Prevalence of *Brachyspira hyodysenteriae* in sows and suckling piglets

Joshua W. Duff, BS; Jeremy S. Pittman, DVM, Diplomate ABVP; J. Mark Hammer, DVM, MS; Joann M. Kinyon, MS

Summary

Objective: To estimate the prevalence of *Brachyspira hyodysenteriae* (B hyo) in breeding animals, lactating sows, and their suckling offspring in swine dysentery- (SD-) positive herds.

Materials and methods: Study 1: lactating sows and suckling piglets. Rectal swabs were collected eight times at 1- to 4-week intervals from an SD-positive breed-to-wean farm. At each sampling, rectal swabs were collected from 60 "sets" of animals (individual swabs from a sow and three suckling piglets). Piglet samples were tested as a litter. Samples were tested by *Brachyspira* species culture and confirmed by culture-based polymerase chain reaction (PCR). Study 2: breeding herds. Five SD-positive sow farms, varying in size, were selected for evaluation of breeding-herd prevalence of B hyo. Rectal swabs were collected once per farm from 150 randomly selected sows. Samples were tested by *Brachyspira* species culture and confirmed by culture-based PCR.

Results: Study 1: lactating sows and suckling piglets. The percentage of sows on a farm that were positive for B hyo ranged from 0% to 5%, with an overall prevalence of 1.04%. The percentage of litters culture-positive and PCR-positive for B hyo ranged from 0% to 5%, with an overall prevalence of 1.88%. Study 2: breeding herds. The percentage of sows positive for B hyo ranged from 0% to 1.33%. Only three of the five farms tested positive.

Implications: Sampling breeding herds and suckling-age piglets could serve as a valuable alternative to traditional surveillance schemes. Understanding the prevalence of SD on endemically infected sow farms could enhance current surveillance programs.

Keywords: swine, *Brachyspira hyodysenteriae*, sows, piglets, prevalence

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Resumen - Prevalencia del *Brachyspira hyodysenteriae* en hembras y lechones lactantes

Objetivo: Estimar la prevalencia de la *Brachyspira hyodysenteriae* (B hyo por sus siglas en inglés) en animales de cría, hembras lactantes, y sus crías en lactancia en hatos positivos a la disentería porcina (SD por sus siglas en inglés).

Materiales y métodos: Estudio 1: hembras lactantes y lechones en lactancia. Se recolectaron hisopos rectales ocho veces a intervalos de 1 a 4 semanas en una granja de cría a destete, positiva al SD. En cada muestreo, se colectaron hisopos rectales de 60 "grupos" de animales (hisopos individuales de una hembra y tres lechones lactantes). Las muestras de los lechones se analizaron como una camada. Las muestras se analizaron por medio del cultivo de especies de *Brachyspira* y se confirmaron por medio de la reacción en cadena de la polimerasa (PCR por sus siglas en inglés) basada en cultivo. Estudio 2: hatos de cría. Se seleccionaron cinco granjas de hembras de diversos tamaños, positivas a la SD para evaluar la prevalencia de B hyo en el hato de cría. Se recolectaron hisopos rectales, una vez por granja, de 150 hembras seleccionadas al azar. Se analizaron las muestras por medio del cultivo de especies de *Brachyspira* y se confirmaron por PCR basado en cultivo.

Resultados: Estudio 1: hembras lactando y lechones lactando. El porcentaje de hembras en una granja positivas a B hyo varió en un rango de 0% a 5%, con una prevalencia total de 1.04%. El porcentaje de camadas positivas al cultivo y positivas a B hyo por medio de PCR basado en cultivo varió en un rango de 0% a 5%, con una prevalencia total 1.88%. Estudio 2: hatos de cría. El porcentaje de hembras positivas a B hyo varió de 0% a 1.33%. Sólo tres de las cinco granjas resultaron positivas.

Implicaciones: El muestreo de los hatos de cría y lechones en edad de lactancia podría ser una valiosa alternativa frente a las estrategias de vigilancia tradicionales. El entendimiento de la prevalencia de la SD en granjas de hembras infectadas endémicamente podría mejorar los programas de vigilancia actuales.

Résumé - Prévalence de *Brachyspira hyodysenteriae* chez des truies et des porcelets à la mamelle

Objectif: Estimer la prévalence de *Brachyspira hyodysenteriae* (B hyo) chez des animaux reproducteurs, des truies en lactation, et des porcelets à la mamelle dans des troupeaux positifs pour la dysenterie porcine (DP).

Matériels et méthodes: Étude 1: Truies en lactation et porcelets à la mamelle. Des écouvillons rectaux ont été prélevés huit fois à des intervalles de 1 à 4 semaines dans une

JWD: North Carolina State University, College of Veterinary Medicine, Raleigh, North Carolina.

JSP: Murphy-Brown, LLC – North Division, Waverly, Virginia.

JMH: Novartis Animal Health US, Inc, Greensboro, North Carolina.

JMK: Iowa State University, Veterinary Diagnostic Laboratory, Ames, Iowa.

Corresponding author: Dr Jeremy Pittman, 434 E Main St, Waverly, VA 23890; Tel: 804-834-1220; Fax: 804-834-8926; E-mail: jeremypittman@murphybrownllc.com.

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ferme de type maternité-naisseur positive pour DP. À chaque échantillonnage, des écouvillons rectaux ont été prélevés de 60 sets d'animaux (un écouvillon individuel d'une truie et trois porcelets à la mamelle). Les échantillons de porcelets ont été testés comme une portée. Les échantillons ont été testés par culture pour les espèces du genre *Brachyspira* et confirmation par réaction d'amplification en chaîne par la polymérase (PCR) sur culture. Étude 2: Troupeaux de reproducteurs. Cinq fermes positives pour DP, variables en taille, ont été sélectionnées pour évaluer la prévalence de B hyo dans les troupeaux reproducteurs. Des écouvillons rectaux ont été prélevés une fois par ferme à partir de 150 truies sélectionnées de manière aléatoire. Les échantillons ont été testés pour les espèces du genre *Brachyspira* et confirmés par PCR sur les cultures.

Résultats: Étude 1: Truies en lactation et porcelets à la mamelle. La proportion de truies sur une ferme qui étaient positives pour B hyo variait de 0% à 5%, avec une prévalence globale de 1,04%. Le pourcentage de portées positive par culture et positive par PCR pour B hyo variait de 0% à 5%, avec une prévalence globale de 1,88%. Étude 2: Troupeaux de reproducteurs. Le pourcentage de truies positives pour B hyo variait de 0% à 1,33%. Seulement trois des cinq fermes étaient positives.

Implications: La prise d'échantillons dans les troupeaux de reproducteurs et chez les porcelets non-sevrés pourrait être une alternative valable aux schémas traditionnels de surveillance. Une connaissance de la prévalence de DP dans les fermes de truies infectées de manière endémique pourrait augmenter les programmes de surveillance actuels.

Swine dysentery (SD), caused by *Brachyspira hyodysenteriae* (B hyo), has worldwide distribution and results in increased production expenses by decreasing feed efficiency, reducing growth rate, increasing mortality, and increasing medication costs.[1] For several decades, SD reached a very low, almost non-existent prevalence in North America; however, in recent years there has been a re-emergence of SD, and a clinically indistinguishable mucohemorrhagic colitis, caused by provisionally named *Brachyspira hampsonii*, in the United States and Canada.[2-4] Historically, SD has been characterized by typhlocolitis and mucohemorrhagic diarrhea, but in modern swine-production systems, clinical signs vary and depend on cofactors such as diet composition, co-infections, immune status, and treatment protocols.[1,5] Currently, most of the epidemiological work on SD has been in grow-finish pigs, while, in contrast, the epidemiology of B hyo in large breeding herds has not been studied extensively. Endemically infected breeding herds are often asymptomatic, in contrast to herds suffering an acute outbreak.[1,5-7] In endemically affected herds, a small percentage of "carrier" sows can transmit B hyo to their piglets during lactation, which allows for maintenance and transmission of disease in groups of weaned or commingled pigs.[8-10] Many commercial breeding herds in modern swine-production systems in North America are large breed-to-wean facilities where pigs are weaned to off-site locations at 3 to 4 weeks of age or less. Separating the susceptible grow-finish animals from the breeding herd often impedes the diagnosis of SD at herd level, since clinical disease is more prominent in locations housing large numbers of immature growing pigs. While SD status of grow-finish pigs is a good predictor of source breeding-herd status,[11] it is not definitive for multi-site production, as infection of grow-finish animals can occur from postweaning facilities (eg, pigs, barns, manure, rodents), contamination via transport units, or lateral introductions (eg, boots, equipment, rodent migration). For the modern integrated swine-production systems, it is imperative that the SD status of breeding herds be known before control or elimination efforts are undertaken. Therefore, a better understanding of SD epidemiology is needed to determine the true status of breeding herds with confidence.

Current breeding-herd SD surveillance programs involve timed and controlled exposure to manure from inventoried breeding females (commonly called "feedback"), clinical evaluation for a period after exposure, and diagnosis at the onset of clinical diarrhea in "sentinel" animals (ie, naive replacement gilts). The goal of the program is to "create a synchronized acute infection in the sentinels to increase the effectiveness of diagnostic testing."[5] Thus, these surveillance protocols are highly dependent on within-herd shedding prevalence of B hyo and the concentration of organisms in manure. In addition, compliance to the program by farm staff, medications being used at the time of exposure, the severity of resultant clinical disease, immune status of replacement breeding stock, sample size and diagnostic test sensitivity, and frequency of sentinel deliveries to the breeding herd can influence successful programs. Diagnosing SD in a sow herd remains difficult, with sows often developing immunity in endemically infected herds.[7,12] Isolation of B hyo from naturally infected breeding stock and suckling-age piglets has not been reported often, and when reported, authors demonstrated variable success.[6-8,13,14]

Songer[8] initially described the isolation of B hyo from asymptomatic adult sows and suckling piglets. Two known positive herds located in Iowa were studied. In Herd A, one of 86 sows sampled (1.2%) and seven of 190 suckling pigs less than 2 weeks of age (3.7%) were positive for B hyo by culture. In Herd B, none of the 42 sows and 76 suckling piglets sampled were positive by culture; however, B hyo was diagnosed in growing pigs. It is important to note that the seven positive suckling pigs in Herd A were asymptomatic and were from a litter of nine piglets from the one positive asymptomatic sow. This finding demonstrated the concept of "carrier sows" and the important epidemiological fact that a small number of carrier animals (sows or piglets) can transmit B hyo to uninfected animals and maintain the infection within herds or recipient herds. Songer[8] commented that this finding may indicate that the "stress of farrowing may be a factor in shedding by carrier animals," but this has not been further reported in the literature.

Windsor and Simmons[15] supported the asymptomatic-carrier theory when they investigated an outbreak of SD in 25 herds in East Anglia and indicated that there was strong evidence in 23 of the herds that the disease had entered in asymptomatic purchased pigs.

Høgh and Knox,[13] using an indirect fluorescent antibody technique (IFAT), demonstrated B hyo from 12 of 543 sows (2.2%) and 136 of 680 weaned pigs (20.0%) from 26 non-clinical Danish herds. The herd or within-herd prevalence, herd size, sow parity, stage of reproductive cycle, or age of the weaned pigs was not reported.

van Leengoed et al[6] characterized the outbreak of SD in a 170-sow breeding herd in which it was suspected that asymptomatic carrier replacement gilts had infected the herd. Clinically significant signs lasted for 3 months. Sows were sampled approximately

10 weeks after a pulse of arsanilic acid in feed (400 g per tonne for 9 days), and 16 of 64 asymptomatic sows (25.0%) were subsequently found positive. In addition, 11 months after the initial infection, B hyo was cultured from three asymptomatic weaned pigs, but the authors did not report the age or total number tested.

Jakubowski et al[14] studied one closed breeding herd in Poland over a 2-year period. Of 317 asymptomatic sows sampled at 14 days prior to farrowing, 29 (9.2%) were positive for B hyo by culture. The authors also found 76 of 481 litters (15.8%) positive for B hyo. After treatment of sows prior to farrowing with olaquindox or ronidazol, 0% of the sows and only one of 317 litters (0.3%) were positive for B hyo, giving support to the idea that effective timing of medication can limit transmission. This finding supports that transmission of B hyo from sow to litter occurs; however, environmental transmission cannot be fully excluded.

Mirko and Bilkei[11] evaluated risk factors for B hyo herd infection in 139 breed-to-finish units in Eastern Europe ranging in size from 101 to 289 sows, with separate breeding and grow-finish facilities. The median number of sow samples positive was five, with a range of zero to nine. Of the 139 farms, 51 (36.7%) were considered positive (at least three positive samples), while in 39 herds (28.1%), test results were inconclusive (one or two positive samples). This study presented a strong association between the B hyo status of the breeding herd and that of the grow-finish herd, further supporting vertical transmission of the organism.

Fellström et al[7] used culture and PCR techniques to evaluate five herds in Sweden that varied in type of production, clinical signs, and SD history. *Brachyspira hyodysenteriae* was diagnosed in three of the five herds (Herds 1, 2, and 4). Only Herd 1 demonstrated B hyo in adults (three of 50; 6.0%) or suckling piglets (four of six; 66.7%), and was also the only herd that demonstrated clinical signs during the study period. In Herd 2, B hyo was diagnosed in on-site 13- to 16-week-old growing gilts, destined for recipient sow herds, on three occasions at 3, 8, and 12 months after the initial study sampling. In Herd 4, only two of 26 weaned pigs (7.7%), 6 to 12 weeks of age, were identified as positive. Herds 3 and 5 never demonstrated a positive B hyo sample.

In the above reviewed cases, it is important to note that many herds had growing pigs on-site, and the largest herd sampled was 289 sows. To the authors' knowledge, no published work has described the prevalence of B hyo in large breed-to-wean herds (eg, > 1000 sows), where disease dynamics and associated management factors are very likely to be different and influential.

A better understanding of within-herd prevalence in breeding animals, lactating females, and suckling piglets in large herds would provide guidelines for appropriate methods of surveillance testing to determine true status with a higher level of confidence. Therefore, a series of cross-sectional studies were undertaken to estimate the within-herd prevalence of B hyo in breeding sows, lactating sows, and 3-week-old suckling piglets on six B hyo-positive breed-to-wean herds. Rectal swabs were collected from selected animals and subsequently tested by *Brachyspira* species culture and confirmed by culture-based PCR.

Materials and methods

Study 1: Lactating sows and suckling piglets

Study 1 was conducted on a 2200-sow breed-to-wean North Carolina breeding farm (Farm A) with an on-site gilt development unit. The sows and pigs utilized in this study were cared for under Pork Quality Assurance Plus (PQA Plus) guidelines (`http://www.pork.org/Certification/2341/pqaPlusMaterials.aspx`). Every 16 weeks, the gilt development unit received replacement breeding stock varying in age from 10 to 24 weeks. Piglets were weaned at approximately 3 weeks of age to an off-site nursery facility. At the time of the study, the farm was being actively depopulated for SD and porcine reproductive and respiratory syndrome virus (PRRSV) elimination, with emphasis placed on rodent control and sanitation. Approximately 1 year prior to the study, in August 2010, six fecal swabs had been collected from replacement gilts in the gilt development unit as part of a sentinel surveillance program.[5] One of six fecal swabs (16.67%) was both culture-positive and culture-based PCR-positive for B hyo at the Iowa State University Veterinary Diagnostic Laboratory, Ames, Iowa (ISU-VDL). A second sampling from the same group of gilts approximately 14 days later again isolated B hyo. Growing pigs originating from this breeding herd also experienced clinical disease, with a confirmed diagnosis of SD in off-site grow-finish units.

At each sampling, 60 lactating sows within a week prior to weaning were randomly selected from the current herd inventory using the random number generator function in Microsoft Excel (Microsoft Corporation, Redmond, Washington). Selected sows, ranging from gilts to 7th parity sows, represented the herd parity distribution at the time of sampling. Sows and gilts received no medications through the water or feed. A convenience sample of three piglets was selected from each litter. Rectal swabs were collected from the lactating sow and three piglets from her litter. Farm protocol allowed for cross-fostering of piglets within 24 hours of birth; therefore, dam origin of each piglet selected was not known, but it was assumed that risk of exposure occurred from the sow from which the piglet predominantly suckled. Rectal swabs were collected eight times over 18 weeks, at weeks 1, 5, 10, 12, 13, 14, 17, and 18 when visits could be scheduled by the authors. At each sampling, rectal swabs were collected from 60 "sets" of animals (based on 95% confidence in detecting at least one positive sample at a disease prevalence of 5%). The sample size for Study 1 was derived by considering low prevalence detection as well as the laboratory and economic constraints at the time of the study.

Study 2: Breeding herds

Study 2 was conducted on five breed-to-wean North Carolina farms (Farms B through F) ranging in size from 2400 to 3600 sows. Each farm had been confirmed positive for B hyo within the previous 12 months by fecal-swab culture and culture-based PCR in replacement gilts using the previously mentioned sentinel program.[5] In addition, growing pigs originating from each of the five breeding herds had expressed clinical SD, with confirmatory diagnosis of B hyo in off-site grow-finish units. Each of the five farms included in this study had an on-site gilt development unit. Sows were randomly selected from the current herd inventory using the random number function in Microsoft Excel (Microsoft Corporation), representing all parities and stages of production (breeding, gestating, lactating) on the farm. Selected sows represented the herd parity distribution. Replacement breeding animals that were on-farm for less than 5 weeks were excluded from the sampling because not all breeding herds have a separate gilt development unit, and the goal of the study was to assess the breeding herd proper. Sows and gilts received no medications through the water or feed. Individual rectal swabs were collected from 150 individual sows (95% confidence in detecting at least one positive sample at a disease prevalence of 2%) at a single point in time at each farm. The Study 2 sample size was based on the Study 1 results, while also accounting for laboratory and economic constraints at the time of the study.

Sampling collection and culture methods

For all studies, a single individual rectal swab (BBL CultureSwab with liquid Stuart medium; Becton, Dickinson and Company, Sparks, Maryland) was collected from each animal. Swabs were sent on ice within 48 hours of collection to the ISU-VDL for *Brachyspira* species culture. Culture was conducted on both colistin-vancomycin-spectinomycin (CVS)[16] and spectinomycin-colistin-vancomycin-spiramycin-rifampicin (BJ)[17] blood agar plates for isolation of *Brachyspira* species. A sample was determined to be culture-positive if *Brachyspira* species growth occurred on either CVS or BJ blood agar plates. The routine use of both media types for *Brachyspira* species isolation balances the more selective properties of the BJ media with the less restrictive properties of the CVS media.[17,18] Results were reported globally as either culture-positive or culture-negative, with no differentiation based on the type of media. For sow samples, half of a culture plate was utilized per sample. In Study 1, piglet swabs were individually streaked on the top, middle, or bottom of the blood agar plates, with results reported on a per-litter basis. A litter was considered positive if at least one piglet from the litter was positive. No distinction was made in the results if more than one piglet from the litter was positive. Plates were incubated anaerobically at 42°C for 6 days. Any strongly beta-hemolytic culture-positive samples were confirmed by PCR for B hyo.[19,20] Isolates in Study 1 that were weakly beta-hemolytic on culture and untypeable by PCR were speciated using 16s ribosomal sequencing. Weakly beta-hemolytic isolates in Study 2 were not further characterized.

Prevalence estimation

To estimate true prevalence, a 95% confidence interval of the group prevalence (Study 1) or farm prevalence (Study 2) was calculated using two different approaches. A frequentist method of prevalence estimation was calculated using AusVet Epi Tools "Estimated true prevalence using an imperfect test" on-line calculator (`http://epitools.ausvet.com.au/content.php?page=TruePrevalence`) based on work by Rogan and Gladen.[21] Sample sizes of 60 (Study 1) or 150 (Study 2) were used along with the following assumptions, as reported by Achacha and Messier:[18] B hyo culture sensitivity of 89.7%, culture specificity of one, and sensitivity sample size of 145. Blaker's exact estimates and confidence limits were utilized.

The Bayesian method of estimation was compared to the frequentist method, since no true "gold standard" test for B hyo exists, and a priori information on culture sensitivity was available. To accomplish this estimation, the AusVet Epi Tools "Estimated true prevalence using one test with a Gibbs sampler" on-line calculator (`http://epitools.ausvet.com.au/content.php?page=OneTest`), based on work by Joseph et al,[22] was used. The required beta distributions were calculated with a priori estimates of prevalence beta ($\alpha = 1$, $\beta = 1$), culture sensitivity beta ($\alpha = 131$, $\beta = 16$) from Achacha and Messier,[18] and specificity beta ($\alpha = 88.28$, $\beta = 1.88$) using the AusVet Epi Tools beta distribution utility with a mode of 0.99 and a 95th percentile of 0.95 to approximate a high culture specificity.

Results

Study 1: Lactating sows and suckling piglets

Over the eight sampling periods, the percentage of sows positive for B hyo ranged from 0% to 5%, with an overall prevalence rate of 1.04%. In three of eight samplings there was at least one positive sow. The percentage of litters positive for B hyo ranged from 0% to 5%, with an overall prevalence rate of 1.88%. In five of eight samplings there was at least one positive litter. Table 1 shows the percentages of sows and litters positive for B hyo by sampling week and total study period, along with the associated estimated true prevalence and confidence intervals using the two methods described. Table 2 shows the distribution of positive sows and litters by parity. Overall, 14 of 960 samples (five sows and nine litter samples; 1.46%) were positive for B hyo. In two of 480 sample sets (0.42%), both the sow and litter were positive for B hyo.

Throughout the study, several weakly beta-hemolytic *Brachyspira* species were identified, including *Brachyspira murdochii*, *Brachyspira innocens*, and *Brachyspira alvinipulli* (data not shown). No strongly beta-hemolytic *Brachyspira* species other than B hyo were isolated in Study 1.

Study 2: breeding herds

The percentage of sows positive for B hyo ranged from 0% to 1.33%. Only three of the five farms demonstrated at least one B hyo-positive culture. Table 3 shows the percentage of sows positive for B hyo and the estimated true prevalence and confidence intervals for each sow farm. Several other weakly beta-hemolytic *Brachyspira* species were identified during sampling. No strongly beta-hemolytic *Brachyspira* species other than B hyo were isolated in Study 2.

Discussion

Brachyspira hyodysenteriae was cultured from rectal swabs of adult breeding and lactating sows and suckling piglets from known SD-positive breeding herds on four of six breed-to-wean farms. However, on two farms known to have SD, our testing method failed to detect B hyo. In addition, for the SD-positive farm in Study 1, our testing method failed to detect B hyo in three of eight sampling points. If broken down further, our testing method failed to detect B hyo in five of eight sampling points in sows, and three of eight sampling points in piglets. Furthermore, in the breeding herds in Study 2 known to be SD-positive, our testing method failed to detect B hyo on two of the five farms. These results highlight the difficulty in determining the true SD status of breeding herds. The methods employed for these two studies – repeated sampling and a large number of samples – are costly and time consuming and require coordination between the veterinarian and the diagnostic laboratory, but do provide a method of detecting B hyo in breeding herds. The results of these studies should be of value to those wanting to explore the true B hyo status of swine breeding herds prior to undergoing a system-level elimination project, evaluating the success of an elimination program (depopulation or medication), or selling breeding stock.

In Study 1, the litters of two of the five sows identified as B hyo-positive were also diagnosed as B hyo-positive, potentially demonstrating the importance of carrier sows transmitting to carrier piglets in the epidemiology of the disease.

Table 1: Percentage of sows and litters positive for *Brachyspira hyodysenteriae* on a 2200-sow, breed-to-wean, North Carolina farm by sampling week and total study period (Study 1, Farm A)*

Sampling week	No. sows positive (%) n = 60	Estimated true prevalence (95% CI)		No. litters positive (%) n = 60	Estimated true prevalence (95% CI)	
		Frequentist†	Bayesian‡		Frequentist†	Bayesian‡
1	1 (1.67)	1.9 (0.1, 9.6)	2.3 (0.1, 9.2)	1 (1.67)	1.9 (0.1, 9.6)	2.3 (0.1, 9.2)
5	3 (5.00)	5.6 (1.5, 15.2)	4.9 (0.4, 13.9)	3 (5.00)	5.6 (1.5, 15.2)	4.9 (0.4, 13.9)
10	1 (1.67)	1.9 (0.1, 9.6)	2.3 (0.1, 9.2)	2 (3.33)	3.7 (0.7, 12.4)	3.5 (0.2, 11.5)
12	0 (0.0)	0.0 (0.0, 6.6)	1.3 (0.0, 6.6)	0 (0.0)	0.0 (0.0, 6.6)	1.3 (0.0, 6.6)
13	0 (0.0)	0.0 (0.0, 6.6)	1.3 (0.0, 6.6)	1 (1.67)	1.9 (0.1, 9.6)	2.3 (0.1, 9.2)
14	0 (0.0)	0.0 (0.0, 6.6)	1.3 (0.0, 6.6)	0 (0.0)	0.0 (0.0, 6.6)	1.3 (0.0, 6.6)
17	0 (0.0)	0.0 (0.0, 6.6)	1.3 (0.0, 6.6)	2 (3.33)	3.7 (0.7, 12.4)	3.5 (0.2, 11.5)
18	0 (0.0)	0.0 (0.0, 6.6)	1.3 (0.0, 6.6)	0 (0.0)	0.0 (0.0, 6.6)	1.3 (0.0, 6.6)
Total N = 480	**5 (1.04)**	**1.2 (0.5, 2.7)**	**0.6 (0.0, 2.1)**	**9 (1.88)**	**2.1 (1.0, 3.9)**	**1.1 (0.0, 3.1)**

* Sixty sows and three pigs from each sow's litter were sampled by rectal swab weekly for 8 sampling weeks (total 480 sows). Swabs were tested by culture for *B hyodysenteriae*. A litter was considered positive if at least one pig tested positive.

† Frequentist approach estimates and intervals calculations based on work by Rogan and Gladen.[21]

‡ Bayesian approach estimates and intervals calculations based on work by Joseph et al.[22]

Table 2: Distribution of sows and litters positive for *Brachyspira hyodysenteriae* by parity on a North Carolina breed-to-wean farm (Study 1; Farm A)*

Parity	No. samples	No. sows positive (%)	No. litters positive (%)
1	119	1 (0.84)	0 (0.00)
2	145	2 (1.38)	4 (2.76)†
3	69	0 (0.00)	1 (1.45)
4	71	1 (1.41)	4 (5.63)†
5	54	0 (0.00)	0 (0.00)
≥ 6	22	1 (4.55)	0 (0.00)

* Study farm and diagnostic testing described in Table 1.

† One sow and litter pair were both positive for *B hyodysenteriae* in each indicated parity grouping.

In both Study 1 and Study 2, there was no effect of parity on culture result. Further studies on parity influences and other potential confounders on B hyo status should be conducted to provide better sampling guidelines for at-risk animals.

One limitation in the methods of this study is the use of culture as a diagnostic test. Sensitivity and specificity of *Brachyspira* species culture has not been studied extensively. One report by Achacha and Messier[18] estimated culture sensitivity at 89.7%, but did not report specificity. Culture has been shown to be more sensitive for detection of *B hyo* than current direct fecal PCR techniques, and culture allows for detection of other *Brachyspira* species (eg, *B hampsonii*) that may be missed by PCR due to primer or probe specificity.[7,23,24] Fecal shedding of *Brachyspira*, especially in recovered carrier animals, may be intermittent, and thus negative culture does not provide information on previous exposure and potential for carrier status.[1] In 1986, Olson and Rodabaugh[25] outlined a procedure by which sodium arsanilate could be fed to pigs at 220 grams per tonne for 21 days in order to induce the asymptomatic SD carrier to show typical clinical signs, thereby increasing the likelihood that carriers could be identified. While this method could help with identification of carriers, it should be noted that at the time of this publication, the sale of sodium arsanilate in the United States and Canada has been voluntarily suspended. In regions where sodium arsanilate is available and its use in sows is legal, its use to assist in the diagnosis of SD should be evaluated. Furthermore, the concept of inducing clinical disease in carrier animals could be further explored through means such as removal of medications[26] or by utilizing feed ingredients that can induce clinical dysentery (ie, nondigestible feedstuffs).[27,28]

Comparison of the frequentist and Bayesian methods of prevalence estimation showed no meaningful differences in the estimated prevalence or confidence intervals. The Bayesian methodology appeared to have a more conservative prevalence and precise confidence interval than the frequentist method; however, the differences were small. For example, the estimated true prevalence when one of 60 sow samples was positive was 1.9 and 2.3 for the frequentist and Bayesian methods, respectively, with confidence intervals of 0.1 to 9.6 and 0.1 to 9.2, respectively. Biologically, this is not a significant difference, and is likely due to the overall low prevalence of the disease in the herds. The estimated upper 95% confidence level of true prevalence in Study 1 was

Table 3: Percentage of sows positive for *Brachyspira hyodysenteriae* for each of five North Carolina sow farms (farms B through F; Study 2)*

Farm	No. positive (%)	Estimated true prevalence (95% CI)	
		Frequentist†	Bayesian‡
B	2 (1.33%)	1.5 (0.3, 5.3)	1.2 (0.0, 4.4)
C	1 (0.67%)	0.7 (0.0, 3.8)	0.8 (0.0, 3.7)
D	0 (0%)	0.0 (0.0, 2.7)	0.5 (0.0, 2.7)
E	0 (0%)	0.0 (0.0, 2.7)	0.5 (0.0, 2.7)
F	1 (0.67%)	0.7 (0.0, 3.8)	0.8 (0.0, 3.7)

* Rectal swabs collected from 150 sows on each farm were cultured for *B hyodysenteriae*.

† Frequentist approach estimates and intervals calculations based on work by Rogan and Gladen.[21]

‡ Bayesian approach estimates and intervals calculations based on work by Joseph et al.[22]

between 6.6% and 15.2% in weekly lactation sows and weaned-pig batches, and in Study 2, between 2.7% and 5.3% in breeding herds. Interpretation of estimated prevalence should consider the characteristics of the sampling and diagnostic methodologies used. Culture only identifies animals shedding above the detection threshold (10^2 colony forming units per g feces) at the time of sampling, and may underestimate the true prevalence of exposure or carrier status.[24] The data presented herein provides veterinarians with a reference for estimated prevalence rates of carrier animals to be used in developing future diagnostic sampling methodologies.

During the course of this study, several weakly beta-hemolytic *Brachyspira* species were identified from breeding animals. Both *B murdochii* and *B innocens* have been shown to cause colitis in swine, but little is understood about the role these isolates may play in breeding-herd enteric infections and immunity.[29-31] The confirmation of *B alvinipulli* by both 16s and *nox* gene sequencing, also isolated from breeding sows in Study 1, represents a unique case in which a *Brachyspira* species seldom reported in swine was isolated (Thomson J, e-mail communication, and Hampson D, e-mail communication, 2012). To the authors' knowledge there have been no reports to date on the impact or significance of *B alvinipulli* in swine, but it has been associated with enteritis in chickens and laying hens, and fibrinonecrotic typhlocolitis in laying geese.[32,33]

Given the results of the studies included herein and the literature currently available, the authors suggest that a multi-tiered approach to diagnosis of *B hyodysenteriae* in breed-to-wean herds be pursued, given the following four assumptions. The use of culture is currently the most sensitive and definitive method of diagnosis for all *Brachyspira* species (especially the pathogenic *B hyodysenteriae, B hampsonii,* and *B pilosicoli*); however, direct fecal PCR and serologic tests could help screen herds and improve laboratory and economic constraints. Apparent prevalence is likely < 5%, due to epidemiology, low shedding of carrier animals, and sensitivity of diagnostic method. True prevalence may vary depending on the time point at which pathogen introduction occurred (endemic versus epidemic). Susceptible populations may be more likely to express clinical disease in endemic herds (ie, higher prevalence in recent gilt introductions, lactating sows, or suckling piglets). Therefore, strategic exposure and sampling of susceptible replacement animals utilizing the sentinel-gilt program, in combination with random sampling of susceptible suckling weaning-aged piglets at a prevalence detection level ≤ 2% over multiple sampling periods, should increase level of confidence in determining the true status of the breeding herd. The true SD status can determine if a farm goes through an expensive elimination program (eg, depopulation, medication program), if those programs were effective, or if animals can be confidently sold to potential markets for growth or genetic replacement. Understanding within-herd B hyo prevalence is necessary in designing effective surveillance protocols. Further research on detection methods for carrier animals (PCR, serology), prevalence estimates of susceptible subpopulations (ie, replacement breeding stock, lactating sows, and suckling piglets), and prevalence within parities would continue to improve upon the surveillance methodologies of *Brachyspira* species in breeding herds.

Implications

- Sampling breeding animals, suckling-age piglets, or both for *Brachyspira hyodysenteriae* could serve as a valuable supplement to the traditional surveillance schemes that utilize sentinel animals.
- A better understanding of the prevalence of *B hyodysenteriae* on endemically infected sow farms should assist veterinarians in developing enhanced surveillance programs.
- Current diagnostic testing methodologies for *B hyodysenteriae* in breeding herds or weaning groups should target low prevalence rates (ie, ≤ 2%).

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Conflict of interest

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