Lyme disease, Olsen and others noted that: "Of particular interest is the finding of suspected cases of Lyme disease in Australia and South Africa, although no Lyme disease-causing spirochete has been isolated from these regions yet. Most of the findings in Australia are based on serological data and clinical cases with symptoms typical of Lyme disease. Our finding of Borrelia DNA in I. uriae ticks obtained from the Crozet Islands and Campbell Island [New Zealand coast] suggests that Lyme disease enzootic foci are present in that part of the world" (1: Pg 3272-3).

There is also the lack of mention of B. Queenslicanda in the report. Whilst not isolated from ticks, in 1962 this species of borrelia was isolated from rats in Richmond (Nth Qld) and was noted as causing " a relapsing type of infection sensitive to antibiotics in laboratory rats and mice". In 1962, Lyme borreliosis had not been "discovered" and the majority of information known about Borrelia was on the species that caused relapsing fever. As these species of borrelia were/are transmitted by the soft ticks, attempts were made to ascertain the vector capabilities of O. *Gurneyi* for the borrelia species found. The attempts to infect O. Gurneyi were unsuccessful, suggesting that they were not the natural vector. Further investigations revealed that the borrelia species was not the same as the borrelia species infecting cattle, or *b.anserina* affecting birds in the region. The conclusion reached was "In view of all these considerations, the name *Borrelia queenslandica* is proposed for this new species isolated from native rats in north-west Queensland" (2: Pg 261).

Whether or not Borrelia queenslandica is a novel Borrelia genospecies in the B. burgdorferi s.l. complex (as opposed to relapsing fever strains/species) is unknown – it was however found to cause a relapsing like illness, and is certainly worthy of a mention and further up to date research with regards to the pathogens and the ticks in this area (Richmond, Nth West Queensland) would certainly be appropriate.

Comment 3 : Page 6, Para 3: "Transmission of Lyme borreliosis is through injection of tick saliva during feeding."

Whilst it is well known regarding the ability of the tick to spread Lyme, it should be noted there is also some evidence that it can be transmitted via other means, which are outlined briefly below.

Blood sucking insects (other than ticks)

In clinical cases of Lyme disease, biting flies (1-3), mosquito's (3,4) and mites (5) are suggested to have been responsible for the infection. The Borrelia bacteria has been found in: numerous species of mites (6); fleas (6-8); biting flies, ie: bot flies, deer flies, horse flies (6,7, 9-11); and mosquito's (8, 9, 11-14), indicating that these insects are capable of maintaining the bacteria and are potential vectors.

Contact transmission

Borrelia spirochetes have been found in the urine of infected dogs (15,16), horses (17,18), cattle (18) and mice (19,20). Studies on mice have found that the spirochetes in urine remained viable for 18-24 hours and concluded that "Urine may provide a method for contact non-tick transmission of B. burgdorferi in natural rodent populations particularly during periods of nesting and/or breeding" (19: pg 40). Evidence for direct contact transmission has been demonstrated in mice (20). These findings suggest that further research is needed to ascertain whether, like the spirochete that causes Leptospirosis, the borrelia spirochete is able to spread by the urine of infected animals to humans.

Human to human transmission

Sexual transmission: There is no direct evidence for sexual transmission, although spirochetes have been found in semen (21), suggesting that it is a possibility. Lyme disease has also been likened to another spirochetal disease, syphilis, which is a sexually transmittable infection (22).

Mother to baby: The possibility of placental transmission is acknowledged, although there are mixed reports regarding exactly what health risk congenital Lyme disease poses to the foetus/newborn. A brief dialogue of various positions:

Allan MacDonald (1989) notes that adverse reactions, such as foetal death and cortical blindness, have been associated with gestational Lyme disease and suggests the need for further research in order to ascertain whether the associations are co-incidental or related to the infection (23).

The International Disease Society of America (IDSA) guidelines downplay any risk, associated with Lyme, and conclude that "there is little evidence that a congenital Lyme disease syndrome occurs" (24).

The Centre for Disease Control (CDC) notes that while "Lyme disease can be dangerous for your unborn child", and "may lead to infection of the placenta and may possibly lead to stillbirth" (25,26), it follows the IDSA guidelines that "favorable outcomes can be expected when pregnant women with Lyme disease are treated with standard antibiotic regimen"; Contrary to this statement, there are reports of adverse outcomes, including the death of newborns, with (27) or without (28) antibiotic treatment of the mother.

CDC Publications include the *Pregnancy Fact Sheet* - "Untreated, Lyme disease can be dangerous to your unborn child. Lyme disease that goes untreated can also cause you to have brain, nerve, spinal cord, and heart problems", and the *Lyme Disease Resource Brochure* - "Prevention and early diagnosis of Lyme disease are important during pregnancy. Rarely, Lyme disease acquired during pregnancy may lead to infection of the placenta and may possibly lead to stillbirth".

The National Institutes of Health puts it short and sweet: "If you are pregnant, be especially careful to avoid ticks in Lyme disease areas because you can pass on the infection to your unborn child" (29: pge 15).

Comment 4: Page 6, Para 3: "The disease is transmitted largely by four species of hard ticks in the *Ixodes ricinus* complex: the major vector in Europe is *I. ricinus* and in Asia is *I. persulcatus*, whereas in the United States the major vector in the north-eastern and mid-western states is *I. scapularis*, and in western US is *I. pacificus* (Stanek et al 2012; Radolf et al 2012). Other hard ticks do not appear to play any significant role in Lyme borreliosis; they are either inefficient in the acquisition of *Borrelia* spirochaetes from blood meals, or they are unable to maintain the spirochaete".

Since these first four ticks, many more species of ticks have been identified as vectors. This includes over a dozen more species of Ixodes ticks, as well as ticks from other Ixodidae genera's including, Amblyomma, Haemaphysalis, Rhipicephalus and Dermacentor. On the following page is a table of tick vectors involved in the transmission and maintenance of Lyme. The table is by no means a fully comprehensive list of tick vectors involved. It does not contain the ticks suspected as being vectors for less studied continents, or countries where Lyme is yet to be acknowledged.

A key to reading the Tick Vectors of Lyme Disease/ Borreliosis Table: The relevant ticks are listed, firstly under the country/continent in which they are found and then under their relevant Ixodidae genera, eg: Ixodes, Amblyomma, Haemaphysalis, Rhipicephalus and Dermacentor. The "scientific" name for the tick is firstly given, with the more common name (if applicable) in brackets; Animal hosts of the ticks are mentioned, with: {I} denoting hosts of Immature ticks ie: larvae and nymphs and {A} for the animal hosts of the adult ticks; If it is a second listing for the tick, ie: the tick is found in more than one continent/country, the animal hosts of the tick are not listed again.

Continent/	Ixodidae	Tick Species and Preferred Hosts
Country	Genera	
North America:	ixodes:	 I. scapularis: Deer Tick / Black-legged Tick: {I} small rodents, reptiles, birds {A} small-medium mammals including dogs and deer NB: In early research this tick is generally reported as I. dammini, before it was realised it was the same species and was re-classified as I. scapularis I.pacificus: Western black-legged Tick: {I} rodents, reptiles, birds {A} large mammals I. dentatus: Rabbit Tick: {I} birds {I A} small rodents, rabbits I. affinis: {I} rodents, birds {A} med-large animals including, moles, squirrels, racoons, deer I. jellisoni: member of I.ricinus complex: {IA} rodents, primarily Californian kangaroo rat I. spinipalpis: Mouse tick: {IA} rodents I. neotomae: {IA} rodents NB: Research in 1997 found that I.neotomae and I.spinipalpis were one species, I. neotomae was subsequently re-classified as I.spinipalpis: I. angustus: {IA} rodents I. minor: {I} birds {IA} rodents; I. muris: {I} birds {IA} rodents
	Amblyomma:	A. americanum: Lone Star Tick: {I} small rodents, birds {A} variety large mammals. The vector of STARI, or Masters disease ("Iyme-like" illness)
	Haemaphysalis:	H. leporispalustris: Rabbit Tick: {I} birds {IA} small rodents, rabbits, hares
Canada:	Ixodes:	I. auritulus {IA} birds I. scapularis ; I. pacificus ; I. spinipalpis ; I. angustus ; I. muris
	Haemaphysalis:	H. leporispalustris
Europe:	Ixodes:	 I. ricinus: Castor Bean/Sheep Tick: {I} small and medium sized mammals, reptiles and birds {A} Med. and large sized mammals including dogs I. hexagonus: Hedgehog Tick/European dog Tick: {IA} main hosts of all stages are hedgehogs and carnivorous mammals of the Mustelidae (eg: badger, ferrets and Canidae (eg; foxes, wolves, dogs) families I. canisuga: Dog/Fox Tick: {IA} Medium to large mammals including dogs, foxes, badgers and cats I. frontalis: Passerine tick: {IA} birds I. trianguliceps: Shrew/Vole Tick: {IA} small mammals such as shrews, rodents
Asia:	Ixodes:	I. ricinus,
лыа.		 I. persulcatus: Taiga Tick: {I} small to med mammals including birds {A} Medium and large sized mammals This tick (I persulcatus) is sometimes included in Europe literature as it is also found in Russia, whose borders span both Europe and Asia I. sinensis: ({I} small to medium mammals {A} larger animals such as goats cows I.ovatus: {I} rodents, hares {A} various large domestic and wild mammals I. nipponensis: {I} small mammals, lizards, birds {A} medium to large mammals I. granulates: {IA} small to medium rodents such as rats, squirrels, rabbits and hares
	Haemaphysalis:	 H flava: {I} birds, small to medium mammals {IA} various, prefer hares and dogs H. bispinosa: {I} birds, {A} various large domestic and wild mammals, ie: dogs, sheep, goats, deer, cattle H. longicornis: {I} birds, hares {A} same as bispinosa: ie: dogs, sheep, deer, cattle
Japan A part of the Asian continent ; though in LD studies; stand-alone island	lxodes:	I. Persulcatus; I. Ovatus; I. columnae: {IA} birds and rodents I. tanuki: {I} rodents {A} small to medium carnivorous mammals such as raccoon dog, weasels and badgers I. turdus: {IA} birds
	Haemaphysalis:	H flava
Worldwide	Ixodes:	I. Uriae (Seabird Tick)

Table : Tick Vectors of Lyme Disease / Borreliosis

*See reference list for source of tick location, animal hosts and journal articles with regards to vector capabilities of each of the above listed ticks (referenced in order of mention).

**Ticks such as *I. jellisoni, I. trianguliceps* and *I. spinipalpis* are known as nidicolous ticks (found in the burrows and nests of their hosts) and as these ticks do not actively look for hosts, their roles as vectors is associated with maintaining the borrelia (and numerous co-infections such as Babesia microti) within the environment, rather than transmitting it to humans (1-3). However, in cases where they do come into contact with people, such as with *I. spinipalpis* (4), transmission to humans may occur.

Comment5 – Covering info on Page 8/9 (below) with regards to ticks and reservoir hosts:

Page 8, Para 2 - No member of the *I. ricinus* complex occurs in Australia, but the most plausible indigenous vector is *I. holocyclus* which is known to parasitise native vertebrate hosts, domestic animals and humans, and is the most common tick biting humans.

No member of the I. ricinus complex is in Australia, this is correct, though the point of the lengthy table above is that it reveals that various genera (not just Ixodes) of the Ixodidae tick family are involved in the Lyme disease/ borreliosis cycle. Ticks on the above table that are in Australia include: *I. uriae (seabird) and I. auritulus (bird) H. bispinosa* and *H. longicornis.* This ticks – along with the reservoir hosts is covered extensively below

Page 8 -9 mentions the tick species that may possibly be investigated as well as the natural reservoirs of Borrelia. The review, research paper I wrote covers most of what I would like to say in regards to this, and due to time constraints I am unable to take out only the relevant bits of information (which around 95% is relevant) and have copied the section titled "Tick Vectors and Reservoir Hosts of Lyme / Borrelia in Australia". I do apologise for not strictly following the requested way to respond in this section, though felt it was better to provide it all, rather than not respond at all.

Page 8, Para 3: The natural reservoirs of Lyme Borrelia species. The reservoirs of Lyme *Borrelia* spp. are small mammals and some birds (reviewed in Piesman and Gern 2004; Rizzoli et al 2011; Franke et al 2013). Deer are not competent reservoirs but are essential in many areas for the maintenance of tick populations because they are one of a few wildlife hosts able to feed sufficient numbers of adult ticks (Stanek et al 2012). Other large domestic animals such as cattle and sheep are also not competent reservoirs.

With regards to the smaller/medium animals, there are over 50 mammalian and avian species that are reservoir hosts of borrelia (31) and include various mammal species such as: mice, rats, voles; hares; rabbits; squirrels; hedgehogs; dogs: as well as numerous species of marine and land birds including puffins, blackbirds and pheasants. There is some question as to whether or not larger mammals, such as sheep, deer, horses and cattle simply serve to amplify the infection within the environment, by providing the tick with a host blood meal or whether they also serve as reservoir hosts of borrelia. In general, the studies show mixed conclusions. Covered extensively below:

Page 8, Para 3: The natural reservoirs of Lyme Borrelia species : Lyme *Borrelia* do not cause disease in reservoir hosts, and other than humans, the only mammals known at this time to show disease symptoms are dogs and possibly horses. Indeed the use of ticks taken from dogs provides a good indication of the presence of Lyme disease in a given location, and dogs are an excellent sentinel species for estimating Lyme disease risk (Hamer et al 2009; Smith et al 2012). Although a broad spectrum of clinical signs have been attributed to infection with *Borrelia* in horses, actual cases of equine Lyme borreliosis are rare if they exist at all (Butler et al 2005).

Besides humans, dogs, cats, horses and cattle appear to be the only animals that may develop a clinical illness due to a borrelia infection. Covered and referenced extensively below.

Page 9, Par 3: It is therefore plausible that certain *B. burgdorferi* s.l. strains could be brought to the Southern Hemisphere and enter local Australian ecosystems through intermingling between seabirds and land-based avian species, but most bird ticks do not bite humans, and if they did, would rapidly drop off before the opportunity to transmit the spirochaete. If cases of human infection were to result, they would be very occasional and localised.

As covered below, tick species such as H. longicornis are mainly associated with mammals, though in early life stages (ie; nymph/larvae) are also associated with birds, including migrating seabirds. I. Uriae has also been associated with biting humans in Faroe Islands. And as can be seen below – foxes and dogs decimated seabird populations on islands that were accessible at low tide. It has been shown (with borrelia responsible for relapsing fever at least), that "rats and dogs can be infected through the consumption of infected rat brains or organs". Denying the possibility of borrelia/human infection in the Australian environment due to the notion that "most bird ticks do not bite humans", seems quite illogical and assumes that birds and the ticks on them are maintained in a closed ecological environment.

Tick Vectors and Reservoir Hosts of Lyme / Borrelia in Australia

The point of the lengthy table above is that it reveals that various genera of the Ixodidae tick family are involved in the Lyme disease/ borreliosis cycle. The existence of Lyme disease in Australia was denied by Russell et al., (1994) and continues to be denied by the NSW Department of Medical entomology (of which Russell was the Director until his retirement in mid 2012), and the NSW Health Director of Communicable Diseases, Dr Jeremy McAnulty, in part due to the fact that Australia does not have any of the first four ticks (ie: *I. scapularis, I. pacificus, I. ricinus, I. persulcatus*) that were initially identified as vectors of Lyme.

As Table above demonstrates, since the early research into Lyme numerous other species of ticks have been found to be implicated in the Lyme transmission cycle. The discussion in this segment further examines:

- Ixodes ticks that are listed in Table Two as capable tick vectors of Lyme and that have been recorded in Australia: *I. uriae, I. auritulus.* As these are both bird ticks, the role of these ticks is discussed in conjunction with bird hosts that have been shown to be either simply hosts/carriers of the tick, or those that are also reservoir hosts of the borrelia bacteria. Also discussed are various birds that have been introduced into Australia, and are known reservoir hosts in the Southern Hemisphere.
- Haemaphysalis ticks that are listed in Table Two as capable tick vectors of Lyme and that have been recorded in Australia: *H. bispinosa* and *H. longicornis*. Whilst the immature (larvae, nymph) tick may feed on birds, these ticks are associated more so with their mammal hosts. These two ticks are discussed in conjunction with mammal hosts that have been shown to be either hosts/carriers of the tick, or those that are also reservoir hosts of the borrelia bacteria. Various mammal species that have been introduced into Australia and are known borrelia reservoir hosts are also examined.
- Rhipicephalus ticks species: *R. sanguineus* and *R. Microplus.* Whilst not listed in the above tick vector table, these species which are also implicated in the borrelia cycle, in that they have been found to carry the borrelia spirochete and are therefore possible vectors.
- Dermacentor ticks: Whilst this family of ticks is not in Australia, this species is also briefly mentioned in order to demonstrate that when looking at the vector competence of a particular species of ticks, that findings on competence may be altered when ticks are examined in co-feeding studies (numerous tick species feeding together which would emulate the natural environment), as opposed to 'traditional laboratory' studies where only one tick species is commonly examined.

Ixodes Species

I. uriae (seabird) and I. auritulus (bird)

The role of *I. uriae* and *I. auritulus* ticks is maintaining/spreading the borrelia bacteria to the animal hosts within their environment. However, unlike nest dwelling ticks whose ecosystem is limited, the fact that birds are the hosts has widespread ramifications. Birds can be both biological carriers (reservoir hosts) and parasitic carriers (eg: ticks) of many different pathogens (1), including borrelia. Anderson and Magnarelli first reported the importance of birds as reservoir hosts and their role in transmitting the borrelia bacteria and ticks into new geographic areas in 1984 (2). In combination this means that not only can birds drop infected ticks into new environments (3-8), but as reservoir hosts, immature ticks that feed on them may become infected and spread the disease to other birds and mammals during their next feed.

Land birds can spread borrelia across continents, whilst migrating seabirds can spread the disease across the Northern and Southern hemispheres (9-16). It must also be noted that whilst the primary role of *I. uriae* appears to be the widespread dispersal of borrelia, these ticks are known to bite humans (17-18) and are the suggested vector for human disease on the Faroe Islands (18).

The Ixodes uriae tick is found Australia-wide, including offshore islands (19). It is prevalent in both the Northern and Southern hemispheres and is "closely associated with many species of colony-nesting marine birds" (20). In 1993 Olsen and others (20) extended on the finding that land-birds as well as mammals could be infected by borrelia, with their research revealing that even in the absence of mammals, borrelia was maintained/amplified by seabirds within the environment. A further study in 1995 (21) revealed "a significant role for seabirds in a global transmission cycle by demonstrating the presence of Lyme disease Borrelia spirochetes in *Ixodes uriae* ticks from several seabird colonies in both the Southern and Northern Hemispheres." It was noted that: "Of particular interest is the finding of suspected

cases of Lyme disease in Australia and South Africa, although no Lyme disease-causing spirochete has been isolated from these regions yet. Most of the findings in Australia are based on serological data and clinical cases with symptoms typical of Lyme disease. Our finding of Borrelia DNA in I. uriae ticks obtained from the Crozet Islands and Campbell Island [New Zealand coast] suggests that Lyme disease enzootic foci are present in that part of the world" (21: Pg 3272-3).

There are numerous species of marine birds that migrate between the Northern and Southern Hemispheres to Australia, as well as birds that migrate between New Zealand and Australia each year. In fact, of the 359 species of marine birds worldwide, 78 different species breed on Australian islands and shores. In comparison to other countries, Australia is second only to New Zealand who, with 84 species has the greatest diversity of marine birds anywhere in the world (22). These marine birds are generally broken down into either seabird or shorebird/wader families (23-25). The seabirds consist of around 20 species and are those that are most commonly found on, over or near the ocean, including shearwaters (more commonly known as mutton birds), albatrosses, penguins, frigatebirds, gulls, cormorants and terns. Some seabirds (such as cormorants) may also be found in other areas surrounding water, such as lakes and wetlands, and can become common in urban areas. Shorebirds/ waders are those which are commonly found on coastal shores, including beaches, rocky shores, mudflats, tidal wetlands and lagoons. These include many species of plovers, sandpipers, stilts, curlews and snipes.

In Australia (and many other countries) seabirds and shorebirds are not restricted to separate areas and share many locations with each other as well as land birds and mammals, including humans: "Some seabird colonies are very accessible to large numbers of people. This is especially true of small islands in mainland estuaries or islands that are linked to the mainland in some way or are close to big cities (26: Pg 74)". The shorebirds from the East Asian-Australasian Flyways alone have 118 internationally important sites that encompass the coastline as well numerous inland areas of Australia (27: Fig 20; pg 210), whilst seabirds nest in many areas on the mainland, as well as on numerous islands off almost every state in Australia. (See Attachment 3 - Seabird areas for more specific locations, including those on mainland Australia)

Seabirds such as the Sooty and Short-tailed Shearwaters, Common and Little Tern, Gulls, and shorebirds such as; Bar tailed Godwits, Red Knots, Sandpipers, Curlews and Snipes migrate to Australia from California, Europe, Asia (including Russia) and Japan (26-34). Lyme disease is endemic in all of these regions. With over 20 million migrating seabirds and 3 million plus shore-birds breeding on Australian Islands and shores each year, it is inconceivable that the health departments of Australia continue to ignore the long established knowledge that "Migrating birds contribute to the spread of B. burgdorferi sl and of infected tick vectors along migration routes" (35).

Along with the seabird tick (*I. uriae*), a number of different ticks have been associated with borrelia and different bird hosts (eg: *I. auritulus, I. dentatus, I. frontalis, H. flava, H. leporispalustris*). Of interest for Australia is the finding that the *I. auritulus* tick is a vector of borrelia (36-38).

The lxodes auritulus is a native tick of Tasmania (39-41). Birds continually spread the known distribution range of ticks (eg: 37-38) and as numerous species of birds, such as the Silvereye (Zosterops lateralis: passerine), migrate from Tasmania and disperse into regions of Victoria, New South Wales and south-eastern Queensland there certainly is the possibility this tick has been spread throughout mainland Australia. The common blackbird (passeriforme) is also abundant in Tasmania (and other areas of Australia), and is a bird that has been regularly identified as a reservoir host of borrelia.

Birds of the Passeriforme order, or passerine birds, are more commonly known as perching or song birds (42), and include over 5000 species grouped into approximately 110 families that may be partially (travelling long distances within the same continent) or fully (travelling across continents) migratory. Numerous passerine species have been identified as reservoir hosts of borrelia and include; Robins, Thrushes, Redstarts (formerly thrush family), Sparrows and Tits (eg:2, 9-11,38-40). Thrushes (Turdiae family) appear to be extremely competent reservoir hosts: Borrelia is thought to have been introduced into Japan from two species of thrush (Turdus cardis and pallidus) that migrate from Asia (43-45), whilst Song thrushes (Turdus philomelos) and the Eurasian/Common Blackbirds (Turdus merula) are consistently found to be competent reservoir hosts of borrelia in Europe (46-49).

Both Song thrushes (Turdus philomelos) and the Eurasian/Common Blackbirds (Turdus merula) have been introduced into Australia: Song thrushes are established in Melbourne after being introduced in the 1850's. The Eurasian/Common blackbirds were introduced into Melbourne and South Australia in the 1860's and 1870s and are now widespread. They range throughout coastal and lower inland regions of South Australia, the whole of Victoria and New South Wales and spread into Queensland in 1986, breeding in regions around Toowoomba and the Highfields (50-52). They are also "abundant in Tasmania

and have successfully colonised offshore islands such as Lord Howe Island, Norfolk Island, Kangaroo Island and Flinders Island" (50: pg 8).

It appears that at least one government department in Australia is aware that Blackbirds can carry borrelia. A risk assessment report from the Queensland State Government (Biosecurity Queensland), examining the potential spread of Blackbirds into Queensland, makes this note with regards to the diseases associated with Blackbirds: "Blackbirds are often infected with intestinal and haematozoan parasites, as well as external parasites such as ticks, which can then infect other blackbirds with illnesses such as Lyme disease" (50: pg 7). Unfortunately, they do not seem to understand the full impact of that statement, which is, that the ticks which feed on both bird and mammal hosts can also spread Lyme disease to other animals within the environment, including humans.

There is the possibility that the borrelia bacteria was brought to Australia with the introduction of blackbirds. However, the presence of the Blackbirds in Tasmania, mainland coastal areas and offshore islands of Australia would no doubt mean that the largest threat of the Blackbirds (and the other reservoir hosts) acquiring and spreading borrelia to other animals and birds would come from sharing the environment with the millions of marine birds that migrate to Australia each year.

In addition to the song thrushes and common blackbirds (Passeriformes), other species of birds that have been introduced into Australia, and are competent reservoir hosts of borrelia, include birds from the order of Galliformes: wild turkeys (53), pheasants (54-55), quails (56) and Anseriformes: Mallard ducks (57).

Haemaphysalis Species

H. bispinosa and H. longicornis (Scrub/bush)

The Haemaphysalis tick species, bispinosa and longicornis have both been recorded in Australia and have been found to be involved in maintaining and transmitting borrelia. Whilst the immature (larvae, nymph) tick may feed on birds, these tick species also have a close association with mammal hosts. Considering this association, these ticks are discussed in conjunction with the mammal hosts that have been shown to be either simply hosts of the tick or those that are also reservoir hosts of the borrelia bacteria. In order to further outline the role that mammals play in the maintenance and spread of borrelia within the environment, the following section also briefly examines clinical illness in animals. This not only serves to give a practical example of which animals are reservoir hosts and can carry borrelia (as well as develop a clinical illness), it also helps to reveal the concerns associated with the introduction and importation of numerous mammal species into Australia.

The H. bispinosa and H. longicornis ticks are very similar, and have the same host preferences. For example, immature ticks feed on birds and hares and hosts of the adult tick include various large domestic and wild mammals such as dogs, sheep, goats, deer, cattle, horses (1-2). Both tick species have been found to be vectors of borrelia in southern China (3-6). Borrelia strains isolated from the *H. longicornis* tick include B.garinii, B. afzelli (5), and B. valaisiana (6). Studies also show that as well as a high infection rate of borrelia, H. longicornis also carries co-infections such as Bartonella, Anaplasma, and Ehrlichia (7-8).

Haemaphysalis bispinosa has been recorded in Australia (9-10). It appears however this species was found to be synonymous with *H. longicornis* (11), and Hoogstraal and others (12) reclassified the species of *H.bisponosa* from Australia and New Zealand as *H. longicornis*. This species is mentioned here due to its original listing as being in Australia, its immense similarities with *H.longicornis*, and that these two ticks are listed as synonymous on many occasions in the literature. There are also other tick vectors of borrelia that have been originally thought to be two separate species, before it was found they were in fact the same species. These include; *I. scapularis* and *I. dammini*: When it was found that they were in fact the same species of tick, *I. dammini* was re-classified as *I. Scapularis*; *I. spinipalpis* and *I. neotomae*: Research in 1997 found that *I. neotomae* and *I. spinipalpis* were actually one and the same species, *I. neotomae* was subsequently re-classified as *I. spinipalpis*.

Haemaphysalis longicornis, is more commonly known as the scrub or bush tick (or cattle tick in New Zealand). It was introduced into Australia on cattle from Northern Japan and was first recognised in 1901 in north eastern New South Wales. It is now established along coastal areas in Queensland, New South Wales, and through north eastern Victoria (esp Murray Valley) and Western Australia (13-14). The bush tick was first recognised at Walpole in Western Australia in 1983, though for how long it had been in the state is unknown. As there have been no reports of the tick in South Australia or the Northern Territory, its presence in Western Australia cannot be attributed to the natural spread of the tick and "The source of introduction to Western Australia has never been traced" (15). Two possible methods of introduction are: Either via cattle transported to the district from states in Australia where the tick is common, or via

migrating birds. In a study of New Zealand tick fauna it was noted that "Haemaphysalis spp. could be introduced ...by migrating birds from Asia, a major source of members of this genus" (16). Walpole is adjacent to Nornalup and Walpole Inlet Marine Parks, home to around 150 bird species including migrating shore and sea birds (17-18).

The hosts of the *H. longicornis* tick (19) include numerous animals that have been found to be reservoir hosts for borrelia and have been introduced or imported into Australia from countries that are endemic for Lyme disease. These animals include; smaller reservoir hosts - mice, rats and hares : domestic animals - cats and dogs : medium to large animals - foxes, cattle, horses, sheep and deer (20) that have varying levels of reservoir competence. Importation of animals carrying borrelia can occur as the animal may show no obvious signs of clinical illness. To examine the very real likelihood of the bacteria underlying Lyme being in Australia, the following extends a little on clinical illness in animals, reservoir competence and the introduction/importation of the aforementioned animals into Australia.

In looking at animals brought into Australia from countries where Lyme disease is endemic, it must be remembered that whilst the first reported cases described as Lyme disease were in the 1970's, DNA studies of ticks from museums has revealed that the borrelia bacteria underlying Lyme has been in the environment since the 1800's (21-24). A study in Europe concluded, "residents of Europe have been exposed to diverse Lyme disease spirochetes at least since 1884, concurrent with the oldest record of apparent human infection" (21), and a study in America revealed, "These studies suggest that the agent of Lyme disease was present in a suitable reservoir host in the United States before the turn of the century and provide evidence against a hypothesis of recent introduction of this zoonotic agent to North America" (23).

Clinical Illness in Animals

Besides humans, dogs, cats, horses and cattle appear to be the only animals that may develop a clinical illness due to a borrelia infection (25). The primary symptom in all these animals is arthritic in nature, where inflammation of joints and limbs may lead to lameness. Dogs are competent reservoir hosts (26) and seem to be the most susceptible to developing a clinical illness (25, 27). As they are generally in close contact with humans, rates of borrelia infection/exposure in dogs has also been studied in order to try and ascertain what the degree of risk of borrelia exposure to humans may be within particular areas/environments (28-30). Apart from lameness (shifting leg lameness in particular), other symptoms in dogs may include; anorexia/weight loss, malaise, neurological dysfunction (25), severe polyarthritis (27), renal lesions (31,32), splenomegaly/ lymphadenopathy, intraocular inflammation (33) abnormal gait and convulsions (34). Cats are more prone to asymptomatic infections (33), though as well as lameness they may develop; fever, anorexia, fatigue (35-36), and kidney problems (37).

Asymptomatic infections seem to be the most common in horses and cattle (38-41), although clinical illness can develop with symptoms in both animals including lameness, uveitis and weight loss (38, 41-43). Other signs in cattle include decreased milk production and abortion (42, 44,45), with head tilt, encephalitis (46,47), aborted, reabsorbed foetuses and foal mortality also being reported in clinical disease in horses (48,49).

Borrelia spirochetes have been found in the urine of infected dogs (31, 50) horses (45, 51) and cattle (45), in both symptomatic and asymptomatic animals. Studies on mice found that the spirochetes in urine remained viable for 18-24 hours and concluded that "Urine may provide a method for contact non-tick transmission of B. burgdorferi in natural rodent populations particularly during periods of nesting and/or breeding" (52: pg 40). Evidence for direct contact transmission has been demonstrated in mice (53) and further studies are required in larger animals to ascertain the potential for the borrelia spirochete to be transmitted simply by being in close contact with an infected animal.

Importation of Animals into Australia

Dogs are currently able to be brought into Australia from numerous countries in Europe, Asia and the United States (54). They are subjected to a 30 day quarantine, with requirements for rabies vaccination and blood tests for various pathogens (ie: ehrlichiosis, brucellosis, leishmaniosis, leptospirosis), though this does not include borrelia infections (55). Red foxes (Vulpes vulpes) are competent reservoir hosts (56-57) and may also carry tick vectors into new geographical areas (58). Foxes were introduced into Australia from Europe in the 1870's. Their range spread across southern Australia in the late 1800s and early 1900s and foxes are now widespread across the continent (59). They are considered a pest in all regions of Australia (eg: 59-60) In NSW they are listed as responsible for the extinction of several species of native fauna including numerous species of ground-nesting birds (59), and on Middle Island in Victoria (home to Little Penguin, Short-tailed Shearwater and Black Cormorant colonies), foxes and dogs that crossed to the island at low tide reduced the penguin numbers from 600 to less than a dozen in between 2000-2005 (61).

Cattle and horses are "low level" reservoir competent hosts, dependent on varying strains of borrelia (62), with reservoir competency still to be assessed with a number of different pathogenic strains. Cattle importation to Australia was suspended relatively recently due to outbreaks of Bovine Spongiform Encephalopathy (BSE) in other countries. Until the BSE outbreaks, cattle were imported from the United Kingdom (UK) until 1988 and from other European countries until 1991, with the suspension being extended to include cattle from Japan in 2001, Canada in 2003 and the United States (US) in 2004 (63-64). Lyme disease has been reported from all of these countries since the late 1970's, and/or early 1980's. Horses are still able to be imported from many countries, including the US and with regards to Lyme disease they only require vet certification that "After due inquiry, for 60 days immediately before export, the horse has not resided on any premises in the United States where clinical, epidemiological, or other evidence of contagiousequine piroplasmosis, horse pox, or Lyme disease has occurred during the previous 90 days" (65). With some animals carrying asymptomatic infections, this certification does not rule out that animals imported will be free of borrelia bacteria.

Sheep and deer may develop antibodies to borrelia infections (66-69), though studies regarding their role as reservoir hosts are mixed, with some studies concluding that they are competent reservoir hosts (67-71), and others finding that their role is limited to that of a host animal supplying a blood meal for the tick (72-74, 62). As with many animals, the differences found in reservoir competency with regards to sheep and deer may be due to species diversity of the animals (eg: there are around 44 recognised species of deer within 17 genera) or borrelia strain differences (ie; lizards are not a competent reservoir hosts for B. burgdorferi, however they are for B. lusitaniae) and needs further examination (62). Currently sheep are only permitted to be imported into Australia from New Zealand, with importations from other countries ceasing in 1952 (64). Deer have been introduced into Australia from Europe since the late nineteenth and early twentieth century's. Whilst over a dozen species of deer have been introduced, only six of these species survived the Australian environment (75). These deer (fallow, red, chital, rusa, sambar, and hog deer) have formed wild populations in Australia, with population numbers estimated to be 200 000 in 2004 (76). Commercial farming of four of these species (rusa, red, fallow, and chital) began in 1971, and in order to increase commercial herd numbers, the importation of a fifth species, the North American elk (wapiti), from Canada began in 1985 (77-78). Apart from varying levels of reservoir competency, the medium to large animals are regarded as maintaining the borrelia bacteria within the environment by providing the tick with a host for a blood meal, with studies finding deer populations correlated with tick density and human incidence of Lyme disease (79-80). The presence of larger host animals may also amplify the borrelia infection within the environment through tick co-feeding (72,81), with one study concluding that sheep "can transmit localized infections from infected to uninfected ticks co-feeding at the same site on the sheep's body" (72: pg 591).

Aside from the introduction/ importation of animals into Australia, it should also be mentioned that a study of Australian animals conducted by Mackerras in 1959 reported that borrelia was found in the blood of cattle, kangaroos, bandicoots and rodents (82). The borrelia in cattle was identified as borrelia theleri (agent of bovine borreliosis), transmitted by the cattle tick (*R. microplus*) (82), whilst the borrelia found in rats in north-western Queensland (Richmond area) was determined to be a new species of borrelia and named B.queenslandica (83). The vector of B. queenslandica was not ascertained (83) and the species of borrelia in kangaroos and rodents not identified (82). Given there is borrelia in the blood of animals in Australia, there must be a vector within the environment spreading/maintaining the bacteria.

Rhipicephalus and Dermacentor Ticks

Borrelia has been found in ticks of the Rhipicephalus and Dermacentor genera, though their competence as vectors (rather than just carriers) is an area of contention that requires much further research. Any research into the competence of particular species of ticks should also be aware of the finding that vector competence studies may be altered when ticks are examined in co-feeding (rather than singular tick species) studies. For example, whilst ticks of the Dermacentor species may be found to be incompetent vectors when feeding alone, in studies where they are co-fed with other species of ticks, they are found to be competent vectors. Whilst there are no ticks of the Dermacentor genera in Australia, this family of ticks examined briefly below due to the significance of these findings. is

Rhipicephalus Species : R. sanguineus (Brown dog tick) and R. microplus (Cattle tick).

Rhipicephalus sanguineus, or the brown dog tick, is located worldwide. In Australia, it is verified as present in every state apart from Tasmania (1: CSRIO info, last updated 2004). It is a tick of "great medical and veterinary significance being the vector and reservoir of many human and animal pathogens" (2: pg 349). Human pathogens include Bartonella, several species of Rickettsia, and Coxiella burnetii (Q

fever). Animal pathogens include; Ehrlichia canis, several Babesia species such as Canis vogelli and gibsoni and is a suspected vector of Anaplasma (2-4). It is also involved in the transmission of Theileria (a protozoa that is closely related to babesia) species such as Theileria parva, otherwise known as East Coast Fever and Theileria ovis (5,6).

Vector competence has not been established with regards to borrelia, although it has been found to harbour borrelia in both America (7) and Europe (8). It is also the suspected vector in Mexico, where a 2008 study in Mexicali, Baja California (a Mexico-US Border City) reported "the existence of B. burgdorferi past/present infection in dogs in an area where the only identified tick is R. sanguineus" (9). This species should be examined both for the borrelia species they may carry and their vector capabilities.

Rhipicephalus microplus (previously known as *Boophilus microplus*), otherwise known as the cattle tick, is considered the most important parasite of livestock in the world (10). It was first introduced into Australia (Darwin) in 1872 on cattle from Indonesia. By 1895 it had spread to Western Australia, reaching Queensland in 1891 and New South Wales in 1906 (11). This tick differs from all other ticks mentioned in the borrelia cycle, in that it is a one host (rather than three host) tick, meaning that it spends its entire life (much shorter cycle than other ticks also) on the one host. As the name suggests, the primary hosts of this tick are cattle, though it may also be found on horses, sheep, goats, camels, alpacas, llamas, deer and dogs (10, 12,13). Although not a common occurrence, these ticks may also attach to humans who come into contact with them (10,12,14). Whilst it may not come into contact with humans on a regular basis, this tick may serve to keep the borrelia cycle active within the environment.

Borrelia burgdorferi has been isolated from *R. microplus* (14-16), though it ability as a vector of this species of borrelia is unclear. It is however a known vector of Borrelia Theileri, the species responsible for bovine borreliosis. "To date, only B. burgdorferi ss and B. garinii have been described in bovine Lyme disease. However, two other spirochetes, B. theileri and B. coriaceae have been described in cattle and considered as the agent of bovine borreliosis and as the putative agent of epizootic bovine abortion, respectively" (17:pg 2). B. theileri has been noted in Australian cattle for over 50 years (18).

DNA sequencing reveals that B. theileri is in the same clade as B. Ionestari and B. miyamotoi, the species of borrelia that are responsible for relapsing fever/ lyme-like disease in humans (19, 20). Indeed, they are that similar it has been postulated that due to the eradication of the *R. microplus* ticks from America, the Ionestari borrelia species that is found in the *A. americanum* tick may have originally been due to the borrelia theileri bacteria relocating from the *R. microplus* tick to the *A. americanum*: "Because white-tailed deer and cattle used to be sympatric throughout the southern USA prior to 1943, which is when cattle ticks were officially eradicated, it has been hypothesized that spirochetes infecting *A. americanum* may represent a host shift of B. theileri as *R. microplus* could have transmitted the spirochete to both ungulate hosts" (14).

With the presence of B. theileri in Australia, combined with the possibility of host shifting of various borrelia species, along with the importations of cattle from countries where Lyme is endemic, then further investigations are certainly warranted. For example, B. miyamotoi was discovered in Japan in 1995, and yet Australia's importation of cattle from Japan didn't cease until 2001. If the cattle, or the ticks these imported animals carried were infected it could have led to an introduction of borrelia miyamotoi in Australia, which may or may not have lead to further adaptations to ticks within the Australian environment.

Dermacentor Species

Species from the Dermecentor genera include those found in America: *D. variabilis* (American Dog Tick), *D. andersoni* (Rocky Mountain Wood Tick), and Europe/Asia: *D. reticulates* (Marsh tick or Ornate cow tick) and *D. marginatus* (Ornate sheep tick).

D. variabilis (American Dog Tick), D. andersoni (Rocky Mountain Wood Tick).

Borrelia has been found in both *D. andersoni* (Rocky Mountain Wood Tick) (1) and *D. variabilis* (American Dog Tick) (1-5). Whilst this indicates their ability to acquire infection from a host animal, whether they maintain that infection through there next molt/life cycle, or are able to pass it on to another host is unknown. Studies on *Dermacentor* ticks are mixed: When the tick is examined in isolation, it is not considered/found to be a competent vector, however, when "they feed in conjunction with lxodes scapularis ticks, the Dermacentor ticks can acquire and transmit Borrelia burgdorferi sensu stricto" (6). The combination of different salivary factors of the ticks feeding in close proximity is believed to be the underlying factor in this finding.

D. reticulates (Marsh tick or Ornate cow tick) and D. marginatus (Ornate sheep tick).

Two Dermacentor species found in Europe / Asia are the *D. reticulates* (Marsh or Ornate cow tick) and the *D.marginatus* (Ornate sheep tick). Both species may feed on humans, particularly the scalp (7), and both have been found to harbour borrelia (8-10). *D. reticulates* has been suggested to be involved in the transmission cycle of borrelia in Europe (11) and a case of human Lyme disease after the bite of a *D.marginatus* in Bulgaria has been reported (12).

As noted in the introduction of this section, there are no ticks of the Dermacentor genera in Australia. They are mentioned here due to the findings that vector competence studies may be altered when ticks are examined in co-feeding (rather than separate genera's) studies, and this may be applicable to other genera (eg; Rhipicephalus) of tick that have also been found to carry borrelia. Considering that in the natural environment many different species of ticks may be found on the host animal, further co-feeding studies of various tick species are warranted and urgently required to further understand the co-feeding phenomenon.

The above mentioned ticks only account for a small number of the approximately sixty known species belonging to the Ixodidae genera in Australia. Other species from this family include the *Ixodes holocyclus* (Paralysis Tick), the *Haemaphysalis bancrofti* (Wallaby Tick) and the *Amblyomma Morelia* (Snake Tick), in which "spirochete-like objects" were cultured from (as well as the *H. longicornis*) in research conducted by Russell et al., in New South Wales (1994). More information on these tick species, as well as a review of the research methods and conclusions drawn about the research findings can be seen in this papers complimentary report, 'Lyme Disease: A Counter Argument of the Australian Government's Denial'.

Comment 6: Page 11, Para 3: As with *all* infectious diseases, infection with *B. burgdorferi* s.l. leads initially to an IgM antibody response, followed 2-4 weeks later by an IgG antibody response. The IgM response tends to be relatively short-lived in most patients, but the IgG response remains for decades following infection (Glatz et al 2008; Kalish et al 2001).

There are numerous papers that suggest that the immune response in Lyme / borrelia infection does not follow "typical patterns". Some references/ info on this I have written previously:

Regardless of whether using American or European Western Blot interpretation, the length of time of IgG responses is not 'set' to the "first few weeks". A few excerpts on this:

Craft, Fischer, Shimamoto and Steere (1986), whose article is referenced in the introduction in the Dressler et al paper that the ICPMR fact sheet refers to: "In 12 patients with early disease alone, both the IgM and IgG responses were restricted primarily to a 41-kD antigen. This limited response disappeared within several months...The IgG response in these patients appeared in a characteristic sequential pattern over months to years.." (13;pg 934).

Strle et al., (1996) "Our work also highlighted the continuing problems associated with use of serological methods for patients with early disease. Fewer than 50% of cases demonstrated seropositivity at any time within the first 2months" (14; pg 64).

Aguero-Rosenfeld et al., (1996) report on the serological results from Culture-Confirmed cases of Lyme: "Although 89% of the patients developed IgG antibodies as determined at a follow-up examination, only 22% were positive by the IgG IB criteria of the Centers for Disease Control and Prevention-Association of State and Territorial Public Health Laboratory Directors. (15; pg 1).

Comment 7: Page 14, Para 3: A major study of 12,000 ticks collected along the coastal strip of NSW was undertaken by Russell and Colleagues to investigate the presence of *Borrelia* species. About 11,000 ticks comprising more than 12 species, especially *Haemaphysalis bancrofti, H. longicornis, I. holocyclus,* and various other *Ixodes* species, were dissected and the gut contents examined by dark field microscopy and, in some cases, culture but no spirochaete of any kind was detected, although spirochaete-like objects were visualised from by dark-field microscopy.

A research article i wrote Lyme Disease: A Counter Argument of the Australian Government's Denial", covers this study. Below is the relevant sections regarding the 12,000 ticks collected. Attached also is a copy of the full Counter Argument.

Vector Studies

DME Website: "There are reports of spirochaetes in Australian native animals, and a local mammal could be a reservoir host for an indigenous spirochaete that occasionally infects humans through a tick vector and produces a clinical syndrome similar to LD; however, no spirochaete was detected in the 12,000 ticks or animals processed".

As discussed in the reservoir host section, the total number of 'animals processed' (processed by, capturing, and taking an 'ear punch biopsy') was seventeen. Ear punch biopsy of 17 vertebrate animals cannot be used to ascertain the presence, or lack thereof, of the borrelia species responsible for Lyme disease in Australia.

The 12,000 ticks processed, is the primary research performed in 1994 by Russel et al., of which the denial of the existence of Lyme in Australia is still based on today. This research is examined in detail below.

Lyme disease: search for a causative agent in ticks in south-eastern Australia. Epidemiology and Infection. Russell RC, Doggett SL, Munro R, Ellis J, Avery D, Hunt C, Dickeson D. 1994. Epidemiology and Infection 112: 375-384.

Abstract: "Attempts were made to identify the causative organism of Lyme disease in Australia from possible tick vectors. Ticks were collected in coastal areas of New South Wales, Australia, from localities associated with putative human infections. The ticks were dissected; a portion of the gut contents was examined for spirochaetes by microscopy, the remaining portion inoculated into culture media. The

detection of spirochaetes in culture was performed using microscopy, and immunochemical and molecular (PCR) techniques. Additionally, whole ticks were tested with PCR for spirochaetes. From 1990 to 1992, approximately 12,000 ticks were processed for spirochaetes. No evidence of Borrelia burgdorferi or any other spirochaete was recovered from or detected in likely tick vectors. Some spirochaete-like objects detected in the cultures were shown to be artifacts, probably aggregates of bacterial flagellae. There is no definitive evidence for the existence in Australia of B. burgdorferi the causative agent of true Lyme disease, or for any other tick-borne spirochaete that may be responsible for a local syndrome being reported as Lyme disease".

Russell et al Pge 377: "The study area comprised the coastal strip of NSW, from the Queensland border in the north to the Victorian border in the south".

Pge 378: "From January 1990 and December 1992, > 20,000 ticks were collected" **Pge 375:** "From 1990 to 1992, approximately 12,000 ticks were processed for spirochaetes".

Counterpoints: There was over 20,000 ticks collected, and approximately 12,000 of these were examined in the study. There is no explanation as to what happened to the other 8,000 or more ticks that were collected, whether they died in storage, or how it was determined which ticks should be utilised.

Of the 12,000 ticks processed, 6,235 of them were questing (looking for a blood meal) larvae. From the numerous journal articles read in relation to this topic, it is typically only the nymph and adult ticks that are utilised in studies examining borrelia rates in the environment as the transoveral infection rate of larvae is less than 1%. "In general, less than 1% of host-seeking larvae are infected, compared with between 10% and 30% of the nymphs and between 15% and 40% of adults" (1: pg13). Indeed, Russell et al., do note in their introduction, "*Transmission to humans will only occur from ticks that feed first on infected reservoir hosts and then on humans*" (pg 376).

From the 12,000 ticks tested, this leaves approximately 5,770 ticks that would have definitely had a chance to acquire borrelia infection/spirochetes via a host/blood meal. Whilst nearly 6,000 ticks 'may be' considered a relatively large number, the figure "12,000" is always brought up in relation to this study, and the denial of Lyme in Australia. On saying 6,000 'may be' considered a relatively large number, what needs to be taken into account is that infection rates of ticks from the same country can vary dependant on, species of tick, stage of tick (ie: larval, nymph, adult), region and environmental area (ie: pastures, mountains, forests, coastal) they are collected from. Differences in infection rates can be tremendous, varying from 0 to <90% (1-6). Therefore, when you take into consideration that the ticks examined in the Russel et al., study were collected from a 2,000km section of Australia's 35,000+ km coastline (one region/state of Australia and one environmental location), and that this tick collection examination/study is the primary basis of denial of Lyme, not just in the State of New South Wales they were collected from, but the whole of Australia, then 6,000 ticks is not a large number by any standard.

PCR testing

Russell et al Pge 378-9: "1038 ticks tested using PCR, no amplification products which would suggest the presence of borrelia were detected".

Russel and others note their own limitations of the PCR testing of ticks in this study: "It is possible that the monoclonal antibodies and PCR primers used in this study may not have been appropriate to identify indigenous Australian spirochaetes. However, the tick gut contents were also negative by culturing and dark field microscopy" (pg 381).

The problems relating to the techniques used and conclusions drawn from the culturing and dark field microscopy examinations is the subject of the next section.

Spirochaete detection and isolation: Darkfield Microscopy and Culture.

Russel et al Pge 378-379: "Between January 1990 and December 1992, > 20,000 ticks were collected. Approximately 11,000, including all stages of four species, Ixodes holocyclus, I. tasmani, Haemaphysalis bancrofti and H. longicornis were dissected for spirochaete isolation. With the additional 1,038 ticks tested using PCR, no amplification products which would suggest the presence of borrelia were detected."

Russell et al Pge 378: "No spirochaetes were detected by dark field microscopy of the gut contents of the unfed ticks..."

Counterpoints: The methods section of Russel et al, explains that the ticks were stored live until processed (pg 377), though doesn't explain the length of time the ticks were actually stored for (ie: were they all stored from 1990, until processed in 1992?), or give any understanding (ie; had 8,000 ticks died

while being stored?) as to why only 12,000 of the 20,000+ ticks collected were subsequently processed in the study.

The time and method of tick storage is very relevant as to whether or not spirochetes may or may not be able to be observed in the gut contents of ticks. In studies that examine poor environmental conditions, such as starvation, it has been observed that motile spirochetes convert into non-motile cyst forms; until such time that their environment is more conducive to their requirements (7-9). The lack of detection of spirochetes in the gut contents of ticks that had been stored live, rather than immediately frozen or stored in ethanol to preserve their contents (10) for an indefinite period of time, cannot rule out the presence of cystic forms of borrelia. Other detection methods, such as indirect fluorescent antibody (IFA), have been shown to be more sensitive in detecting the presence of spirochetes (11).

Culture: Spirochaete like Objects (SLO's)

Russell et al Pge 379: Spirochaete-like objects (SLO's)... were revealed by dark field microscopy in 92 cultures.." "Purified SLO's were obtained with 0.45um filters, but it was not possible to subculture them in the absence of bacterial contaminants..."

Background of culture medium and counterpoints: BSKII medium is a specialized growth medium that may be used for culturing spirochetes, though as the quality of the medium is variable due to variations of medium components such as bovine serum albumin, rabbit serum and yeast extract and each batch mixed requires special care in preparation, filtering, and screening for its ability to support the growth of borrelia (12). Considering this variability, specialist laboratories examine each batch of medium prepared to assess its viability to maintain spirochetal growth. There is no indication that the batch of medium prepared by Russel et al., (a laboratory with no prior experience in culturing borrelia spirochetes) was tested for its ability to maintain viable spirochetal growth prior to use in this study.

The use of 0.45um filter paper is ideal to obtain purified spirochetes as unlike most other bacteria, leptospires and treponemes are able to migrate through filter papers (13). Whilst Russell et al., concluded that what they obtained from the cultures were SLO's, the fact that the bacteria that was isolated was able to migrate through the filter paper is highly suggestive of the fact that they were indeed spirochetes.

As purified SLO's were obtained via filtration methods, it is feasible to assume that the bacterial contaminants in the subculture were more than likely due to the BSK II medium, rather than contaminants from the ticks bloodmeal.

Whilst the use of 0.45um filter paper has been found to be one way of culturing purified spirochetes, another method known to rid the culture of contaminants is the addition of antibiotics such as Rifampin, Phosphomycin, Amphotercin B (12,14) that borrelia are resistant to. It has also been found that BSK medium containing Co-trimoxazole (15) or Rifampin, is "more efficient for spirochete isolation than unsupplemented BSK medium" (11). It is not possible to determine from the methods section of the Russel et al., paper whether the use of antibiotics such as those previously mentioned was employed. The methods section does however mention the use Skirrows supplement, which is an antibiotic supplement recommended for selective isolation of campylobacter species and contains three antibiotics, Vancomycin, Polymixin and Trimethoprim (16), Spirochetes are susceptible (killed) to both Vancomycin (17,18) and Trimethoprim (19), rendering the choice of Skirrows supplement a less than ideal additive, considering the aim was to culture/grow (rather than kill) spirochetes.

Molecular identification & description of culture products

Russel et al Pge 379 : "While a few positive results were obtained by IFAT using polyclonal antibodies, the results were both variable and inconsistent for the 18 SLO's tested."

Counterpoint: Variability of positive IFAT results should be cause for further investigation: The quality of medium has been found to, alter gene expression patterns (20), effect the morphology (length and number of coils) and motility of spirochetes as well as alter the results of IFAT tests (21).

Russel et al Pge 380: "PCR ... successfully amplified a 950bp fragment in 92 of 92 SLO cultures, however the fragments amplified produced characteristic enzyme digestive products of a Bacillus sp. and not a Borrelia sp."

Russel et al Pge 380: ".. the SLO's appeared straight, rigid and uniformly coiled, varied in length (10-300um)* and had 2-40 complete coils; all appeared to be non-motile."

*It is assumed that 300um is a typographical error and should read *30um. This would be in line with the picture (pg 380) showing a 50um bar for comparison.

Counterpoints: The 950bp fragments amplified by 16S rDNA cannot be interpreted as being able to rule out Borrelia species as enzyme digestive products with a 950bp have also been identified for B. burgdorferi ss (22). "...heterogeneities between 16S rRNA genes seems to be a common phenomenon and, that for species identification, 16S rDNA analysis has to be interpreted with care" (23: Pg 2246).

Bacterial species cannot be defined by DNA similarities alone, (24), and what is more descriptive here is the appearance of the SLO's. Bacillus species are rod like and 5-10um in length. Borrelia species are spiral shaped and 10-30um. The pictures provided in the journal article (pg 380) and the description of the SLO's are more representative of the appearance of the Borrelia species rather than bacillus.

Russel et al Pge 381 : "Electron micrographs showed that these SLO's had no distinct cellular structure but were composed of fibre-like subunits, and were not spirochaetes."

Counterpoint: Spirochetes do not have a distinct cellular structure and are composed of axial filaments which have one or more fibrials. The three brief quotations below expand on this:

"The outer sheaths of S. plicatilis, all Borrelia species, and T. phagedenis strains so far examined are characterized by a lack of structural detail" (25 :pg 118).

"Ultrastructural examination of spirochetes has established their procaryotic nature and the one ultrastructural feature-the axial fibril-that sets them apart from other prokaryotes" (25: pg 152).

"Spirochetes consist of three main structures: aprotoplasmic cylinder, an axial filament (consisting of one or more fibrils), and an outer envelope..." (26 pg: 1087).

Whilst the conclusion was drawn by Russel and others that the 'objects' cultured from some of the ticks were spirochete-like objects (SLO's), the following section is based on the assumption that they were in fact more than likely spirochetes and examines the tick species they were cultured from briefly below.

Tick Species Spirochete-Like Objects (SLO's) Cultured from

Russel et al Pg 379: "The tick species yielding these SLO's were I.holocyclus, H.bancrofti, H.longicornis and Amblyomma morelia."

Paralysis Tick (*Ixodes holocyclus*)

I. holocyclus is more commonly known as the paralysis tick as bites from this tick can cause paralysis in animals and humans. It is found in Queensland, New South Wales, Victoria and Tasmania. The *holocyclus* range of hosts is extremely wide and includes both native and introduced animals, including birds and reptiles. The mammalian hosts range from rodents, to animals in the wild such as kangaroos, koalas, bandicoots, to domesticated and farm animals such as dogs, cats, cattle, horses, pigs and sheep. Humans may occasionally become accidental hosts (27-29).

The *I. holocyclus* is the tick "presumed" most likely to be the vector for borrelia in Australia, and as such is the only tick species to have been examined in relation to its capability of transmitting borrelia. In 1991 Piesman and Stone (30) conducted a study that examined the ability of *I. holocyclus* to acquire, maintain and transfer the borrelia burgdorferi ss species. It was found that while larval *I. holocyclus* could ingest the spirochetes, the infectivity was not maintained once the tick had "moulted" to its next cycle, the nymphal stage. The conclusion was, "These experiments should be repeated with Australian strains of spirochetes" (30). However, in the 21 years since; no further studies have been performed. Whilst there has been no Australian spirochete identified (due to no ongoing research), studies to ascertain the ability of transmission with European species of borrelia, would have been possible to conduct.

Taking into consideration the knowledge that certain tick species may only transmit species of borrelia (eg 31) common to their country of origin, it is not appropriate to rely on one study (30) that examined an indigenous Australian tick species ability to transmit a borrelia species most common to America. As Piesman and Stone (30) concluded, additional research should be performed. Along with the fact that SLO's were cultured from this species of tick, further information adding to the argument for additional research on this species ability as a vector is that borrelia-like spirochetes were also cultured from *l.holocyclus* ticks collected from the Manning River district of NSW in research conducted by Wills and Barry in 1991 (32). Additionally, very many of the animal hosts of *l. holocyclus* are capable reservoir hosts for borrelia, for example mice, rats, cats, dogs, cows, horses and birds, adding even more reason to conduct further research with regards to what pathogens the ticks may carry, and the ability of this tick to carry/transmit borrelia species that are more common in Europe and Asia.

Wallaby Tick (Haemaphysalis bancrofti)

H. bancrofti is informally known as the wallaby tick as their principle hosts are wallabies. This species has also been collected from kangaroos, bandicoots and other mammals and livestock including cattle and sheep. *H. bancrofti* is found in Queensland, New South Wales and on Kangaroo Island, off South Australia (33, 34).

As *H. bancrofti* is only found in Australia and New Guinea, countries that have not typically been associated with borrelia, there does not appear to have been any research to determine its capabilities as a vector. What is known is that *H. bancrofti* is a vector of Theileria (piroplasm) (35-37), and this tick species is thought to be involved in the transmission of severe outbreaks of the disease which resulted in the death of over 800 in cattle on NSW farms in 2008 (37, 38). In research across the world there has been found to be an association between ticks that transmit piroplasms and borrelia (eg:39, 40). Considering this association, as well as the fact that SLO's were cultured from this tick species, it would seem apparent that further research on this tick species vector capabilities would be appropriate. This is especially so when you also add in the information that in 1991 Wills and Barry cultured borrelia-like spirochetes from *Haemaphylsalis* species of ticks, and many of the animal hosts of *H. bancrofti* are capable reservoir hosts for borrelia, including cattle, kangaroos, bandicoots and rodents in which a 1962 study (41) reported borrelia in the blood of these Australian animals.

Scrub/Bush Tick (Haemaphysalis longicornis)

The *H. longicornis* is more commonly known as the scrub or bush tick. It was introduced into Australia on cattle from Northern Japan and was first recognised in 1901 in north eastern New South Wales. This tick species is now established along coastal areas in Queensland, New South Wales, and through north eastern Victoria (esp Murray Valley) and Western Australia (42-44).

The hosts of the *H. longicornis* tick (45) include numerous animals that have been found to be reservoir hosts for borrelia and have been introduced or imported into Australia from countries that are endemic for Lyme disease (45,46). These animals include the smaller reservoir hosts listed previously in this counter-argument, ie: mice, rats and hares, as well as domestic animals such as dogs and cats (47-52) and medium to large animals such as foxes (53,54), cattle, horses (55-62), sheep and deer (63-65) that have been introduced into Australia and have varying levels of reservoir competence for borrelia.

Examination of *H. longicornis* as a possible vector of Lyme in Australia is warranted for numerous reasons. This includes knowledge about its role in the borrelia cycle in China (66-70). Combine this information with the fact that SLO's were cultured from this tick species, and the knowledge that this tick species was the one infesting cattle in cases of suspected Lyme disease in cattle at Camden NSW in 1989, in which positive IFAT serology for Borrelia burgdorferi was reported: "the herd from which these cases came was heavily infested with the Bush tick, *Haemophysalis longicornis*, at the times of presentation..." (71: pg 298), then it would seem apparent that research on this tick species role in the borrelia cycle in Australia is long overdue.

NB: Due to its known role of in China, the *H. longicornis* tick is covered in more depth in this counterarguments complimentary report, Lyme Disease / Borreliosis: An overview of Lyme and direction for further research required in Australia.

Snake Tick (Amblyomma morelia)

Amblyomma morelia is more commonly known as the snake tick. Whilst its preferential host is snakes, they are also found on various reptiles such as lizards and monitors (72). In Australia it is found in Queensland, New South Wales, Victoria, and the Northern Territory (73).

Whilst snakes and lizards were initially thought to be incompetent reservoir hosts of borrelia, one species, *B. lusitaniae* has been associated with lizards in several studies (74,75). Further examination of *A. morelia* is warranted, especially considering that even though the number of this tick species in this study was limited to 14 (4 nymphs and 10 adults), SLO's were cultured. Also of interest would be the examination of smaller rodents such as mice and rats, which due to their close natural environmental coexistence, the larvae may have initially fed on.

As well as the above mentioned ticks, there are also numerous other species from the *Ixodidae* genera in Australia. This includes the Seabird tick (*Ixodes uriae*) and a Bird tick (*Ixodes auritulus*) that are known vectors of borrelia (76-80). The *I. uriae tick* is found worldwide, including Australia and its offshore islands (81). The role that migrating seabirds and the *I. uriae* tick play in spreading borrelia has been known of

since the early 1990's (82). In a 1993 study by Olsen et al., Borrelia DNA was found in *I. Uriae* ticks from Crozet and Campbell Islands, off the New Zealand coast suggesting Lyme is indeed in the Southern hemisphere (83). The *I. auritulus* is a native tick of Tasmania (84-85). The first reports of borrelia being found in this tick species were from Canada in 2005 (80). *I. auritulus* attach to bird hosts such as the European blackbird and song thrushes. Both of these bird species have been introduced into Australia (86-88), and both are known reservoir hosts of borrelia (89-92). These two tick species as well as others from the *Ixodidae* genera, are covered in more detail in, 'Lyme Disease / Borreliosis: An overview of Lyme and direction for further research required in Australia'.

Comment 8: Page 17, Para 3: Co-transmission of tick-borne organisms

<u>Babesia</u>. Bovine babesiosis is a significant disease of cattle in Australia, having been introduced as early as 1829 by cattle imported from Indonesia, It currently costs the industry as much as \$29 million each year in lost production. Two species of *Babesia* cause bovine tick fever, *B. bovis* and *B. bigemina*, and they are transmitted by *R.microplus*. The former is by far the most important causing 80% of outbreaks. Considerable work on *B. bovis* was undertaken by CSIRO scientists in the 1940s through to the 1960s, especially in livestock (J. Curnow, personal communication). The first report of locally-acquired case of human babesiosis, caused by *Babesia microti*, was in a 56 year old man who had never travelled and had no history of blood transfusions (Senanayake et al 2012). The origin of the aetiological agent is uncertain; it is most closely related to North American strains, and the patient was either bitten by an imported tick or a local tick might have transmitted an autochthonous infection, presumably originating from one or more species of introduced rodent. If it was a local tick, the most likely candidate would be *I. holocyclus* as *Ixodes* species are the usual vectors overseas.

As noted – many co-infections/multiple pathogens need to be tested for – in particular Babesia – which is noted in all other parts of the world (besides Australia) as able to cause human infection. A little with regards to Babesia/ tick species below:

Co-infections/pathogens carried by H. longicornis:

Many ticks carry numerous pathogens, and as with other ticks associated with borrelia (eg: *I. ricinus* in Europe and *I. scapularis* in America), this is also the case for the *H. longicornis* tick. As well as its role in borrelia, it is a known vector for: bacterial infections such as Bartonella; Rickettsial infections including human rickettsiosis (R. japonica), Anaplasma and Ehrlichia; Protozoal infections Thelieria and Babesia. Of the protozoa, *H. longicornis* is a vector for a number of species including : East Asian bovine theileriosis (T. buffeli), Theileria Equi, Bovine babesiosis (B. ovata) and Canine babesiosis (B. gibsoni) (5-8,85-91).

Theileria is closely related to Babesia, and generally tends to be separated on the phylogeny tree/chart by organism size and phylogenetic analysis (91-95). There are over 100 species of Babesia, with some of the "large" Babesia species including: B. divergens, B. bovis and B. canis. The smaller babesia species include, B. microti, B. gibsoni, and B. gondii. The Theileria species "sit" within the smaller Babesia tree and include, T.equi, T. annulata, T. parva, T. buffeli, and T. sergenti (94: pge 13). Due to phylogenetic analysis, what was once designated Babesia equi, is now termed Theileria equi. It has also been noted that what is commonly called Babesia microti, would be more appropriately designated to that of human theileriosis/babesiosis (95).

Of the Babesia species, B. microti (typically a rodent parasite), and the bovine parasites, B. divergens and B. bovis are the main species to cause human infections (91-95), with other species such as B. duncani and B. bigemina also implicated in cases of human infections. Whilst B. bovis and B. bigemina are believed to have been introduced into Australia around 1872, the same time as the cattle tick (96,97), the first known case of Babesia microti in Australia came to light as the result of the infection and subsequent death of a 56yo NSW male in April 2011 (98,99). With its known vector capability with regards to some theileria and smaller babesia species, examination of the capability of *H. longicornis* in Australia to carry and/or transmit B. microti and other babesia species, would be highly appropriate and long overdue.

Co-infections/pathogens carried by R. Microplus:

As well as borrelia, *R. microplus* is the vector for many zoonotic pathogens; including those responsible for "Tick Fever"; Babesia bovis, B. bigemina and Anaplasma marginale, which may result in sickness and death in cattle (10,12-14, 21) as well as humans, particularly those that are immune-compromised (10,22). It is also suspected as a vector of Theileria equi (10), previously known as Babesia equi, and has been found to carry Ehrlichia, Wolbachia, and Coxiella burnetti (14).

As noted above with regards to babesia; B. divergens and B. bovis are the main species to cause human infections (22-26), with other species such as B. duncani and B. bigemina also implicated in some infections. It is known that over 80% of tick fever outbreaks in Australia are due to B. bovis (21) and it is long overdue that the health departments in Australia communicate information acknowledged in the rest of the world by updating the information such as that found on the Queensland Government: Agriculture, Fisheries and Forestry website: "People can find cattle tick on themselves after working with cattle or other animals. The ticks are easily removed and cause no lasting affect apart from the site itching for a few days" (12).

It urgently needs to be acknowledged that the Babesia protozoa these ticks can carry can be passed on to humans and result in clinical illness. It must also be noted that Babesia is able to be survive in the blood and be passed on through a blood transfusion (27).

There were numerous other sections I could have commented on – but again, health and time constraints prevented this. With regards to the scoping study points, I believe the Lyme Disease Association covered these in great depth, and again reiterate my support of their submission.

References:

As noted above, due to health decline and time restraints, I have been unable to format the references into a more flowing format for this response. And as much of the writing in this response comes from my research papers, I have therefore left the numbering/referencing in the sections originally. Sorry for the unconventional reference style. Whilst I would have dearly loved to provide a more professional looking response, for me, having the chance to offer input on this scoping study was much more important.

Comment 1 : Page 5, Para 1

CDC Press Release, August 19, 2013 : http://www.cdc.gov/media/releases/2013/p0819-lyme-disease.html

Comment 2 : Page 6, Para 2:

(1) Olsen B, Duffy DC, Jaenson TG, Gylfe A, Bonnedahl J and Bergstrom S (1995) Transhemispheric exchange of Lyme disease spirochetes by seabirds. J Clin Microbiol; 33:3270-3274. http://www.ncbi.nlm.nih.gov/pubmed/8586715

(2) Carly JG and Pope JH (1962) A new species of Borrelia (B. queenslandica) from Rattus villosissimus in Queensland. Aust J Exp Biol Med Sci; 40:255-61. <u>http://www.ncbi.nlm.nih.gov/pubmed/13876596</u>

Comment 3 : Page 6, Para 3:

(1) Luger SW (1990) Lyme Disease Transmitted by a Biting Fly. N Engl J Med; 322(24):1752. http://www.ncbi.nlm.nih.gov/pubmed/2342543

(2) Herzer P, Wilske B, Preac-Mursic V, Schierz G, Schattenkirchner M and Zollner N (1986) Lyme arthritis: clinical features, serological, and radiographic findings of cases in Germany. Klin Wochenschr ; 64(5):206-15. <u>http://www.ncbi.nlm.nih.gov/pubmed/3702279</u>

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This Publication appears to have been removed from the National Institute of Health Website: http://www.niaid.nih.gov/topics/lymeDisease/Documents/lymedisease.pdf

The full publication can still be viewed (correct as at June 2012) at the Town of Boxborough, Massachusetts website: <u>http://www.town.boxborough.ma.us/NIHLymeDisease.pdf</u>

Comment 4: Page 6, Para 3:

Lyme Disease Tick Vectors

I. scapularis and I. pacificus: Well known vectors

I. dentatus:

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Comment 8: Page 17, Para 3: Co-transmission of tick-borne organisms

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