

Spring 2018

Characterization of isolates and whole samples from turkeys infected with bordetellosis

Alexandra Lovrinic
James Madison University

Follow this and additional works at: <https://commons.lib.jmu.edu/honors201019>

 Part of the [Animal Diseases Commons](#), [Bacterial Infections and Mycoses Commons](#), and the [Respiratory Tract Diseases Commons](#)

Recommended Citation

Lovrinic, Alexandra, "Characterization of isolates and whole samples from turkeys infected with bordetellosis" (2018). *Senior Honors Projects, 2010-current*. 514.
<https://commons.lib.jmu.edu/honors201019/514>

This Thesis is brought to you for free and open access by the Honors College at JMU Scholarly Commons. It has been accepted for inclusion in Senior Honors Projects, 2010-current by an authorized administrator of JMU Scholarly Commons. For more information, please contact dc_admin@jmu.edu.

Characterization of isolates and whole samples from turkeys infected with Bordetellosis

An Honors College Project Presented to
the Faculty of the Undergraduate
College of Integrated Science & Technology and
College of Science & Mathematics
James Madison University

by Alexandra Lovrinic

Accepted by the faculty of the College of Integrated Science & Technology and College of Science & Mathematics, James Madison University, in partial fulfillment of the requirements for the Honors College.

FACULTY COMMITTEE:

HONORS COLLEGE APPROVAL:

Project Advisor: Louise Temple,
Professor, ISAT

Bradley R. Newcomer, Ph.D.,
Dean, Honors College

Reader: Pradeep Vasudevan,
Assistant Professor, Biology

Reader: Kyle Seifert,
Professor, Biology

PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at

Biosymposium on April 13, 2018 .

Table of Contents

List of Figures.....	3
Acknowledgments.....	4
Abstract.....	5
Introduction.....	6
Methods.....	7
Results.....	12
Discussion.....	17
Bibliography.....	20

List of Tables and Figures

Table 1: Identification of isolates spanning >40 years.....	13
Table 2: Identification of turkey isolates (representative sample)	13
Table 3: Characterization of 14 ambiguous isolates.....	14
Figure 1: Replicate plating of whole swabs (representative sample).....	15
Table 4: Results from replicate plating of whole swabs.....	16

Acknowledgements

I would like to thank my advisor, Dr. Louise Temple. Constantly advocating for women in science, she has inspired and pushed me to reach my potential as a young researcher. This research and where I have gotten in my academics could not have been possible without her. I would also like to thank Dr. Kyle Seifert and Dr. Pradeep Vasudevan for all the hard work they put in on my honors committee. Thank you to all the members in the lab that were in collaboration with this research, especially Madison Himelright.

I would like to acknowledge the College of Integrated Science and Technology, the College of Science and Mathematics, and Butterball Turkeys, Inc. for providing the opportunity and funds to conduct research.

Abstract

Bordetellosis is a disease in turkeys attributed to the Gram-negative bacterium, *Bordetella avium*. The closely related species, *B. hinzii*, is known to colonize in turkeys as well but was not thought to cause disease. However, over the past few years, *B. hinzii* has been isolated from turkeys diagnosed with bordetellosis. The presumed identification of isolates obtained from Butterball farms in different locations was conducted through standard identification tests, including hemagglutination, PCR of *B. avium* specific genes, 16s rDNA PCR, carbohydrate utilization tests, and complete genome sequencing of a few strains. Out of ~100 isolates tested, 14 yielded contradictory results. Sequencing of the 14 isolates will be completed to determine if these contradictions were the result of a polymorphism in genes encoding the tested phenotypes or if they represent novel species. The presence of *B. avium* and *B. hinzii* together in turkeys diagnosed with bordetellosis has never been determined. To study this, swabs from turkey tracheas were obtained, and their ability to form colonies was tested through replicate plating on citrate and MacConkey media. A mixture of results from replicate plating, including growth of all colonies on citrate, no colony growth on citrate, and mixed colony growth on citrate, as well as the emergence of contradictory isolates, suggest that *Bordetella* species infecting and causing disease in turkeys are evolving. Despite the use of antibiotics and vaccines, bordetellosis continues to cause financial losses in the poultry industry from death of young turkeys and sickness in adults. Therefore, ongoing research with detailed analysis of these isolates could provide useful information to improve intervention, treatment and prevention.

Introduction

Bordetellosis is a bacterial infection in turkeys that causes upper respiratory disease. *Bordetella avium* is a Gram negative, non-fermentative, motile, aerobic bacterium that causes bordetellosis. *B. avium* attaches and kills ciliated epithelial cells in the turkey trachea, leading to sickness among adult turkeys and death in young turkeys (Hopkins et al., 1990). The incubation period of the infection is seven to ten days, and symptoms include sinusitis, a snick or cough, difficulty breathing, foamy-watery eyes, and altered vocalization. It is a highly contagious disease that is transmitted between domesticated and wild turkeys through shared water sources, air, food, and litter (Raffel, et al., 2002). The negative impact on the turkey industry is significant, leading to several million dollars of loss each year from turkeys infected with bordetellosis (Jackwood and Saif, 2008).

Prior to 2014, limited diagnostic tools were used for identifying the causative agent of bordetellosis. Common in the industry practice, tracheal swabs from sick turkeys were cultured on MacConkey agar, and those colonies with typical morphology were reported as *B. avium* with no further testing. This led researchers to believe that *B. avium* was the sole causative agent of bordetellosis. However, because colony morphology is indistinguishable between *B. avium* and *B. hinzii*, some isolates were misidentified, as shown by previous work in the lab. In 2014, veterinarians at Butterball LLC. noted changes in symptoms associated with bordetellosis and questioned if there was a different causative agent. Several samples were identified by a commercial lab as *B. hinzii*, which was known to colonize in turkey tracheas but never thought to cause disease (Vandamme et al., 1995). To investigate this issue, Butterball veterinarians collected pure cultures for further testing.

Bordetellosis continues to plague the turkey industry, even with a vaccine in use in some parts of the country, causing tremendous financial loss in the industry. The goal of this study was to differentiate isolates as *B. hinzii* or *B. avium* in recent bordetellosis outbreaks. Additionally, this study sought to investigate the likelihood that the turkeys were concomitantly infected with both species. Identifying isolates will provide a better understanding of the emerging presence of *B. hinzii* as a causative agent of bordetellosis in turkeys.

Methods and Materials

Growth of Strains:

Strains used in this study were isolates from infected turkeys and are shown in tables 1, 2, and 3. To obtain individual isolated colonies, bacteria samples were T-streaked using sterile wooden tooth picks on MacConkey agar, and incubated at 37°C for 48 hr. Samples were maintained on MacConkey and or stored at -70C in glycerol.

Bacterial suspensions were made in sterile tubes containing 5mL of phosphate buffer saline (PBS). Using a spectrophotometer set to 600 OD, bacteria samples cultured on a MacConkey agar were added to the tube with a swab until 1.0 OD was reached.

Hemagglutination:

Sheep's blood was mixed by inversion, and centrifuged in a fixed angle rotor floor centrifuge at 2000 RPM for 5 minutes. Once centrifuged, 4-5 mL of the supernatant was removed, and 100µL of the RBC's was diluted in 10 mL of PBS, resulting in a RBC suspension of 1%.

50 μ L of saline was added to each well of a pointed or round-bottom 96-well plate, except columns 1 and 7. 100 μ L of the bacteria samples were added to the first corresponding column, and serial two-fold dilutions were performed using 50 μ L of the RBC suspension (1%) . Contents were gently mixed in wells, covered with parafilm, and incubated at 4 °C for 4 hr.

Hemagglutination was used to test for red blood cell pellet formation. The presence of hemagglutination is the formation of a blood pellet at the bottom of the well. *B. avium* receptors stick to RBC preventing it from forming a blood pellet, having a negative result, while *B. hinzii* receptors do not have this affect leading to a positive result. Wells were considered *B. hinzii*, if there was hemagglutination, or *B. avium*, if there was not.

DNA Extraction (Blood and Tissue Kit, Qiagen):

Microcentrifuge (MC) tubes with 1mL of bacterial suspensions were centrifuged at full 1300rpm for 2 minutes. The supernatant was removed and the bacterial pellet was re-suspended in 180 μ L of Buffer ATL. 20 μ L of Proteinase K was mixed in by vortexing. To lyse the cells, MC tubes were incubated in a water bath at 56°C for 5 minutes. After which, 200 μ L of Buffer AL was added to the sample, incubated in a water bath at 70°C for 10 minutes, and then centrifuged at 1300rpm for 1 minute. 200 μ L of ethanol (100%) was added to the sample and centrifuged at 1300rpm for 1 minute. The mixture was pipetted into a spin column filter, centrifuged at 8000 rpm for 1 minute, and the spin column was then placed in a new collection tube. 500 μ L of Buffer AW1 was added to the spin column, centrifuged at 8000 rpm for 1 minute, and again, the spin column was placed in a new collection tube. 500ul of Buffer AW2 was added and centrifuged at 1300rpm for 3 minutes. The final product transferred to a new MC tube and

stored in the freezer at -20°C (<https://www.qiagen.com/us/shop/sample-technologies/dna/genomic-dna/dneasy-blood-and-tissue-kit/#orderinginformation>).

16S rDNA analysis:

To help with isolate identification, 16S rDNA analysis was done using a standard protocol (Cai et al., 2003). DNA used in 16S PCR was extracted using the methods above discussed in DNA extraction section.

The following was mixed in a 0.2mL PCR tube with a total volume of 25µL; 2.5 µL of isolate DNA template, 2.5 µL of 16s Forward Primer, Bac8f, 2.5 µL 16s Reverse Primer, Univ 1492r, 12.5 µL AmpliTaq Gold master mix, containing *Taq* enzyme, dNTPs, MgCl₂, and buffer, and 5 µL sterile deionized water.

Tubes were run in a thermal cycler at 95°C for 5 minutes, and 35 cycles of 95°C for 1 minute, 50°C for 30 seconds, 72°C for 1.5 minutes. After completion, products were analyzed and sequenced (Elim Biopharmaceuticals).

***B. avium* specific PCR analysis:**

Specific primers, forward primer, N-*avium*, and reverse primer, C-*avium*, were used to amplify a unique *B. avium* gene region of 528pb (Register and Yersin, 2005).

The following was mixed in a 0.2mL PCR tube with a total volume of 25µL; 2.5 µL of isolate DNA template, 2.5 µL of Forward Primer, N-*avium*, 2.5 µL Reverse Primer, C-*avium*, 12.5 µL AmpliTaq Gold master mix, containing *Taq* enzyme, dNTPs, MgCl₂, and buffer, and 5 µL sterile deionized water.

Tubes were run in a thermal cycler 95°C for 5 minutes, and 35 cycles of, 95°C for 1 minute, 52°C for 30 seconds, 70°C for 1 minute. Products were analyzed using gel electrophoresis. Results were positive when there was a 528bp band.

Gel Electrophoresis and DNA Database Comparison:

A 1% agarose gel was made mixing 0.5g agarose and 50mL 1X Tris-acetate-EDTA (TAE) buffer, microwaving for one minute, and adding 1 μ L of 10mg/mL ethidium bromide. The mixture was poured into the gel setup to solidify, and then covered with 1X TAE buffer. Samples were prepared with 5 μ L loading dye and 8 μ L of the PCR sample. The gel was run at 110 volts for 40 minutes. The gel was imaged on a Bio-Rad ChemiDoc MP imager.

16s rDNA amplicons were sequenced by Elim Biopharmaceuticals. Sequences were analyzed using BLASTn and the 16srDNA database in GenBank.

Tracheae Attachment Assay:

The tracheae attachment assay was performed by infecting turkey tracheal rings with varying strains of *B. avium* or *B. hinzii*.

Bacterial samples were suspended in EBSS at an OD of 0.5 and diluted 1/10 to achieve $\sim 2 \times 10^8$ CFU/ml. Those bacterial samples were then serially diluted with 4.5mL PBS, and 100 μ L of each dilution was plated onto MacConkey, and incubated overnight. The number of colonies formed were used to calculate the inoculum.

Turkey tracheas were then dissected from turkeys 2-3 days before hatching, and cut into 2mm rings. Trachea rings were washed with EBSS 3 times and placed into 1mL of the bacterial sample and incubated at 42 °C for 1 hr on a rocker°. After incubation, rings were individually

placed in a tube with 1 ml PBS, 1% triton and held at 4 °C for at least 1 hr. After vortexing, the bacteria released from the rings were diluted 10 fold. Then, 100µL of appropriate dilutions were plated onto MacConkey and incubated at 37°C for 1-2 days. After incubation, the number of colonies formed were used to calculate the number of bacteria attached. The percent adherence was calculated from the number of colonies was used as a measure of the rate of infection.

Simmon's Citrate and Malonate Slants:

Isolates from the MacConkey agar were inoculated onto the media and incubated at 37°C for 24 hours. A positive result consisted of a pH change (media changed from green to blue) or bacterial growth. A negative result was consistent with no growth. *B. hinzii* has positive results for both testing, including a pH change. *B. avium* has a negative result for malonate, and is thought to grow on citrate but not cause a pH change. Results were labeled *B. hinzii* for a positive result or *B. avium* for a negative result in malonate and lack of pH change in citrate.

Growth and Testing of Trachea Swabs:

Swabs were taken from sick turkey tracheas. In order to get individual colonies, 50µL of bacterial sample was diluted with 450µL dH₂O to the 10⁻⁵, 100 µL of each dilution was then plated on MacConkey, and incubated at 37 °C for 16 hr. MacConkey plates were then replicate plated using velvets onto a citrate plate, and then onto another MacConkey plate to serve as a control. Colonies that did not grow on citrate were confirmed by picking the corresponding colonies from the original MacConkey plate and inoculating on citrate and again on MacConkey.

Results

Identification of Isolates:

Isolates previously tested before 2004 were re-tested to confirm identity. Out of 21 isolates collected before 2004, 18 were identified as *B. avium*, and 3 were identified as *B. hinzii* (Table 1). After re-testing of those isolates, it was found that 13 were *B. avium*, and 8 were *B. hinzii* (Table 1), thus 5 isolates were wrongly identified as *B. avium*. From 2014-2015, out of 36 isolates, 6 were confirmed *B. avium*, and 30 were confirmed *B. hinzii* (Table 1).

The most accurate and reliable characterization tests, hemagglutinin, *B. avium* specific PCR, biochemical testing, and chromosomal sequencing, were used to confirm the identity of isolates. Isolates were confirmed as *B. avium* or *B. hinzii* when 3 of the tests matched (Table 2). 24 were identified as *B. avium*, 40 identified as *B. hinzii*, and 14 were considered ambiguous (Table 2).

Table 1. Selection of isolates spanning >40 years. Older isolates were re-tested using several methods as described showing 5 that were originally mis-identified. A number of recent isolates were definitively identified, with about 80% of new isolates as *B. hinzii*.

Year of Isolation	Location	#	Initial ID	New ID
Prior to 1980	NC, GA	9	<i>B. avium</i>	<i>B. avium</i>
		3	<i>B. avium</i>	<i>B. hinzii</i>
		3	<i>B. hinzii</i>	<i>B. hinzii</i>
1980-2004	NC, VA, NJ	4	<i>B. avium</i>	<i>B. avium</i>
		2	<i>B. avium</i>	<i>B. hinzii</i>
2014-2015	NC, MO	6	NA	<i>B. avium</i>
		30	NA	<i>B. hinzii</i>

Table 2. Identification of Turkey Isolates (representative samples). Hemagglutination, species specific PCR (Register, 2005), carbohydrate utilization tests, and chromosome sequencing were done by standard published methods. Chromosome sequencing was done at CDC. Identification of isolates was considered definitive when three tests matched the expected and contradictory results. ND= not determined.

HA	BAV PCR	Biochemical Test	Chromosome Sequenced	Presumptive ID	Case Number	Year
Positive	Positive	Negavtive	ND	<i>B. avium</i>	14-824	2014
Positive	Positive	Negavtive	Yes	<i>B. avium</i>	14-4061	2014
Positive	Positive	Negavtive	Yes	<i>B. avium</i>	Ba 002	1980
Negative	Negative	Positive	ND	<i>B. hinzii</i>	14-4002	2014
Negative	Negative	Positive	Yes	<i>B. hinzii</i>	14-3425	2014
Negative	Negative	Positive	Yes	<i>B. hinzii</i>	SV-2	2006
Positive	Negative	Positive	ND	Results Contradictory	14-4467	2014
Positive	Negative	Negative	ND	Results Contradictory	Ba 4141	2014
Negative	Positive	Negative	ND	Results Contradictory	14-C0319 Cook	2014
Positive	Negative	Positive	ND	Results Contradictory	14-4410	1980

Characterization of Ambiguous Isolates:

Isolates were considered ambiguous if more than one of the identification tests did not match (Table 2), where fourteen strains could not be definitively identified (Table 3). These individual isolates had characteristics indicative for both *B. avium* and *B. hinzii* depending on which identification test was used. Since isolates showed positive characteristics for both species, they could not be confirmed as either *B. avium* or *B. hinzii*, and they were deemed ambiguous. There were no clear patterns between isolates to explain contradictory results.

Table 3. Characterization of the 14 isolates with ambiguous isolates. Isolates were considered contradictory if one or more tests did not coincide with each other. Results indicating *B. avium* and *B. hinzii* are listed for reference. ND= not determined.

Case Number	Hemagglutination	BAV PCR	Biochemical Test	Year
<i>B. avium</i> Expected	Positive	Positive	Negative	
<i>B. hinzii</i> Expected	Negative	Negative	Positive	
14-4407	ND	Positive	ND	2014-15
14-4410	Negative	Negative	Positive	2014-15
14-4467	Positive	Negative	Positive	2014-15

14-C0319 Cook	Negative	Positive	Negative	2014-15
1419078 TR	Negative	Positive	Positive	2014-15
1422161 TR A	Positive	Negative	Positive	2014-15
15-1089	Positive	Negative	Negative	2014-15
15-1091	Negative	Negative	Positive	2014-15
3394 TR	Negative	Positive	Positive	2014-15
3396 TR	Negative	Positive	Positive	2014-15
Ba 4143	Positive	Negative	Negative	1980
15-1088	Negative	Negative	Negative	2014-15
15-354	Neg then Pos	ND	Negative	2014-15
15-524	Negative	Negative	Positive	2014-15

Swab Replicate Plating:

In order to investigate whether both *B. avium* and *B. hinzii* might infect a single turkey, swabs from turkey tracheas were obtained from Butterball in North Carolina. This was different from the usual protocol where isolates from single colonies were grown on MacConkey agar. Bacteria in these potentially mixed cultures were analyzed based on colony growth causing a pH change on citrate and compared to growth on MacConkey agar (Figure 1).

Of the 12 samples, 2 samples resulted in colonies that were all positive (Table 4), indicating that all the colonies that grew on MacConkey, also grew and caused a pH change on citrate (Figure 1C). Six samples resulted in colonies that were all negative (Table 4), meaning that none of the colonies that grew on MacConkey had clearly visible growth or a pH change on citrate (Figure 1B). Four samples resulted in a mix culture, colonies that were both negative and positive (Table 4), meaning that some colonies that grew on MacConkey cause a pH change while others did not (Figure 1A). These results indicate that the causative agent for bordetellosis may involve both *B. avium* and *B. hinzii*.

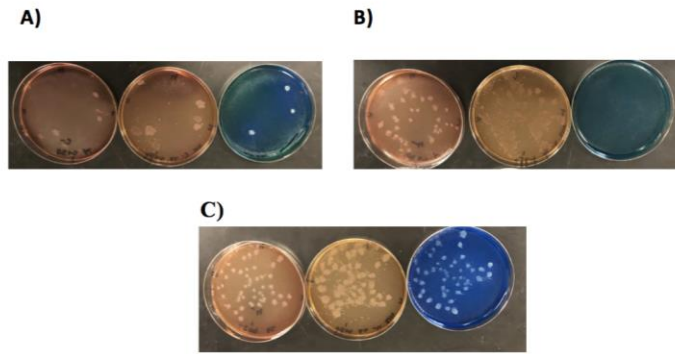


Figure 1. Tracheal samples were diluted and plated on MacConkey agar, then replicate plated to citrate and MacConkey. An all positive result is represented in panel A, an all negative result is represented in panel B, and a mixture of positive and negative result in panel C.

Table 4. Number of colonies that grew from replicate plating of swabs from tracheas of sick turkeys. Samples consisted of colonies that were either all negative, all positive, or a mix of both positive and negative. Positive results were indicative of a pH change (blue color).

Strain	Citrate	MacConkey
180-0309	52	52
18-0560	40	40
18-0299	0	35
18-0825	0	83
18-0855	0	132
18-0520	0	4
18-0527	0	32
18-0312	0	72
18-0594	9	296
18-0493	1	54
18-0522	3	7
18-0523	29	49

Tracheal Attachment Assay:

The rate of attachment from the trachea assays from strains of *B. avium* and *B. hinzii* were compared. It was found that the two species attach at the same rate. *B. avium* and *B. hinzii* had strains had an average attachment rate of 2-10%.

Discussion

In this study, isolates from sick turkeys were identified through extensive testing, and swabs were obtained to look at the different species that inhabit individual turkeys. Although, some isolates were mis-identified in the past, the majority of isolates prior to 2014 appear to be *B. avium*. However, starting in 2014, more tests indicated that *B. hinzii* is cultured from sick turkeys in the majority of cases. In order to confirm the identification of each isolate, the characterization tests used for comparison were chosen based on reliability and repeatability. The identification test used were hemagglutination, *B. avium* specific PCR, biochemical testing (malonate and citrate utilization), and chromosomal sequencing. 16S PCR was not used as a comparative tool when identifying isolates due to the similarities in sequences between *B. avium* and *B. hinzii* they are too similar in sequence to be useful. A dramatic increase in the amount of *B. hinzii* present in sick turkeys since 2014 was indicated by thirty-six samples identified as *B. hinzii*, showing. Fifteen of the isolates had conflicting test results. No clear pattern between the ambiguous isolates was identified to further explain why testing was contradictory. Both the increase of *B. hinzii* present and the findings of ambiguous isolates presents unforeseen issues towards combatting bordetellosis in turkeys.

Using tracheal swabs rather than single colonies was a starting point to begin investigating the hypotheses mentioned above. The purpose of this testing was to reveal if *B. avium* and *B. hinzii* are present in the same sick turkey. The collection of samples did not give a definitive answer, but rather included every possible combination, which consisted of all positive colonies, all negative colonies, and mixed colonies containing both positive and negative colonies. It is thought that *B. avium* can grow on citrate but does not cause a change in pH. Colonies that grew on citrate but did not cause a pH change were considered a possible indication of *B. avium*. However, most of the colonies that did not cause a pH change, had little to no growth on citrate, but had morphological features of bordetella. While this could be an indicator of *B. avium* since growth is not prominent due to lack of pH change, it was also possible that this was a new species of bordetella that does not grow on citrate. Even though this data did not directly show if *B. avium* and *B. hinzii* colonize together or not, the mixture of results indicates that the cause of infection is more complex than previously thought. Future work should investigate the metabolic pathways of *B. avium* in citrate to understand the genetic basis of malonate and citrate utilization in bordetella. Work with whole swabs from sick turkeys should continue to be done to clarify any patterns with attachment.

Several hypotheses can be put forward from this data that should be thoroughly examined in the future. The first hypothesis is that *B. hinzii* has gained the ability to infect and cause sickness in turkeys, suggesting that, *B. hinzii* and *B. avium* can cause sickness independently of one another. This would mean that a vaccine targeting both species of bordetella would need to be created. The trachea assay results indicate that, at least in vivo, *B. hinzii* can colonize the trachea in a rate similar to *B. avium*.

Another possibility is that *B. hinzii* does not infect independently, but aids *B. avium* in attachment through the use of its biofilms. *B. hinzii* produces much greater biofilms than *B. avium*. *B. hinzii* and *B. avium* could have co-evolved to work together to infect turkeys. This possibility has not been tested until this study, where tracheal swabs were tested for mixed cultures of the two species.

It is also thought that this could be a new strain of bordetella that closely resembles *B. avium* and *B. hinzii*. Recently, a new species of bordetella, *B. pseudohinzii*, was isolated from sick mice (Ivanov et al, 2003), however, no studies have been done to determine whether this species infects birds. As indicated by the presence of isolates that could not be clearly identified, these isolates could in fact be a different, related species.

Genome analysis has been done comparing *B. avium*, *B. hinzii* and *B. pseudohinzii*. Several ambiguous strains reported here have recently been sequenced. To determine if they resemble known species or are completely different from anything seen before, comparison of these genomes to known *B. avium*, *B. hinzii* and *B. pseudohinzii* will be conducted.

Continuation of this work is crucial to figuring out the causative agent to bordetellosis. Without proper identification of the bordetella strain(s) involved, an effective treatment is not possible, and the turkey industry will continue to suffer financial losses.

Bibliography

- Animal Science Database. (n.d.). *Bordetella hinzii* Can Cause Disease in Turkeys. Retrieved from <http://www.cabi.org/animalscience/news/19628>
- Cai, H., Archambault, M., & Prescott, J. F. (2003). 16S ribosomal RNA sequence – based identification of veterinary clinical bacteria. *Journal of the American Veterinary Medical Association (JAVMA)*, 9, 465–469.
- Hopkins, B. a, Skeeles, J. K., Houghten, G. E., Slagle, D., & Gardner, K. (1990). A survey of infectious diseases in wild turkeys (*Meleagris gallopavo silvestris*) from Arkansas. *Journal of Wildlife Diseases*, 26(4), 468–472. <https://doi.org/10.7589/0090-3558-26.4.468>
- Ivanov, Y. V., Linz, B., Register, K. B., Newman, J. D., Taylor, D. L., Boschert, K. R., ... Harvill, E. T. (2016). Identification and taxonomic characterization of *Bordetella pseudohinzii* sp. nov. isolated from laboratory-raised mice. *International Journal of Systematic and Evolutionary Microbiology*, 66(12), 5452–5459. <https://doi.org/10.1099/ijsem.0.001540>
- Jackwood, M.W., Saif, Y. M. (2013). Bordetellosis (turkey coryza). *Diseases of poultry* (13th ed., pp. 705-715)
- Kerstens, K., Hinz, K., Hertle, A., Segers, P., Lievens, A., & Ley, J. D. E. (1984). *Bordetella avium* sp . nov ., Isolated from the Respiratory Tracts of Turkeys and Other Birds. *International Journal Of Systematic Bacteriology*, (64), 56–70. <https://doi.org/10.1099/00207713-34-1-56>
- McLaughlin, K., Folorunso, A. O., Deeni, Y. Y., Foster, D., Gorbatiuk, O., Hapca, S. M., ... Spiers, A. J. (2017). Biofilm formation and cellulose expression by *Bordetella avium* 197N, the causative agent of bordetellosis in birds and an opportunistic respiratory pathogen in humans. *Research in Microbiology*, 168(5), 419–430. <https://doi.org/10.1016/j.resmic.2017.01.002>
- Miyamoto, D. M., Ruff, K., Beach, N. M., Stockwell, S. B., Dorsey-Oresto, A., Masters, I., & Temple, L. M. (2011). *Bordetella avium* causes induction of apoptosis and nitric oxide synthase in turkey tracheal explant cultures. *Microbes and Infection*, 13(10), 871–879. <https://doi.org/10.1016/j.micinf.2011.04.011>
- Raffel, T. R., Register, K. B., Marks, S. A., & Temple, L. (2002). Prevalence of *Bordetella avium* infection in Selected Wild and Domesticated Birds in the Eastern Usa. *Journal of Wildlife Diseases*, 38(1), 40–46. <https://doi.org/10.7589/0090-3558-38.1.40>
- Register, K. B., Kunkle, R. A., Register AC, K. B., & Kunkle B, R. A. (n.d.). Strain-Specific Virulence of *Bordetella hinzii* in Poultry. <https://doi.org/10.1637/8388-070108-Reg.1>

- Register, K. B., & Yersin, A. G. (2005). Analytical verification of a PCR assay for identification of *Bordetella avium*. *Journal of Clinical Microbiology*.
<https://doi.org/10.1128/JCM.43.11.5567-5573.2005>
- Spears, P. A., Temple, L. M., Miyamoto, D. M., Maskell, D. J., & Orndorff, P. E. (2003). Unexpected similarities between *Bordetella avium* and other pathogenic bordetellae. *Infection and Immunity*, 71(5), 2591–2597. <https://doi.org/10.1128/IAI.71.5.2591-2597.2003>
- Temple, L. M., Miyamoto, D. M., Mehta, M., Capitini, C. M., Von Stetina, S., Barnes, H. J., ... Orndorff, P. E. (2010). Identification and characterization of two *Bordetella avium* gene products required for hemagglutination. *Infection and Immunity*, 78(6), 2370–2376. <https://doi.org/10.1128/IAI.00140-10>
- Temple, L. M., Weiss, A. A., Walker, K. E., Barnes, H. J., Christensen, V. L., Miyamoto, D. M., ... Orndorff, P. E. (1998). *Bordetella avium* virulence measured in vivo and in vitro. *Infection and Immunity*, 66(11), 5244–5251.
- Vandamme, P., Hommez, J., Vancanneyt, M., Monsieurs, M., Hoste, B., Cookson, B., ... Blackall, P. J. (1995). *Bordetella hinzii* sp. nov., isolated from poultry and humans. *International Journal of Systematic Bacteriology*, 45(1), 37–45. <https://doi.org/10.1099/00207713-45-1-37>