NATIONAL VETERINARY LABORATORY

P.O. Box 239, 1Tice Road
Franklin Lakes, NJ 07417
877-NVL-LABS (877-685-5227)
www.natvetlab.com

NEWSLETTER

Literature Review: A Scientific Potpourri©

Evelyn E. Zuckerman, Editor **Spring 2019** **Vol. 18, Number 2**

In This Issue:

The Spring 2019 issue of the NVL Newsletter will review the general scientific literature as it relates to One Health and the veterinary profession. We will review some U.S. and global scientific and cultural policy decisions, environmental publications and, of course, recent *Bartonella* papers, some of which veterinarians are unlikely to have seen.

One Health:

Wadman, M. Closure of U.S. Toxoplasma lab draws ire. Science 364: 109, 2019.

On April 2, the head of a small research lab in Beltsville's Maryland, Dr. Jitender Dubey, learned only from media reports, that the laboratory was being closed. The laboratory had been operated by the USDA for the past 37 years and contributed significantly to the research into *Toxoplasma gondii*. The USDA, after pressure from animal rights groups, abruptly announced it was shutting down the lab saying the program, which cost only $625,000 annually to operate, had "reached its maturity" and "achieved" its agricultural research goals. The USDA declined to answer questions submitted from Science magazine, and even denied a request to interview the head of the laboratory, Dr. Dubey.

Numerous outside scientists disagree with the closing of the laboratory indicating that it has made major contributions in *Toxoplasma* research. The lab developed a blood test that can differentiate whether a person is infected by eating infected undercooked meat versus ingesting the parasitic oocytes shed from cats. *Toxoplasma gondii* parasites infect more than 1 billion people globally causing death, birth defects, and blindness. The lab used a few dozen cats or kittens annually infected with *Toxoplasma* to harvest the parasitic oocytes, the infective hardy forms of the organism, from their feces.

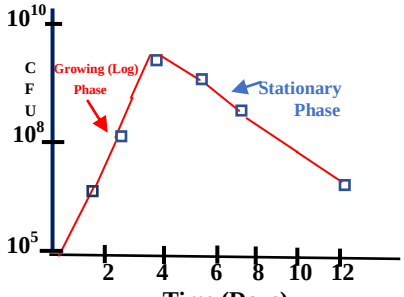
The cat is the only known animal to shed the infective *Toxoplasma* oocytes after completing its lifecycle. *Toxoplasma gondii* causes about 190,000 babies to be born with defects each year in the United States. In addition, there are about 1 million new infections annually and it is the second leading foodborne killer causing about 750 human deaths.

Collaborating scientists, utilizing the oocytes produced by this laboratory, had been working on a vaccine to protect cats from *Toxoplasma gondii* and to develop better methods for detecting the parasitic oocytes in drinking water and soil. Other studies, that will be curtailed, focused on keeping the parasite out of the food and water supply. Researchers fear the fate of the more than 1,000 *Toxoplasma gondii* strains and the numerous tissue samples and reagents that are housed by the now closed laboratory. It may be time for veterinarians and healthcare workers, in a One Health effort, to urge their Congress person to rethink this decision. This is an important zoonotic organism which has major public health implications around the world.

Bartonella:

Li, T., Feng, J, Xiao, S., Shi, W., Sullivan, D., and Zhang, Y. Identification of FDA-Approved Drugs with Activity against Stationary Phase *Bartonella henselae*. Antibiotics 2019, 8, 50; doi:10.3390/antibiotics8020050.

Treatment of *Bartonella* infections in animals and humans is challenging and may be due to the phenomenon known as persister (stationary phase) bacteria. Bacterial persistence is well known for diseases (infections) like tuberculosis, brucellosis, Q-fever and Lyme disease. Stationary phase bacteria are those that grow in liquid culture in log phase to a certain concentration but then become stationary in number as shown in the figure below.



Hypothetical growth curve in liquid media of *Bartonella henselae* over 12 days

This may occur if nutrients are depleted in the cultures or if crowded contact or, as with *Bartonella*, clumps of bacteria form which signal growth to slow or become dormant. The *in vivo* persister phenomenon does not appear to be the generation of antibiotic resistance during therapy, but rather may be selection of preconditioned groups of bacteria found in nature and transmitted by infected vectors. *B. henselae* colonize erythrocytes and endothelial cells, as well as being found free in the blood. Future studies are needed to determine if the persister *Bartonella* are mostly found intracellularly in infected animals and humans. Persister *B. henselae* may be the reason that therapy of *Bartonella* infections are problematic in people and cats and may be responsible for recurrences in both species.

This group studied the 110 FDA-approved drug-library for activity against stationary phase *Bartonella henselae*. The results of select commonly used *B. henselae* antibiotics are shown in Tables 1 & 2. The most active against the growing log phase *B. henselae* are rifampin, azithromycin (the 2 best) and doxycycline, those that we have recommended since our inception of *Bartonella* testing in 1999.

Table 1

Minimum inhibitory concentrations (MICs) of the best drugs against growing, log phase *B. henselae*

Antibiotics	MIC(µg/ml)
Rifampin	<0.01
Azithromycin	0.04-0.08
Pyrvinium pamoate	0.04-0.08
Methylene blue	0.08-0.16
Doxycycline	0.08-0.16
Erythromycin	0.08-0.16

However, these were not among the most effective in killing the stationary, non-growing *Bartonella*. Here the best were pyrvinium pamoate (an antihelmintic drug) and methylene blue. Unfortunately, pyrvinium pamoate is poorly absorbed, a reason for the antihelmintic effectiveness. Six agents were found to be very effective against stationary (persister) *Bartonella* (Table 2): pyrvinium pamoate, daptomycin, methylene blue, clotrimazole, gentamicin, and streptomycin. Both gentamicin, and streptomycin have been shown to be effective clinically for treating *Bartonella*.*

Table 2

Drugs with activity against, non-growing, stationary phase *B. henselae*

Antibiotics (50µM)		Viable Residual %
Pyrvinium pamoate	1	0%
Daptomycin	2	21%
Methylene blue	3	25%
Clotrimazole	4	27%
Gentamicin*	5	32%
Streptomycin*	6	39%
Rifampin	Poor	59%
Azithromycin	Poor	42%

Future studies of combination therapy for drugs to treat both the growing and stationary phases of *Bartonella* infection may improve therapy for animals and humans.

Cheslock, M.A. & Embers, M.E. Human Bartonellosis: An Underappreciated Public Health Problem? Trop Med & Infect Dis. 4: 69; doi:10.3390/tropicalmed4020069, 2019.

This review details the risks to humans from *Bartonella species* from various hosts, the most important being the pet cat. It remains perplexing to realize than many veterinarians and physicians still do not consider feline *Bartonella* significant pathogens. We hear that some veterinarians "do not believe in *Bartonella*," despite the **thousands of publications on PubMed**, of the diseases caused by these bacteria in cats and humans. Similar opinions have occurred for years with Lyme Disease and *Helicobacter pylori* etiologies in humans. The AAFP and the CDC also seem ambivalent regarding *Bartonella* importance in veterinary and human medicine. In addition, after interacting with thousands of veterinarians during the past 19 years, we still feel that many in our profession do not fully appreciate the importance of *Bartonella* in cats or in people. Likewise, after interviewing more than 500 people infected with *Bartonella*, it is clear that a substantial proportion of physicians do not know much about *Bartonella* or are dismissive of their clinical importance.

Selective Human *Bartonella* Diseases Caused by Feline *Bartonella**:

General Inflammatory Diseases:
Cat Scratch Disease, Fever, Lymphadenopathy, Mononucleosis-like syndrome. **Ocular Disease:** Parinaud's oculoglandular syndrome, Uveitis, Chorioretinitis, Neuroretinitis, Optic neuritis, Conjunctivitis, Blepharitis, **Heart Diseases:** Endocarditis, Valvulitis, Myocarditis, Stroke. **Neurologic Diseases:** Encephalitis, Meningoencephalitis, Meningitis, Seizures, Status Epilepticus, Coma, Headaches- Encephalalgia, Hemiparesis, Cognitive dysfunction, Brain fog, Agitation, Dementia. **Major Organs Involvement: Liver:** Peliosis hepatis, Granulomas. **Spleen:** Splenic bacillary angiomatosis, Splenomegaly. **Kidney:** Necrotizing glomerulonephritis. **Intestines:** Inflammatory bowel disease, Bacillary angiomatosis. **Respiratory Diseases:** Pulmonary granuloma, Pulmonary infiltrates. **Musculoskeletal Diseases:** Myositis, Arthralgia, Arthritis polyarthritis, Osteomyelitis, Myalgia. **Skin Disease:** Bacillary angiomatosis, Cutaneous rash- Henoch-Schenlein Purpura, Cutaneous granuloma annulare. **Other:** Co-infection with Lyme**,** Mononucleosis-like syndrome**,**
***Many similar diseases have been found in *Bartonella*-infected cats.**

Larson, H.L. and Schulz, W.S. Reverse global vaccine dissent. How did vaccine reluctance and refusal become such a major risk? Science 364: 105, 2019.

The World Health Organization has named vaccine hesitancy as one of the top 10 global health threats along with climate change, antimicrobial resistance, the Ebola virus, and of course, the next influenza pandemic. Anti-vaccine sentiment has been around for more than 100 years. In the early 2000's a vaccine boycott in Nigeria allowed the transmission of polio across multiple African countries and as far as Indonesia. The reason, unfounded rumors of the vaccine containing anti-fertility agents. Vaccination with the human papilloma virus decreased severely in Japan when young women complained of movement disorders and chronic pain, they thought caused by the vaccine. The sentiment spread to many other countries. In Indonesia, Muslim leaders issued a fatwa against the measles vaccine saying it contains pork compounds. And over 20 years ago, the British physician Andrew Wakefield spread doubts about the safety of the MMR (measles, mumps, rubella) vaccine suggesting a link between the vaccine and autism. This has thoroughly been discredited by numerous studies.

Veterinarians have also faced a similar reluctance to vaccinate cats against the feline leukemia virus, due to the poor performance of the initial vaccines and the threat of developing sarcomas at the vaccine sites. Presently, we are undergoing a measles epidemic due to the resistance to vaccination in many parts of the world. Much of these problems are now associated with, and magnified, by social media platforms such as Facebook, YouTube, Twitter and Instagram with their many chatrooms offering incorrect anti-vaccine sediment. In the One Health spirit, technology experts, physicians, veterinarians, social scientists, and public health experts must come together to counteract the inaccurate information on these platforms.

Corals are Dying!

Cornwall, W. The Reef Builders. Science 363: 1264-1269, 2019.

Along with pollution, global warming, over-fishing, ocean oil drilling and boating, the coral reefs of the world are dying at an alarming pace. I was fortunate to have been able to dive and photograph many of the underwater sites for the past 50 years. I saw most reefs in their majestic splendor. I, along with Dr. Bob Jack, co-founded The Society of Aquatic Veterinary Medicine in 1976, which has enabled veterinarians to have ship-board seminars and to dive the wonderful worldwide coral reefs.

Coral reefs develop by each generation building on top of the previous generation. Many reefs have grown, over millions of years, hundreds of feet from the ocean floor. One marine scientist calculated an interesting historic timeline: coral reefs, 20 feet down Columbus may have seen, 85 feet down was the time of Christ, and 180 feet down was the time of the pyramids. Now, coral reefs, the only living things visible from space, are under severe threat of extinction.

More than 50% of the coral on the Great Barrier Reef in Australia has recently died. When ocean temperatures increase, the coral polyps eject their symbiotic algae, which produce food for the polyps, and eventually die.

Great Barrier Reef from space,
The only living structure visible from space
Credit: NASA/GSFC/LaRC/JPL,MISR team

Great Barrier Reef, vibrant and full of life, 1985

Dead bleached coral

Total reef destruction

But there is hope! The Australian government recently committed $300 million to coral research and restoration. In addition, the late Paul Allen, co-founder of Microsoft, gave $4 million dollars to coral geneticist, Dr. Madeleine van Oppen at the University of Melbourne who is trying to re-engineer corals using techniques as old as plant domestication and as new as gene-editing with the CRISP-Cas9 tool. What makes this work more difficult, corals only spawn once a year driven by seasonal cycles and a full moon. The male and female polyps release their sperm and eggs together, 5 days after a full moon, and for the next few hours the eggs and sperm must form gametes or they must wait another year.

Coral polyps spawn once a year, depending on seasonal cycles, 5 days after a full moon

Dr. Van Oppen collects the spawning eggs and sperm and is trying to create corals, that are tolerant of higher water temperatures, by three methods: 1) Cross-breeding to create hybrids. They are also maintaining corals at gradually higher temperatures to see if they adapt. 2) Manipulating the microbiome of corals to determine if they will become heat resistant by gradually increasing water temperature and by the CRISP-Cas9 gene editing tool. 3) Adapting the symbiotic algae, that live in the coral polyps and produces the polyp's food, to higher temperatures and by gene editing. If the research is successful, scientists hope to re-seed the baron reefs with heat-tolerant corals which will hopefully restore this productive and beautiful habitat. Coral reefs are the nurseries for many food marine species.

Planting coral on an artificial reef

Conclusion: As veterinarians we should embrace the One Health movement to limit diseases of plants, animals and humans and we MUST embrace movements to protect our beautiful earth and all its creatures.