Peck Farm Phase 4.5 Report

This Phase 4.5 Research report will utilize past research generated as a foundation in a continued effort to build upon the understanding of how negative associated environmental organisms, when exposed to deer / elk are causal for the decline of the deer's immunity. By identifying the negative organisms, we can now continue to understand this disease associated health conditions in deer / elk alike. These organisms, left unchecked, in turn will lead to development of either an acute or chronic condition negatively impacting your deer / elk health and consequently your farms bottom line. Some of these organisms are treatable with the help of your farm veterinarian while other organisms identified are more antibiotic resistant pose a greater concern. Once antibiotic resistant organisms or pathogen loads gain a foot hold in deer / elk, they will lead to a further diminished health consequence for which could lead to the development of the neurodegenerative disease we only have come to know as Chronic Wasting Disease (CWD). Historically these organism types have never been reported in cases of CWD positive detected deer / elk upon death on the farm or in the wild. These current findings will help the Cervid farm industry and wildlife personnel alike in the understanding of how these environmental organisms in their survival in deer / elk contribute to the development of CWD.

Background

The first index CWD case on this farm was in early January 2016. Purple 1 (focus of this report) was rectally tested in the spring of 2016, 2017, 2018 and 2019 testing as non-detect by IHC methods by NVSL. From the start of this study farm records show Purple 1 (doe) had not produced a fawn in the spring of 2015, 2016, 2017 or 2018. Since prior rations were used on each of the 3 farms in this study helped generate baseline findings for each these individualized farms. In the fall of 2018 an improved ration was designed and started on the quarantined farm as well as both control farms in this study. Switching to a common ration was a way to provide a more complete picture of dietary similarities on 3 different farms in 3 different geographical locations under different health status' (CWD exposed / non-CWD exposed) of deer / elk. This new ration was designed to re-establish reproduction through improved health promotion via inflammation reduction due to negatively associated environmental organisms considered to diminish deer / elk health status. All deer on each farm started this new ration in the fall of 2018 prior to conception in the fall breeding season. Purple 1 delivered a buck fawn (Purple 2) on August 15, 2019 her first known fawn since the start of this study in spring 2016. By maintaining all deer on this ration Purple 1 subsequently produced a second buck fawn (Purple 3) on July 1, 2020 (45 days sooner vs. 2019 birth date) for which both buck fawns are still alive as of this report 4/2021.

Purple 1 was found deceased on November 19, 2020. Observations upon sample collection noted that her right side diaphragm was ruptured into the lung area. This could be an inflicted wound from the buck being aggressive going into the onset of the breeding season. Samples of brain, lung, liver and Kidneys were collected for testing and comparison to previously reported clinical findings that included deer from this farm which were deceased with CWD or deceased without detected with CWD (negative). Control deer samples were also collected to compare like samples (lung, liver, fecal) in the first comparison of a wild deer harvested from the CWD endemic zone within 1 mile of the quarantined farm as well from a deer harvested in Northern Wisconsin during the 2020 hunting season (lung, liver, kidney). Samples collected from Purple 1will provide an end point health status and contribute to our knowledge base, to date, of deer health while under quarantine for CWD.

Results

Water

Well water bacteria were compared to all deer samples harvested. In the past and current review, note there is a continued seasonal fluctuation of bacteria dependent on geographical location. We found more well water bacteria in the fall / winter seasons than the early spring (Table 1) from this 200+ ft. deep artisanal well.

Water Bacteria	Total Count	Like Bacteria	Bacteria / Purple 1	Percent	Bacteria / Controls 2	Percent
Farm Water Fall / Winter	370	370	75	60	15	42.5
Farm Water Spring	225	71	38	30	13	37.5
Farm New Water Spring	42	14	13	10	7	20
Water Related			126	100	35	100
Non water Related	0	0	384		78	
Total organisms			510	5X	103	

Table 1. Fall / winter farm well water supply shows higher bacterial organism count vs. early spring. Purple 1 also shows an overall increased (5X) bacterial load vs the 2 control deer (bacteria / Control 2 , northern and south west wild deer).

Compared to fall / winter bacterial counts, the top 100 organisms identified showed only 42 bacterial organisms were found in the spring sampling. Only 5 like organisms (Table 2.) were found in the farm new water supply taken at the same time in the spring. This new water supply will provide insight on clean farm water management that supports our bio-security plan for providing clean drinking water sources for deer / elk. Reducing bacterial loads from drinking water should help both on farm and wildlife production alike.

Top 20 Farm Water Bacteria Load - Deer Lung	Farm Fall / Winter	Farm Spring	Farm New Spring	Control Deer North	Control Deer SW	Purple 1
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	36	0	46,757
Fusobacteria; Fusobacterium necrophorum	570	0	0	0	0	3,529
Proteobacteria; Thiothrix	4	0	0	0	0	3,265
Bacteroidetes; Alistipes	0	0	0	0	0	753
Firmicutes; Clostridium butyricum	0	0	0	0	0	630
Firmicutes; Paeniclostridium	1,434	0	0	0	0	211
Firmicutes; Candidatus Stoquefichus	0	0	0	0	0	112
Firmicutes; Clostridium innocuum group	0	0	0	0	0	76
Firmicutes; Acetitomaculum	0	0	0	0	0	70
Bacteroidetes; Barnesiella	0	0	0	0	0	45
Firmicutes; uncultured Mogibacterium	0	0	0	0	0	44
Bacteroidetes; Porphyromonas	2,284	889	0	0	0	39
Synergistetes; Fretibacterium	0	0	0	0	0	38
Firmicutes; Ruminococcaceae	654	604	0	0	0	37
Firmicutes; Marvinbryantia	0	0	0	0	0	27
Firmicutes; Subdoligranulum	0	0	0	0	0	24
Archaea; Methanosphaera	0	0	0	0	0	23
Firmicutes; Clostridium botulinum	124	4	4	9	2	22
Firmicutes; Clostridiales vadinBB60 group	0	0	0	0	0	18
Firmicutes; Ruminococcaceae UCG-014	0	0	0	0	0	18

Table 2. Most bacteria organisms present in the fall/winter sampling had higher enrichment counts vs. bacteria organisms in the spring. If left un-checked, could have a negative consequence on the health and production schedules on your farm.

Lung Tissue

Test results from well water (Table 3) were compared to the lung tissue collected from Purple 1 to help indentify contributing factors leading to her death. We compared lung tissue samples from 2 control deer harvested during the fall hunting season. One deer was harvested from northern (Control North) Wisconsin (non- CWD affected County) and southwest (Control SW) Wisconsin (CWD endemic county). The deer from the south west harvested area was sourced within 1 mile of the quarantined farm for this study.

Review of the top 20 water bacteria demonstrates 7 of these were identified in the lungs of Purple 1. There was 1 bacterial organism identified in only 1 of the 2 control deer lung tissue tested. The top 1 bacterial organism identified in Purple 1 was also identified in the control northern deer lung tissue but at a low enrichment count. This leading organism though lower in the spring testing timeframes show quite clearly it's established growth in the lungs of Purple 1. There was only 1 like bacterium of low enrichment count found in both control (control northern / control SW) deer lungs as found in the lung sample of Purple 1.

Top 20 Farm Water Bacteria Load - Deer Lung	Farm Fall / Winter	Farm Spring	Farm New Spring	Control Deer North	Control Deer SW	Purple 1
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	36	0	46,757
Fusobacteria; Fusobacterium necrophorum	570	0	0	0	0	3,529
Proteobacteria; Thiothrix	4	0	0	0	0	3,265
Bacteroidetes; Alistipes	0	0	0	0	0	753
Firmicutes; Clostridium butyricum	0	0	0	0	0	630
Firmicutes; Paeniclostridium	1,434	0	0	0	0	211
Firmicutes; Candidatus Stoquefichus	0	0	0	0	0	112
Firmicutes; Clostridium innocuum group	0	0	0	0	0	76
Firmicutes; Acetitomaculum	0	0	0	0	0	70
Bacteroidetes; Barnesiella	0	0	0	0	0	45
Firmicutes; uncultured Mogibacterium	0	0	0	0	0	44
Bacteroidetes; Porphyromonas	2,284	889	0	0	0	39
Synergistetes; Fretibacterium	0	0	0	0	0	38
Firmicutes; Ruminococcaceae	654	604	0	0	0	37
Firmicutes; Marvinbryantia	0	0	0	0	0	27
Firmicutes; Subdoligranulum	0	0	0	0	0	24
Archaea; Methanosphaera	0	0	0	0	0	23
Firmicutes; Clostridium botulinum	124	4	4	9	2	22
Firmicutes; Clostridiales vadinBB60 group	0	0	0	0	0	18
Firmicutes; Ruminococcaceae UCG-014	0	0	0	0	0	18

Table 3. There were 7 of the top 20 well water supplied bacterial organisms identified in the lungs of Purple 1 with only 1 bacterial organism identified in one of the 2 control deer lung tissue.

There were 31 total bacterial organisms with a low enrichment count (1- 44) identified in the control north deer lung tissue as compared to 8 low bacterial organisms with low enrichment counts (2- 10) found in the control south west deer lung tissue. There was only 1 identified bacteria organism from the farm water supply that was identified in lung tissues of each deer tested (Clostridium botulinum). There was 1 bacterial organism identified in the control northern deer lung at a low enrichment count (Firmicutes: clostridium sensu strict 1) that was also found at a high enrichment count identified in Purple 1's lung tissue. There were no other shared bacteria organisms identified between the control northern deer and the control south west deer lung tissues (Table 4).

Top 19 Farm Water Bacteria Load - Deer Lung	Farm Fall / Winter	Farm Spring	Farm New Spring	Control Deer North	Control Deer SW	Purple 1
Proteobacteria; Succinivibrionaceae UCG-002	0	0	0	44	0	0
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	36	0	46,757
Bacteroidetes; uncultured Muribaculaceae	3878	708	5	22	0	0
Proteobacteria; Rhodoferax	0	0	0	17	0	0
Chloroflexi; KD4-96; Ambiguous taxa	0	0	0	10	0	0
Bacteroidetes; Pedobacter	0	0	0	10	0	0
Firmicutes; Clostridium botulinum	124	4	4	9	2	22
Acidobacteria; Subgroup 6	0	0	0	9	0	0
Proteobacteria; Beijerinckiaceae	0	0	0	8	0	0
Firmicutes; Peptostreptococcus	532	0	0	7	0	0
Proteobacteria; Desulfovibrio	0	0	0	7	0	0
Proteobacteria; Haliangium	0	0	0	7	0	0
Synergistetes; Fretibacterium	0	0	0	0	10	0
Bacteroidetes; Hymenobacter	0	0	0	0	8	0
Proteobacteria; Pseudomonas	0	0	0	0	5	0
Firmicutes; uncultured Acetitomaculum	0	0	0	0	5	0
Firmicutes; Ruminococcaceae UCG-014	0	0	0	0	3	0
Firmicutes; Marvinbryantia	0	0	0	0	2	0
Actinobacteria; Coriobacteriales Incertae Sedis	0	0	0	0	2	0

(Table 4) Bacterial organisms identified in lung tissues of control deer north and control deer SW show low cross over bacterial organisms shared between wild and farmed deer in this study.

Liver Tissue

Well water bacteria were compared to the liver tissue collected from Purple 1 and the 2 control deer. Purple 1 had 52 overall identified bacteria from her liver where as the 2 control deer had only 24 (control deer north) and 6 (control deer sw) bacteria organisms respectively. Of the top 19 bacterial organisms identified in the liver of Purple 1, (Table 5) there were 2 common well water associated bacteria identified in the livers of the 2 control deer (Clostridium sensu stricto 1and Clostridium botulinum) but at a low enrichment amount.

Top 19 Farm Water Bacteria Load - Deer Liver	Farm Fall / Winter	Farm Spring	Farm New Spring	Control Deer North	Control Deer SW	Purple 1
Firmicutes; Paeniclostridium	1,434	0	0	0	0	141,031
Proteobacteria; Thiothrix	4	0	0	0	0	2,959
Bacteroidetes; Alistipes	0	0	0	0	0	1,825
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	31	5	1,379
Firmicutes; Clostridium butyricum	0	0	0	0	0	371
Fusobacteria; Fusobacterium necrophorum	570	0	0	0	0	266
Firmicutes; uncultured Mogibacterium	0	0	0	0	0	240
Firmicutes; Blautia	4,441	4,626	0	0	0	101
Bacteroidetes; Barnesiella	0	0	0	0	0	90
Firmicutes; Acetitomaculum	0	0	0	0	0	78
Firmicutes; Candidatus Stoquefichus	0	0	0	0	0	50
Firmicutes; Ruminococcaceae	654	604	0	0	0	39
Bacteroidetes; Porphyromonas	2,284	889	0	0	0	36
Firmicutes; Clostridium botulinum	124	4	4	9	67	36
Synergistetes; Fretibacterium	0	0	0	0	0	34
Firmicutes; Pygmaiobacter massiliensis	0	0	0	0	0	34
Firmicutes; Ruminococcaceae UCG-014	0	0	0	0	0	34
Firmicutes; Subdoligranulum	0	0	0	0	0	24
Firmicutes; Selenomonas 1	0	0	0	0	0	19

(Table 5) Top 13 bacterial organisms identified in the liver of Purple 1 where as 7 were from the farm water source.

Since the 2 control deer had 2 different sets of bacterium (geographic locations) in their liver, they were comparred to the well water and Purple 1. Of the top 16 bacterial organisms identified in the liver of the control deer north there were only 3 like well water associated bacteria (Clostridium sensu stricto 1, Bacteroidetes; uncultured Muribaculaceae and Clostridium botulinum) and 9 non-associated bacteria identified to farm water (Table 6) . In the control deer SW there were 2 of 6 bacterial organisms identified common with the farm water and Purple 1. Only 1 common bacteria identified in the farm water was common each deer.

Top 16 Farm Water Bacteria Load - Deer Liver	Farm Fall / Winter	Farm Spring	Farm New Spring	Control Deer North	Control Deer SW	Purple 1
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	31	5	1,379
Bacteroidetes; uncultured Muribaculaceae	3,878	708	5	29	0	5
Proteobacteria; Succinivibrionaceae UCG-002	0	0	0	22	0	0
Proteobacteria; Rhodoferax	0	0	0	20	0	0
Firmicutes; Christensenellaceae R-7 group	0	0	0	14	0	0
Actinobacteria; Friedmanniella	0	0	0	13	0	0
Firmicutes; Clostridium botulinum	124	4	4	9	67	36
Bacteroidetes; Rikenellaceae RC9	0	0	0	9	0	0
Bacteroidetes; uncultured Bacteroidetes	0	0	0	8	0	0
Bacteroidetes; Prevotella 1	0	0	0	7	0	0
Bacteroidetes; Prevotellaceae UCG-001	0	0	0	6	0	0
Firmicutes; Butyrivibrio 2	0	0	0	6	0	0
Firmicutes; Fusicatenibacter	0	0	0	0	5	0
Firmicutes; Marvinbryantia	0	0	0	0	4	0
Firmicutes; uncultured Quinella	0	0	0	0	4	0
Proteobacteria; Sphingomonas	0	0	0	0	3	4

Table 6. Both control deer liver bacteria showed low enrichment counts as compared to Purple 1. This would show control deer not having access to the bacterial load exposure provided by the farm well water supply as in Purple 1.

Kidneys Tissues

The kidneys are likely to give us information that help frther understand CWD. Various anatomic structures may handle bacterial loads differently. To help pintpoint a location in the kidney where bacterial contamination would be problematic, we looked at 3 different anatomical structures of both kidneys from each of 2 deer (Table 7) . These 3 structures will provide, for the first time, a timeline for future sampling refinements for developing antimortum test detection of negative bacteria contributing to CWD in deer / elk. The kidneys from the control SW deer were unavailable for this part of the study. Well water bacteria identified from Purple 1 were compared to the control northern deer (Table 8). In the cortex area of the kidney Purple 1 had 9 identified bacteria in this reqion of her kidneys where as only 5 of these bacterias were found in the farm water source.

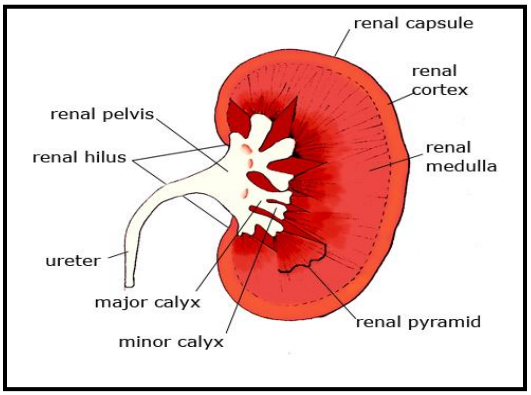

Table 7. Kidney structure diagram notes locations for cortex , hilus, and medulla for bacterial sampling.

The most enriched bacteria found in the cortex region (C1/C2) of the kidney of Purple 1 was identified as not being associated with any well water source. The control northern deer cortex did not test positive for any like bacterial organisms present as identified in Purple 1.

Top 9 Farm Water Bacteria Load - Deer Kidney Cortex	Farm Fall / Winter	Farm Spring	Farm New Spring	Control North - C1	Purple 1 - C1	Control North - C2	Purple 1 - C2
Bacteroidetes; Rikenellaceae; Alistipes	0	0	0	0	70,064	0	44,551
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	0	772	0	12,110
Fusobacteria; Fusobacterium necrophorum	570	0	0	0	4,105	0	4,484
Firmicutes; Paeniclostridium	1,434	0	0	0	11	0	920
Firmicutes; Blautia	4,441	4,626	0	0	56	0	241
Proteobacteria; Thiothrix	4	0	0	0	121	0	101
Firmicutes; Clostridium butyricum	0	0	0	0	48	0	21
Kiritimatiellaeota; uncultured WCHB1-41	0	0	0	0	0	0	4
Firmicutes; Christensenellaceae	0	0	0	0	0	0	3

Table 8. Cortex region of kidneys (C1 / C2) in Purple 1 shows high bacterial enrichment vs. no enrichment of like bacteria organisms found in both kidney cortex regions (C1 / C2) from the control deer north.

The cortex regions of the control north (C1/C2) deer kidneys identified only 1 bacterial organism in 1 of the cortex regions that was identified in the farm water supply at a very low enrichment count. Other bacteria identified were unique to the control northern deer were not associated with the farm water sources(s) nor identified in connection to bacterial organisms from Purple 1 (Table 9).

Top 14 Farm Water Bacteria Load - Deer Kidney Cortex	Farm Fall / Winter	Farm Spring	Farm New Spring	Control North - C1	Purple 1 - C1	Control North - C2	Purple 1 - C2
Proteobacteria; Rhizobiales; uncultured A0839	0	0	0	7	0	0	0
Chloroflexi; KD4-96;Ambiguous taxa	0	0	0	4	0	0	0
Firmicutes; Clostridium botulinum	124	4	4	3	0	0	0
Actinobacteria; uncultured Microbacteriaceae	0	0	0	3	0	0	0
Planctomycetes; Pir4 lineage	0	0	0	3	0	0	0
Acidobacteria; Subgroup 6	0	0	0	2	0	0	0
Actinobacteria; Nakamurella;Ambiguous taxa	0	0	0	2	0	0	0
Proteobacteria; Myxococcales; Blfdi19	0	0	0	0	0	8	0
Proteobacteria; Ruminobacter	0	0	0	0	0	6	0
Bacteroidetes; Prevotella 1	0	0	0	0	0	6	0
Bacteroidetes; uncultured F082 bacterium	0	0	0	0	0	4	0
Bacteroidetes; Prevotella 1	0	0	0	0	0	0	0
Firmicutes; Ruminococcaceae UCG-004 bacterium	0	0	0	0	0	2	0
Firmicutes; Veillonellaceae	0	0	0	0	0	2	0

Table 9. Bacteria organisms identified from both cortex regions of the control deer kidneys from northern Wisconsin show no connection to Purple 1.

The hilus region showed a more enriched location of the kidney (Table 10) for identifying bacterial presence from both Purple 1 and the northern control deer. The hilus regions identified 19 bacterial organism from Purple 1 (H1/H2) where as 8 were linked to the farm water source. The control northern deer hilus (H1/H2) only identified 1 like bacterial organism but at a very low enrichment that was associated to the farm water supply and Purple 1 (Table 11). There were 7 other identified bacterial organisms in the control northern deer hilus that were not associated with the farm water supply or Purple 1.

Top 19 Farm Water Bacteria Load - Deer Kidney - Hilus	Farm Fall / Winter	Farm Spring	Farm New Spring	Control North - H1	Purple 1 - H1	Control North - H2	Purple 1 - H2
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	0	62,113	0	75,852
Bacteroidetes; Alistipes	0	0	0	0	27,704	0	47,722
Fusobacteria; Fusobacterium necrophorum	570	0	0	0	9,943	0	16,053
Proteobacteria; Thiothrix	4	0	0	0	5,073	0	3,990
Firmicutes; Clostridium butyricum	0	0	0	0	1,154	0	527
Firmicutes; Blautia	4,441	4,626	0	0	3,606	0	298
Firmicutes; Paeniclostridium	1,434	0	0	0	3,436	0	267
Firmicutes; Candidatus Stoquefichus	0	0	0	0	125	0	72
Firmicutes; Clostridium botulinum	124	4	4	0	59	5	53
Firmicutes; Acetitomaculum	0	0	0	0	52	0	26
Firmicutes; Clostridiales vadinBB60 group	0	0	0	0	32	0	24
Firmicutes; Marvinbryantia	0	0	0	0	96	0	24
Synergistetes; Fretibacterium;Ambiguous taxa	0	0	0	0	226	0	22
Firmicutes; Ruminococcaceae	654	604	0	0	884	0	20
Firmicutes; Christensenellaceae R-7 group	0	0	0	0	24	0	13
Bacteroidetes; Porphyromonas	2,284	889	0	0	339	0	12
Firmicutes; Subdoligranulum	0	0	0	0	33	0	12
Actinobacteria; Intrasporangiaceae	0	0	0	0	19	0	12
Firmicutes; Lachnospiraceae	0	0	0	0	9	0	7

Table 10. Bacteria organisms identified from both hilus regions of Purple 1 where as 4 were identified to the farms water supply. Only 1 organism at a low enrichment was identified in the control northern deer hilus regions of both kidneys.

Top 8 Farm Water Bacteria Load - Deer Kidney - Hilus	Farm Fall / Winter	Farm Spring	Farm New Spring	Control North - H1	Purple 1 - H1	Control North - H2	Purple 1 - H2
Firmicutes; Lachnospiraceae NK3A20 group	0	0	0	2	0	0	0
Firmicutes; Oscillibacter	0	0	0	2	0	0	0
Firmicutes; Clostridium botulinum	124	4	4	0	59	5	53
Firmicutes; uncultured Quinella	0	0	0	0	0	4	0
Proteobacteria; Beijerinckiaceae	0	0	0	0	0	3	0
Firmicutes; Ruminiclostridium 9	0	0	0	0	0	3	0
Firmicutes; Veillonellaceae UCG-001	0	0	0	0	0	3	0
Bacteroidetes; Prevotella 1	0	0	0	0	0	2	0

Table 11. The hilus region of the control northern deer only identified 1 bacterial organism associated to the farm water and Purple 1.

The medulla region of both kidneys of Purple 1 identified enriched bacteria presence (Table 12) in this region that were also identified to the farms well water source. There were only 2 like low enriched bacterial organisms present in the medulla region of one of 2 kidney structures of the northern deer also identified as from a well water source from the quarantined farm.

Top 7 Farm Water Bacteria Load - Deer Kidney - Medulla	Farm Fall / Winter	Farm Spring	Farm New Spring	Control North - M1	Purple 1 - M1	Control North - M2	Purple 1 - M2
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	0	1,265	0	18,344
Bacteroidetes; Alistipes	0	0	0	0	38,463	0	10,582
Firmicutes; Paeniclostridium	1,434	0	0	0	3,183	0	4,306
Fusobacteria; Fusobacterium necrophorum	570	0	0	0	3,050	0	2,200
Proteobacteria; Thiothrix	4	0	0	4	209	0	38
Firmicutes; Clostridium butyricum	0	0	0	0	41	0	5
Firmicutes; Eubacterium brachy group	208	19	3	0	0	0	3

Table 12. Bacteria organisms identified from both medulla regions of Purple 1 where as 5 were identified to the farms water supply. Only 1 organism at a low enrichment was identified in the control northern deer medulla regions of both kidneys.

The medulla regions of the control northern deer kidneys identified 15 total organisms of low enrichment counts (Table 13). Only 2 bacterial organisms were identified as being present in the farm water supply. This similarity of like organisms found in these same structures of the deer on the farm and in the wild deer notes these to be environmental across a wide geographical region since the control north deer (control north) is distanced by approximately 300 miles and the deer from the south west (control SW) wild deer does not have access to drinking water from the deer farms water supply. There was 1 higher enriched bacteria organism found in the farms water supply that was not identified in Purple 1 but was identified in the control northern deer at a low enrichment. The control northern deer shows a different bacterial presence than that of Purple 1.

Top 15 Farm Water Bacteria Load - Deer Kidney - Medulla	Farm Fall / Winter	Farm Spring	Farm New Spring	Control North - M1	Purple 1 - M1	Control North - M2	Purple 1 - M2
Bacteroidetes; uncultured Muribaculaceae	3,878	708	5	0	0	11	0
Actinobacteria; Friedmanniella	0	0	0	0	0	8	0
Chloroflexi; KD4-96; Ambiguous taxa	0	0	0	0	0	6	0
Acidobacteria; Subgroup 6	0	0	0	0	0	5	0
Proteobacteria; Thiothrix	4	0	0	0	209	4	38
Bacteroidetes; Rikenellaceae RC9 gut group	0	0	0	0	0	4	0
Firmicutes; Clostridium botulinum	124	4	4	0	11	3	0
Proteobacteria; Beijerinckiaceae	0	0	0	0	0	3	0
Proteobacteria; Rhodoferax	0	0	0	0	0	3	0
Firmicutes; Lachnospiraceae NK3A20 group	0	0	0	0	0	3	0
Firmicutes; Ruminiclostridium 9	0	0	0	0	0	3	0
Synergistetes; uncultured Fretibacterium	0	0	0	0	0	3	0
Bacteroidetes; Bacteroides	0	0	0	0	0	2	0
Actinobacteria; uncultured Atopobium	0	0	0	0	0	2	0
Firmicutes; uncultured Quinella	0	0	0	0	0	2	0

Table 13. 15 bacteria organisms identified in the control north kidneys had only 3 like bacteria as in the farm water supply.

Brain

The brain was submitted for identification of bacteria as in past brain tissues of deceased deer from this quarantined farm (Phase 3.5) that tested either negative detect or positive for CWD. These specific regions were chosen as to their specific locations of known prion accumulation. As in past, CWD positive brain region reviews the identification and presence of negative associated bacteria load in these regions of the brain are responsible for further brain dysfunction. The current results (Table 14) show an early disease process where the brain sections have not been fully integrated with these negative bacteria as in other regions such as her lungs, liver and kidneys.

Top 10 Farm Water Bacteria Load - Purple 1 Brain Regions	Farm Fall / Winter	Farm Spring	Farm New Spring	Cerabellum	Cerebrum	Thalamus	Hypothalamus
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	14	0	0	0
Bacteroidetes; Alistipes	0	0	0	8	0	0	0
Firmicutes; Veillonella	13	0	0	5	0	0	0
Firmicutes; Paeniclostridium	1,434	0	0	4	0	0	0
Firmicutes; Bacillales	0	0	0	4	0	0	0
Proteobacteria; Bibersteinia	0	0	0	3	0	0	0
Cyanobacteria; Chloroplast	4,600	0	0	0	0	5	0
Bacteroidetes; Porphyromonas	2,284	889	0	0	0	2	0
Bacteroidetes; Butyricimonas	0	0	0	0	0	2	0
Firmicutes; Clostridiaceae 1	0	0	0	0	5	0	0

Table 14. Brain regions of Purple 1 identified 10 identified bacterial organisms with 5 identified from the farms water source.

Though she tested positive for CWD by NVSL in both lymph nodes and obex her earlier death was attributed to a perforated diaphragm on her right side in the lung region possibly from receipt of an aggressive buck during this early part of the breeding season.

This untimely death would suggest that though she was positive in both lymph nodes and obex regions, demonstrates an earlier underlying health progression of this bacterial integration to infections of the brain tissue. It also suggests she was in an earlier phase the of the disease process versus the more advanced clinical symptoms seen in Yellow 2 (brain and physical wasting of body muscle and fat reserves-phase3.5 of this study). This earlier stage of the disease process would be consistent with farmers and hunters alike that are stumped or surprised by the healthy robust outward body condition of a deer / elk as "looking healthy" only to their dismay their deer / elk was detected positive for CWD.

Fecal

This part of the study was to examine the potential connections between wild deer and farmed deer in shared organisms which could negatively both. This reviews both the south west wild deer as a control in on either side of the fence. This is an important consideration since farmers, hunters, wildlife agencies and lawmakers have always held the belief that nose to nose contact is a way that deer exchange "prions" across a single fence though this has never been truly diagnosed / confirmed. The farms water supply comes from an artisan well that is 180 feet in depth that free flows 365 / 24 / 7 without the aide of an electrical pump. This deep well draws its water from a deep aquifer that is supplied from surface water from the surrounding geographical terrain. Other wells in this area are shallower in nature that would typically be 30 feet deep as a "sandpoint" type of installation. This is doable in this region due to the sandy soil conditions along with a high water table.

Though the southwest deer has no access to the farm water supply, it suggests that organisms that are acquired by the wild deer would come from a water source on the landscape or in close proximity to the farm. These water sources could be from a pond, creek or other areas of a deer's' habitat. The fecal sample of the southwest wild deer identified 300 bacteria organisms with 10 bacteria (Table 15) that were consistent with the farm water supply.

Farm Water Bacteria Load - Control South West Deer Fecal	Farm Fall / Winter	Farm Spring	Farm New Spring	Fecal
Firmicutes; Clostridium sensu stricto 1	13,930	1,375	0	1,588
Cyanobacteria; Chloroplast	4,600	0	0	4,400
Firmicutes; Blautia	4,441	4,626	0	2,361
Bacteroidetes; uncultured Muribaculaceae	3,902	708	5	1,498
Firmicutes; Streptococcus	3,559	125	0	381
Firmicutes; Desulfosporosinus	881	0	0	50
Firmicutes; Ruminococcaceae	654	604	0	83
Firmicutes; Clostridium botulinum	124	4	4	61,253
Firmicutes; Terrisporobacter	20	0	0	391
Bacteroidetes; Bacteroides	8	0	0	716

Table 15. Fecal bacteria identified in the control south west deer that was associated with the farm water supply.

There are 15 other bacteria of interest that were found in the fecal tested that were not associated with the farms water supply (Table 16). There are 5 of these 10 bacteria organism that were associated with the farm water supply that were also identified in the farm deer tissue samples including the lung, liver, kidneys and brain.

Bacteria Load - Control South West Deer Fecal	Farm Fall / Winter	Farm Spring	Farm New Spring	Fecal
Fusobacteria; uncultured Caviibacter	0	0	0	10,360
Bacteroidetes; uncultured Porphyromonas	0	0	0	5,595
Bacteroidetes; Prevotella 7	0	0	0	2,401
Proteobacteria; Desulfovibrio	0	0	0	1,916
Archaea; Methanobrevibacter	0	0	0	1,851
Actinobacteria; Trueperella	0	0	0	1,341
Firmicutes; human gut metagenome	0	0	0	1,330
Firmicutes; Staphylococcus	0	0	0	1,228
Firmicutes; Lachnospiraceae	0	0	0	1,215
Actinobacteria; Bifidobacterium	0	0	0	1,108
Nitrospirae; uncultured Sphingobacteriales	0	0	0	1,034
Firmicutes; Erysipelotrichaceae UCG-003	0	0	0	1,025
Firmicutes; Fusicatenibacter	0	0	0	972
Firmicutes; Lachnospiraceae NK3A20 group	0	0	0	972
Firmicutes; Ruminococcus 2; Ambiguous taxa	0	0	0	960

Table 16. Fecal bacteria identified in the control south west deer that was not associated with the farm water supply.

In Southwest Wisconsin there is an ongoing study of the 3 counties (Iowa, Grant and Lafayette) that are specific to surface water quality for drinking water from shallow wells (32ft.). In the most recent update of the report, table 1 notes only results for identified coliform, ecoli and high nitrate forms of water contamination (Table 17). Our study looks deeper into water quality for bacterial organisms that are not covered by the multi - county surveillance study.

Table 1. Percentage of wells positive for total coliform, *E. coli*, and high nitrate* for two sampling events.

	November event (301 wells tested)				April event (539 wells tested)			
County	Total coliform	*E. coli*	High Nitrate*	Total coliform or High Nitrate*	Total coliform	*E. coli*	High Nitrate*	Total coliform or High Nitrate*
Grant	38	7	12	43	14	1	14	25
Iowa	26	3	13	33	14	1	13	25
Lafayette	40	3	27	55	23	4	21	36
All	**34**	**4**	**16**	**42**	**16**	**2**	**15**	**27**

*High nitrate exceeds the health standard of NO_3-N > 10 mg/L

Table 17. Water test results of an ongoing multi county well water testing for drinking water contamination.

In table 2 of the multi - county study, it shows what shared pathogens and viruses are identified by both sources of animals and humans (Table 18). These findings are consistent with home owner well water supply and or the septic systems in close proximity to the well water supply are in need of review and repair.

In the state of Wisconsin there is no law mandating well monitoring for private wells such as those found in rural communities such as farms and other non- county public water supply systems.

Table 2. Results from the first round of well testing for pathogens and microbial source tracking. Only organisms we detected are listed; see complete list in Table 3.

Microbe group	Microorganism	No. Positive Wells
Human-specific pathogens	*Cryptosporidium hominis*	1
	Human adenovirus groups A-F	2
	Human enterovirus	1
Human or livestock pathogens	*Cryptosporidium parvum*	2
	Cryptosporidium spp.	4
	Rotavirus group A (NSP3 gene)	3
	Rotavirus group A (VP7 gene)	1
	Salmonella (invA gene)	7
	Salmonella (ttr gene)	5
Human wastewater	*Bacteroidales*-like Hum M2	6
	Human *Bacteroides*	29
Bovine manure	Bovine polyomavirus	1
	Ruminant *Bacteroides*	16
Swine manure	Pig-1-*Bacteroidales*	3
	Pig-2-*Bacteroidales*	3
Pathogen*		13
Any microorganism*		32

*The value for Pathogen and Any microorganism are less than the sum of individual microorganisms because some wells were positive for more than one microorganism.

Table 18. Shared bacterial and virus' found in some private drinking well water in the 3 county study online review known as the "Update on the Southwest Wisconsin Groundwater and Geology Study, August 1, 2019.

Discussion

This current review of Purple 1 adds to the base information that has been gleaned from the past reviews of the study. In the findings we know she was detected CWD positive in both lymph nodes and obex by NVSL. The review of her tissues submitted for bacterial identification revealed that the well water supply was the point source of water born organisms and were present in different concentrations seasonally. These organisms loads found in the farm water supply were not found in the tissues of the 2 control deer at the concentrations of Purple 1 who was found positive for CWD.

In this study we are compiling data in the review of the bacterial origins of the gut microbiota of deer for which are dietary related such as feed and water sources. The microbiota communities are complex and they consist of all bacteria, viruses, fungus, and protozoa living in the intestine supporting the deer's immune system that have co-evolved in a symbiotic relationship since the origin of the deer's immune system. The bacterial community forming the microbiota plays an important role in the regulation of multiple aspects of the deer's immune system as laid out in the Immune System Modulations by Products of the Gut Microbiota (1.)

In another recent published study of chronic wasting disease (CWD) it is noted that the prion ingestion is fatal, contagious and a neurodegenerative disease affecting both free-ranging and captive cervid species.

Science has only explored CWD being spread via direct or indirect contact or by oral ingestion of prions. The study reviews the possible ingestion of prions into the gastrointestinal tract, where prions then enter the lymph system through microfold cell (M-cells) locations of the gut region where the abundance of these cells can be influenced by the gut microbiota. So to explore these links between the gut microbiota and CWD, the study collected fecal samples from farmed and free-ranging white-tailed deer (*Odocoileus virginianus*) around the Midwest. It is noted that farmed deer fecal samples originated from farms that were depopulated due to CWD here in Wisconsin where as free-ranging deer were sampled during annual deer harvests in a non- CWD affected area as described in Alterations in gut microbiota linked to provenance, sex, and chronic wasting disease in White-tailed deer (*Odocoileus virginianus*)(2).

In our study from the beginning we first reviewed our quarantined farm as to what may have led to the deer to contract CWD in the first place. This is noted in the Phase 1 thru Phase 4 reports generated to date. What we have learned to date is that it is imperative to provide clean feed and water for healthy cervid production cycles. The quality of the feed and water sources need to be balanced in a way that does not provide for an inflammatory process to escalate leading to diminished animal health. Water (a nutrition source) should be tested for providing clean fresh water on a consistent basis. In ruminants, another key nutrient in supplied feed comes from the fat type, source and load of the feed. If a ruminant is fed too high of grain products during their lifecycle (fawn / adult) could lead to damage of the ruminal wall lining which would restrict the proper gut microbiota from forming supporting rumen health, nutrient transfer and immune system support. A pro - inflammatory status could further complicate a cervids ability to fight off a health challenge due to poor feed, water quality or other environmental exposures. Feeding balanced types of fats in a ration is also important for ruminant health as balanced rations provide the ruminant with key cellular lipid (fat) properties supporting overall health and immunity. In our current study we looked specifically at the bacterial properties identified in the well water and their negative impact on Purple 1 leading to her health decline.

To really understand the first line of a defense in mammalian cell health, one needs to know that the overall cell system is comprised of the cell membrane that contains an essential structure, called the lipid bilayer. This lipid bilayer (derived by dietary fats) supports two main types of interaction between the cell membrane and protection from bacterial toxins. Bacterial toxins can alter the lipid bilayer and subsequently the cells integrity thus crossing the cell membrane to reach and modify an intracellular target. Pore formation (hole in the cell membrane) is a result of common bacterial toxin mechanisms that alters the cell membrane (lipid bilayer). Usually, when a bacterial toxin hijacks a cell physiological (normal cell function) process for their mode of action to the cell, pore formation continues its onslaught in to the cell by creating β-barrel structures that are unique process' used by these bacterial toxins. The resulting enzymatic process of these B- barrel structures have been found to be inserted into the cells wall (lipid) membranes through one or several transient transmembrane creating a β- sheet structures as described in Bacterial protein toxins and lipids: pore formation or toxin entry into cells (3).

In furthering your understanding of amyloid diseases it is important to understand the nature of the disease process itself. In an example, the description of "unstructured polypeptides" form pathogenic amyloid aggregates known as AB peptides. These AB peptides are present in the A.) brain of patients affected by Alzheimer's Disease (AD), B.) the Prion protein, responsible for the "madcow" disease and C.) IAPP, which is also the protein component of type 2 diabetes-associated islet amyloid. On this basis, peptide binding and penetration to the lipid membrane is proposed as the major driving force to explain how the Aβ conversion from a soluble, unstructured conformation to a potentially toxic β-sheet rich form. This cell membrane disruption process first allows the misfolding of soluble peptide monomers that then starts the aggregation process via the formation of β-sheet rich protofibrils where the next step is the clustering of these proteins on the cell membrane surface induces their assembling into β sheet-rich aggregates as described in Amyloid growth and

membrane damage: Current themes and emerging perspectives from theory and experiments on Aβ and hIAPP (4, figure1).

In our past research review bacterial contamination of buck Blue 1 (control negative for CWD) and that of CWD positive Yellow 2 (Phase 3.5) Aeromonas was one of the leading bacterial organisms identified in the brain section of Yellow 2. With this bacterial presence it's mode of action upon the cell allows for a process that allows the cleavage and polymerization of the cellular prion protein upon conversion to the scrapie isoform. Similarly, the amyloid precursor protein has been found to be localized to the caveolae structure of the cell where developing lipid rafts might be the privileged location on a cell outer membrane for the process of these cleavages and polymerization. In addition, it is also noted that aerolysin (bacterial toxin) has evolved to interact selectively via a class of proteins that is only expressed on the apical surface of polarized epithelial cells. Since Aeromonas infections are increasingly associated with food-borne infections where the toxin is most likely secreted inside the gut whereas after binding to cell surfaces and maturation, the toxin produced leads to a potassium efflux and lowering of the membrane potential as described in A Pore-forming Toxin Interacts with a GPI-anchored Protein and Causes Vacuolation of the Endoplasmic Reticulum(5).

In our current study there were other bacterial species identified that produce pore forming toxins. Pore forming toxins (PFTs) also have the ability to disrupt the host (deer / elk) immune responses. In some cases, PFTs cause an exacerbated inflammatory response that leads to extensive host tissue damage. In many cases, PFTs impair immune defenses, and this is accomplished through several different mechanisms. These include allowing bacteria to physically hide from the immune system surviving the phagocytosis processes as described in Role of Pore-Forming Toxins in Bacterial Infectious Diseases (6, figure 1&2).

Additionally, all pore forming toxins undergo a functional metamorphosis that leads inactive, soluble and monomeric proteins (single proteins) to assemble into conductive transmembrane pores (formed by bacterial toxins) at the target cell membrane. Upon oligomerization (toxin function) and membrane insertion, they undergo a prion-like α-helix-to-β-strand transition forming a prion structure as described in Pore-forming toxins: ancient, but never really out of fashion (7, figure1).

When pore forming toxins make their cellular entry into the cells membrane the properties they generate are called protofibrils. Protofibrils are suggested that by targeting neurons of the cells could kill cells by unregulating the cell membrane permeabilization (leakiness), by a type of protofibril referred to as the 'amyloid pore' as described in Are amyloid disease caused by protein aggregates that mimic bacterial pore forming toxins? (8).

In our current identification of bacterial organisms from well water (Table 2) we identified several clostridial organisms present along with others at a high enrichment count in tissues of Purple 1 (Table 3,8,10,12). There are several known clostridium species involved in infectious diseases that are known to be pathogenic while others are considered emerging pathogens. The bacteria essentially produce potent toxins that are responsible for life-threatening diseases in humans and other animals alike. Interestingly, these species produce the highest number of toxins of any type of bacteria, and the genetic characteristics of toxigenic clostridia supports a horizontal toxin gene transfer as described in Clostridium butyricum: from beneficial to a new emerging pathogen (9).

In the clostridia pore-forming toxin gene evolution it notes that many clostridial toxins (almost one third) as well as a large number of other bacterial toxins are pore-forming toxins. Most of them form pores through insertion of structures called "amphipatic hairpins" organized in a B-barrel structure inserted into the cell membrane that are called B-pore-forming toxins (B-PFTs). The largest B-pore forming toxin family is the

cholesterol-dependent (fat) cytoslysin (CDC) family, which encompasses toxins from at least 9 Clostridium species such as C. perfringens perfringolysin (PFO), C. botulinum botunolysin, and C. tetani tetanolysin and toxins from more than 15 other Gram positive bacterial species (Streptococcus, Bacillus, Listeria, etc. as outlined in the Genetic Characteristics of Toxigenic Clostridia and Toxin Gene Evolution (10).

Some other organisms identified were clostridium tetaniis which are capable of producing the two exotoxins, tetanolysin and tetanospasmin – Tetanolysin causes lysis of red blood cells while tetanospasmin is a neurotoxin that causes the clinical manifestations of tetanus. Another bacterium identified was clostridium botulinum (Table 15) which produces neurotoxins (which are the most potent natural poisons known) that cause botulism. Strains of C. baratii and C. butyricum have also been implicated as causative agents of botulism as they also produce the types F and E neurotoxins as described in the UK Standards for Microbiology Investigations - Identification of *Clostridium* species (11).

One area of interest has always been how does the central nervous system (CNS) and the brain of deer / elk become compromised in the development of and detection for CWD in the lymph nodes and obex. Once clostridia toxins are ingested from any food or water source and then allowed to multiply or escape the intestinal tract they commence with their destructive abilities. These bacteria types traffic their neurotoxins through the circulatory system and over time eventually into the brain regions by various pathways causing damage to multiple brain regions as demonstrated in Clostridial Toxins (12, figure 6)

Summary

In our current farm review we identified water born bacterial organisms that were being supplied in the deer's drinking water. The increased bacterial load supplied via water for the deer in the fall/winter season was more enriched than in the spring time water supply. There were other bacterial organisms found to be enriched in the deer that were not associated with the deer's water supply as noted by the control deer used in this study from different geographical areas. The bacterial organisms of concern found in the tissues of a deceased whitetail deer were directly associated with the farm's water supply. In reviewing of the past research relating to this quarantined farm, our current findings have now identified certain bacterial organisms and their capabilities to diminish deer / elk health. These bacteria identified have the capabilities and pathways to generate pore forming toxins. These bacteria use these toxins to hijack a cell's physiology and in this process they create B-barrel structures that when inserted into the cells lipid membranes create B- sheet structures in similarity if not identically the same as a conformational protein change in what we only know about prion creation to date.

Future, by the farm management first identifying, then reducing or eliminating these identified bacterial sources from the farm chain of supplies would provide for a higher level of bio-security. This improved surveillance and actions taken is needed to reduce the bacterial burden loads in deer / elk. Other bio-security health measures should include the consideration of where products brought to the farm including other transferred deer / elk from other geographical locations. By continually developing your farms best management practice through the use of an effective vaccine program should provide for a risk reduction in the exposure to these bacteria with capabilities to produce pore forming toxins. This new information provides the farmer tools for the continuing health management of their deer / elk from potentially developing B-sheet structures from these bacterial toxins that are found to be consistent with that of the hallmark signature of the prion B-sheet structure in the disease we have come to only know as CWD.

Submitted by: Jerome Donohoe,
Agricultural Omega Solutions, LLC
ag_o3@earthlink.net

To review past research reports please visit or contact your Board members supporting our CWD research**.**
WCDEFA wcdefa@gmail.com, NADeFA schafer@nadefa.org , or DBC cati@dbcdeer.com

References:

1. **Immune System Modulations by Products of the Gut Microbiota**
Thierry Chénard, Karine Prévost, Jean Dubé and Eric Massé *, Biochemistry and Functional Genomics Department, University of Sherbrooke, Sherbrooke, QC J1E 4K8, Canada; thierry.chenard@usherbrooke.ca (T.C.); Karine.Prevost@USherbrooke.ca (K.P.); Jean.Dube@USherbrooke.ca (J.D.) * Correspondence: eric.masse@usherbrooke.ca
2. **Alterations in gut microbiota linked to provenance, sex, and chronic wasting disease in White-tailed deer (*Odocoileus virginianus*)**
David Minich, Christopher Madden , Morgan V. Evans, Gregory A. Ballash, Daniel J. Barr, Keith P. Poulsen, Patricia M. Dennis, Vanessa L. Hale , The Ohio State University College of Veterinary Medicine, Columbus, OH
Veterinary Preventive Medicine, The Ohio State University, Columbus, OH, Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin-Madison, WI. , Cleveland Metroparks Zoo, Cleveland, OH
3. **Bacterial protein toxins and lipids: pore formation or toxin entry into cells**
Blandine Geny and Michel R. Popoff1 Unite´ des Bacte´ ries Anae´ robies et Toxines, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris cedex 15, France
4. **Amyloid growth and membrane damage: Current themes and emerging perspectives from theory and experiments on Aβ and hIAPP**
Michele F.M. Sciaccaa, Carmelo Temprab, Federica Scollob, Danilo Milardia, Carmelo La Rosab, a Istituto CNR di Biostrutture e Bioimmagini- Sede Secondaria di Catania, Via Paolo Gaifami 18, 95126 Catania, Italy b Università degli Studi di Catania, Dipartimento di Scienze Chimiche, Viale Andrea Doria 6, 95125 Catania, Italy
5. **A Pore-forming Toxin Interacts with a GPI-anchored Protein and Causes Vacuolation of the Endoplasmic Reticulum**
Laurence Abrami,* Marc Fivaz,* Pierre-Etienne Glauser,* Robert G. Parton, and F. Gisou van der Goot*
*Department of Biochemistry, University of Geneva, 1211 Geneva, Switzerland; Center for Microscopy and Microanalysis, Department of Physiology and Pharmacology, and Center for Molecular and Cellular Biology, University of Queensland, Queensland 4072, Brisbane, Australia
6. **Role of Pore-Forming Toxins in Bacterial Infectious Diseases**
Ferdinand C. O. Los,a Tara M. Randis,a Raffi V. Aroian,b Adam J. Ratnera, Department of Pediatrics, Columbia University, New York, New York, USAa; Division of Biological Sciences, Section of Cell and Developmental Biology, University of California San Diego, La Jolla, California, USA
7. **Pore-forming toxins: ancient, but never really out of fashion**
Matteo Dal Peraro1 and F. Gisou van der Goot2
8. **Are amyloid disease caused by protein aggregates that mimic bacterial pore forming toxins?**
Hilal A. Lashuel1* and Peter T. Lansbury Jr.2*, Laboratory of Molecular Neurobiology and Neuroproteomics, Brain Mind Institute, Ecole Polytechnique Federale de Lausanne (EPFL), CH-1015 Lausanne, Switzerland 2 Harvard Center for Neurodegeneration and Repair, Center for Neurologic Diseases, Brigham and Women's Hospital and Department of Neurology, Harvard Medical School, 65 Landsdowne St, Cambridge, MA 02139, USA
9. **Clostridium butyricum: from beneficial to a new emerging pathogen**
N. Cassir, S. Benamar and B. La Scola Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes (URMITE), UM63 CNRS 7278 IRD 198 INSERM U1095, Facultés de Médecine et de Pharmacie, Aix-Marseille Université, Marseille, France
10. **Genetic Characteristics of Toxigenic Clostridia and Toxin Gene Evolution**
Michel R. Popoff * and Philippe Bouvet Institut Pasteur, Unité des Bactéries anaérobies et Toxines, Paris, France
11. **UK Standards for Microbiology Investigations - Identification of *Clostridium* species**
12. **Clostridial Toxins**
Michel R. Popoff * and Philippe Bouvet Institut Pasteur, Unité des Bactéries anaérobies et Toxines, Paris, France