Evaluation of protective immunity in pigs following oral administration of an avirulent live vaccine of *Lawsonia intracellularis*

Jeremy J. Kroll, BS; Michael B. Roof, PhD; Steven McOrist, BVSc, PhD

Objective—To evaluate the efficacy of an orally administered avirulent live vaccine to protect pigs against challenge exposure with virulent *Lawsonia intracellularis*.

Animals—108 weaned 3-week-old pigs (35 in experiment 1 and 73 in experiment 2).

Procedure—2 experiments were conducted. On day 0, vaccinates were orally administered vaccine via drench or in drinking water, whereas challenge-control pigs were administered cultured medium. On day 21, pigs were challenge exposed with a virulent heterologous isolate of *L intracellularis*. Clinical observations, weights, seroconversion, and fecal excretion of *L intracellularis* were measured until day 42. At study termination, pigs were euthanatized and examined for *L intracellularis*-specific lesion development of the ileum and colon.

Results—Pigs receiving a single dose of vaccine were protected when challenge exposed with virulent *L intracellularis* (at least 10^{7} $TCID_{50}$/dose). In experiment 1, vaccinates had significantly less fecal excretion (47% and 40% for days 35 and 42, respectively), compared with challenge-control pigs. In experiment 2, vaccinates had significantly less fecal excretion (50% and 58% for days 35 and 42, respectively), compared with challenge-control pigs. Significant reductions in lesion development were evident in the ileum of vaccinated pigs (70% and 56% at day 42 for experiments 1 and 2, respectively), compared with challenge-control pigs.

Conclusions and Clinical Relevance—Oral administration by drench or via drinking water of an avirulent live vaccine against *L intracellularis* resulted in substantial protection against proliferative enteropathy among vaccinates and offers a better way to reduce stress of pigs during vaccine administration. (*Am J Vet Res* 2004;65:559–565)

Proliferative enteropathy is a common enteric disease of pigs after weaning that is caused by the obligate intracellular bacterium, *Lawsonia intracellularis*.[1,2] The characteristic pathologic feature of the disease in all species is proliferation of immature epithelial cells in the crypts of the ileum, large intestine, or both, leading to macroscopic thickening of the mucosa.[3] *Lawsonia* organisms are invariably found in the apical cytoplasm of these proliferative enterocytes.[2,3] Genome and protein analysis of this bacterium indicates a remarkably homogenous single strain, suggesting that this agent only recently evolved as a result of a large genetic shift from its ancestors in the Desulfovibrio family.[1,4] Clinical signs in affected pigs after weaning can include diarrhea and uneven weight gain.[5] In pigs > 12 weeks old, there can be a more acute clinical form of the disease, with the proliferative lesions accompanied by rapid onset of diffuse hemorrhage from the affected mucosa, leading to melena and sudden death.[5] Estimates of the annual economic losses attributable to the clinical and subclinical effects of this disease are approximately $100 million for the US swine industry alone.[6]

In vitro culturing and the primary causative role of *L intracellularis* in all forms of the disease in pigs were established in 1993.[2,7] Before that time, an on-farm trial of a formalin-killed bacterin developed against a common secondary agent, *Campylobacter mucosalis*, resulted in equivocal results.[8] It was concluded that an orally administered avirulent vaccine was the formulation most likely to be effective against *L intracellularis*, which is an obligate intracellular pathogen. Ingestion of *L intracellularis* by intestinal mucosal macrophages, specific humoral responses, and antigen-related mitogenesis of porcine lymphocytes all are evident following oral infection.[9-12] Pigs exposed orally to raw *L intracellularis*-infected fecal material were judged to have developed protective immunity in on-farm studies.[2,4] Also, protective immunity to infection by other pathogenic intracellular bacteria, such as *Brucella* spp or *Chlamydia* spp, has only been documented following appropriate delivery of whole live-attenuated bacteria.[12-15] Killed or subunit vaccines developed against these agents have been far less successful, despite numerous attempts.[12,15]

In the study reported here, we evaluated the efficacy of an avirulent live vaccine developed by use of *L intracellularis* (isolate B3903) and various methods of oral administration followed by challenge exposure with a virulent heterologous *L intracellularis* isolate of differing origin from the vaccine's parent isolate. Clinical evaluation of this vaccine was performed in 2 experiments: the first was to ensure proof of concept of the efficacy of the vaccine and the second to establish equivalence for the various methods of oral administration to ensure it could be practical and acceptable to the pork industry for use in the vaccination of pigs.

Materials and Methods

Animals—Weaned 3-week-old pigs (n = 108) were used in the study. All pigs were confirmed with negative results for

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From the Department of Biological Research and Development, Boehringer Ingelheim Vetmedica Inc, 2501 N Loop Dr, Ames, IA 50010 (Kroll, Roof); and the Department of Clinical Development, QAF Meats, Bunge, Australia (McOrist).
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Address correspondence to Mr. Kroll.

L intracellularis by use of a **polymerase chain reaction (PCR)** assay conducted on fecal samples as well as analysis of serum samples prior to inoculation. Both experiments were conducted in accordance with established guidelines for good clinical practices.[16]

Experimental protocol—Two experiments were conducted. Both experiments were conducted by use of a double-blind approach in which the group identity for each pig was not disclosed to the study monitor or investigators until processing of samples, laboratory testing, compilation of data, and statistical analysis were completed.

Preparation of the vaccine—*Lawsonia intracellularis* isolate B3903 was originally isolated by use of routine coculture methods[6] from the ileum of a sow in Denmark in 1998. That sow had acute hemorrhagic proliferative enteropathy, as confirmed by the use of routine histologic techniques and **immunohistochemical (IHC)** staining methods. The vaccine used in both experiments was manufactured commercially, placed in sterile 100-mL glass bottles, lyophilized, and stored at 4°C. The master seed of the vaccine was tested independently at 3 laboratory sites[a-c] by use of routine cell culture and bacteriologic methods to ensure that it did not contain other adventitious viruses or bacteria.

Preparation of challenge-exposure inoculum—A pure culture of virulent *L intracellularis* used as the challenge isolate in both experiments was acquired from a 12-week-old pig from the United States that was affected by acute hemorrhagic proliferative enteropathy. This challenge isolate (*L intracellularis* N101494) was extracted from the severely infected ileum of the pig and cocultured by use of methods described elsewhere.[6] Prior to the day of challenge exposure (day 21 of each experiment), routine tissue culture and bacteriologic methods[2,6,17] were used to propagate the challenge isolate in vitro. On the day of challenge exposure, each active culture was harvested and pooled with frozen-thawed concentrated (10X to 20X) stocks of low-passage *L intracellularis* N101494. Each challenge inoculum was administered within 1 hour after preparation.

Bacterial quantification—Quantification of the vaccine and challenge inoculum N101494 was accomplished by performing a $TCID_{50}$ endpoint assay[18] on a representative 1-mL aliquot. The vaccine was reconstituted with sterile water and serially diluted 10-fold in Dulbecco modified Eagle's medium fortified with Ham's F12[d] with heat-inactivated 5% newborn bovine serum.[c] The total of live *L intracellularis* ($TCID_{50}$/dose) was calculated by use of the Reed-Muench method.[19] Vaccinated pigs received a dose ($10^{4.9}$ $TCID_{50}$) of the vaccine *L intracellularis* (isolate B3903). Pigs in challenge-exposed groups each received a dose ($10^{7.7}$ $TCID_{50}$) of a pure culture of virulent *L intracellularis* N101494.

Experiment 1 (proof of concept)—The objective of the first experiment was to determine the efficacy of an orally administered avirulent live vaccine for *L intracellularis* (isolate B3903) in pigs for use in protecting against challenge exposure with a virulent heterologous strain. Thirty-five weaned 3-week-old pigs with negative results when tested for *L intracellularis* were randomly allocated to 3 groups (15 vaccinates; 10 unvaccinated challenge-control pigs; and 10 unvaccinated, unchallenge-exposed, negative-control pigs). To avoid cross-contamination, each group was housed in a separate room but with the same conditions (temperature, ventilation, and pen size). Pigs in each room were housed in the same pen.

On day 0 of the experiment, each of the 15 vaccinates was orally administered a single 2-mL dose of commercially available *L intracellularis* vaccine[f] in accordance with label instructions. Each dose was applied directly to the caudal portion of the oral cavity of each pig by use of a sterile plastic 10-mL syringe. Also on day 0, challenge-control and negative-control pigs were each orally administered a 2-mL dose consisting of uninfected tissue culture cells suspended in growth medium.

On day 21, vaccinates and challenge-control pigs were challenge exposed with a single dose of virulent *L intracellularis* isolate N101494 containing $10^{7.7}$ $TCID_{50}$; challenge exposure was achieved by use of oral gavage. The remaining 10 negative-control pigs did not receive vaccine and were not challenge exposed during the study.

All pigs were examined daily to monitor clinical signs (including diarrhea), behavior, and body condition. Mean clinical score of 1 to 4 was calculated for each group on each day (1, clinically normal; 4, severe illness). An indirect fluorescence antibody test[18] was conducted on serum extracted from blood samples that were collected weekly from each pig from days 0 to 42 of the experiment; results of the antibody test were used to determine rate of seroconversion to *L intracellularis*. Fecal samples were collected weekly from days 0 to 42; these samples were analyzed with a PCR assay that used *L intracellularis* primers and methods described elsewhere.[18,20,21] The *L intracellularis*-specific primers used in this PCR assay cannot differentiate between various *L intracellularis* isolates. Extraction and purification of bacterial DNA from fecal samples were conducted by use of a rapid DNA extraction kit.[g]

Weight measurements were obtained on days 0, 21, and 42 to determine mean daily weight gain of each group of pigs. Mean initial weights were uniform among all groups (variation of < 0.2 kg/pig). Weight gains were calculated for 2 distinct phases of the experiment. The first phase (days 0 to 21) represented the period between inoculation and challenge exposure and was used to evaluate immediate effects of inoculation with the vaccine. The second phase (days 21 to 42) represented the period between challenge exposure and end of the experiment and was used to evaluate the effect of challenge exposure with a virulent heterologous organism.

On day 42 of the study, all pigs in all 3 groups were euthanatized. Postmortem examinations were performed to determine the extent of macroscopic and microscopic lesions in the ileum and colon of the pigs. Macroscopic lesions in the ileum or colon were scored on the basis of the severity of mucosal thickness (1, normal; 2, mild thickening; 3, moderate thickening-inflammation; and 4, severe thickening-inflammation-mucosal hemorrhaging or necrosis).

Samples of ileum and colon (2 to 4 cm in length), tonsils, and mesenteric lymph nodes were collected, fixed by immersion in buffered formalin, and processed for detection of microscopic lesions. This included staining with H&E and IHC staining by use of specific *L intracellularis* monoclonal antibodies.[4,22] Immunohistochemical analysis by use of *L intracellularis*-specific monoclonal antibodies is currently considered the criterion-referenced test for assessment of the actual infection status of a pig.[23] Microscopic lesions in IHC-stained tissues were scored separately on the basis of severity of *L intracellularis*-specific cell proliferation (0, normal; 1, mild-focal; 2, moderate-diffuse; and 3, severe-diffuse). Mean scores for microscopic lesions and the frequency of lesions detected in the affected tissues were calculated for group comparisons.

In addition, 6 to 10 cm of fresh tissue samples from the ileum, colon, tonsils, and mesenteric lymph nodes was collected from each pig. Each fresh sample was subjected to extraction of bacterial DNA and the *Lawsonia*-specific PCR assay described previously to determine the extent of possible tissue colonization.

Experiment 2 (bioequivalence of oral administration)—The objective of this experiment was to evaluate the efficacy of orally administering the avirulent live vaccine (*L intracellularis* isolate B3903) by 2 methods, followed by challenge exposure with virulent heterologous organisms. Seventy-three weaned 3-week-old pigs with negative results when tested for *L intracellularis* were randomly allocated to 4 groups (20 vaccinates with vaccine orally administered via water delivery; 20 vaccinates with vaccine orally administered via a drench; 20 unvaccinated challenge-control pigs; and 13 unvaccinated, unchallenged, strict-control pigs). Housing and measures to ensure biosecurity for each group were similar to those described for experiment 1.

On day 0, pigs in the water-delivery vaccinate group were orally vaccinated by ingesting the vaccine that was added to the drinking water. The reconstituted vaccine was added to a volume of water that we estimated would be consumed by 3-week-old pigs within a 4-hour time period (ie, 2 L of vaccine-containing water in a clean trough); it was the sole water source available to the pigs. The amount of vaccine added to the group's drinking water was calculated to deliver 1 dose/pig. These pigs were not subjected to withholding of water prior to this vaccination process.

Pigs vaccinated orally via drench were each administered a single 2-mL dose of the vaccine. Each dose was applied directly to the caudal portion of the oral cavity of each pig by use of a plastic 10-mL syringe with a plastic tip canula. Pigs in the challenge-control group were administered 2 mL of the tissue culture growth medium directly to the caudal portion of the oral cavity. Pigs designated as strict-control pigs were not administered vaccine or tissue culture growth medium and thus were unvaccinated, unchallenge exposed during the study.

On day 21, both vaccinate groups and the challenge-control group were challenge exposed via oral gavage with a single dose of a virulent culture of *L intracellularis* isolate N101494, as described for experiment 1. Collection of samples and clinical monitoring were conducted as for experiment 1. On day 42, pigs were euthanatized and postmortem examinations were conducted. All examinations were conducted in the same manner as for experiment 1, except for the scoring system for macroscopic lesions. In experiment 2, this system for scoring macroscopic lesions was modified to increase scrutiny among degrees of lesion severity as follows: 0, normal; 1, mild thickening; 2, moderate thickening-inflammation; 3, severe thickening-inflammation; 4, severe thickening-inflammation-edema-mucosal hemorrhaging; and 5, necrosis. Mean scores for macroscopic lesions were calculated for group comparisons.

Statistical analysis—All key variables were analyzed statistically. Macroscopic lesions, microscopic lesions, clinical scores, and body weight gains were analyzed by use of a 1-way ANOVA or Kruskal-Wallis test, whereas tissue colonization (PCR assay), fecal shedding (PCR assay), and seroconversion were analyzed by use of χ^2 or Monte-Carlo tests.[24,25] For experiment 1, the primary indicator of vaccine efficacy was considered to be a significant ($P < 0.05$) reduction in the prevalence and severity of macroscopic and microscopic lesions in the ileum and colon. This was used to document the degree of protection achieved for administration of the vaccine. For experiment 2, the primary indicator used to document efficacy of the 2 administration methods was a significant ($P < 0.05$) reduction in prevalence and severity of macroscopic and microscopic lesions in the ileum and colon.

Results

Safety of the vaccine and administration procedures—We did not detect adverse reactions attributable to the vaccine or vaccination procedure among any vaccinated pigs throughout the study.

Experiment 1—Analysis of clinical observations did not reveal significant differences between vaccinates and unvaccinated challenge-control pigs for diarrhea, behavior, and body condition. Mean initial weight for vaccinates, challenge-control pigs, and negative-control pigs was 8.2, 8.0, and 8.0 kg/pig, respectively, whereas mean final weight for vaccinates, challenge-control pigs, and negative-control pigs was 32.9, 31.0, and 32.5 kg/pig, respectively. Average daily weight gain did not differ significantly among any of the groups during the first 21 days of each experiment. After challenge exposure (ie, days 21 to 42), average daily weight gain did not differ significantly between vaccinates (0.73 kg/d) and negative-control pigs (0.74 kg/d); however, the challenge-control pigs had a significantly lower average daily weight gain (0.66 kg/d), compared with values for the vaccinates and negative-control pigs.

During the period before challenge exposure (days 0 to 21), *Lawsonia*-specific PCR analysis of fecal samples revealed that none of the groups had positive results for *L intracellularis* (**Fig 1**). Initial PCR detection of *L intracellularis* in the feces of pigs in any of the groups was on day 28 of the study (data not shown). On day 35 (14 days after challenge exposure), a significantly lower proportion of vaccinated pigs (5/15, 33%) had PCR-positive fecal samples, compared with the proportion in the challenge-control group (8/10, 80%). On day 42 (termination of experiment), a significantly higher proportion of pigs in the challenge-control group shed *L intracellularis* (4/10, 40%), compared with the proportion for the vaccinated group (0/15, 0%).

The pattern of seroconversion during the course of the experiments was similar to the rates of fecal shedding among vaccinates and challenge-control pigs (**Fig 2**). All pigs were seronegative on the basis of analysis of indirect fluorescence antibody testing of sera obtained during the 3 weeks before and on the day of challenge exposure (ie, days 0 to 21). On day 35

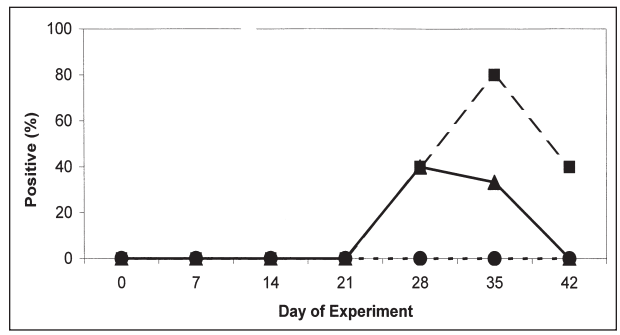

Figure 1—Fecal shedding of *Lawsonia intracellularis* in pigs orally vaccinated with an avirulent live vaccine and unvaccinated challenge-control pigs that were challenge exposed 21 days later with virulent *L intracellularis* during experiment 1. Fecal shedding was determined on the basis of positive results for a polymerase chain reaction assay. Fifteen pigs were vaccinated and challenged exposed (triangle), 10 pigs were unvaccinated and challenge exposed (square), and 10 pigs were unvaccinated and not challenge exposed (cirle). Day 0 is the day of oral administration of vaccine or culture medium.

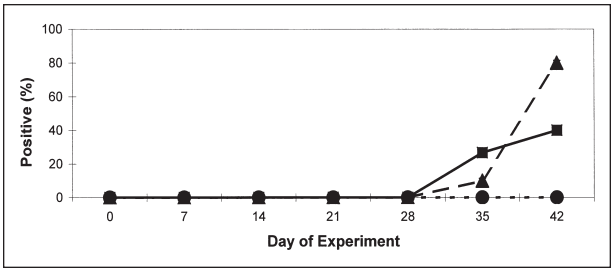

Figure 2—Seroconversion to *L intracellularis* in pigs orally vaccinated with an avirulent live vaccine and unvaccinated challenge-control pigs that were challenge exposed 21 days later with virulent *L intracellularis* during experiment 1. Seroconversion was determined on the basis of results for an indirect fluorescence antibody assay. *See* Figure 1 for key.

(14 days after challenge exposure), 4 of 15 (27%) vaccinated pigs was seropositive for *L intracellularis*, whereas only 1 of 10 (10%) pigs were seropositive in the unvaccinated challenge-control group. On day 42 (termination of experiment), 6 of 15 (40%) vaccinates were seropositive, whereas 8 of 10 (80%) challenge-control pigs were seropositive. We did not detect significant differences in the pattern of seroconversion among treatment groups at any time period throughout the experiment.

Lawsonia-specific PCR analysis of various tissues revealed a higher proportion of mesenteric lymph nodes with positive results in the challenge-control group (3/10, 30%), compared with the vaccinates (0/15, 0%). Tissues obtained from the tonsils of all pigs were PCR-negative for *L intracellularis* DNA during postmortem examination. Positive results by use of the PCR assay were less frequently found in tissues of the ileum and colon obtained from vaccinates (2/15 [13%] and 1/15 [7%], respectively), compared with results for ileum and colon obtained from challenge-control pigs (4/10 [40%] and 3/10 [30%], respectively).

Evaluation of the variables used to determine primary efficacy revealed that the mean scores for macroscopic lesions were significantly lower for the ileum ($P < 0.001$) and colon ($P = 0.05$) in the vaccinates (1.5 and 1.0, respectively) than in the unvaccinated challenge-control group (3.6 and 2.0, respectively). Mean score for macroscopic lesions of the ileum and colon in the vaccinates was similar to the score in the unvaccinated, unchallenged, negative-control group (**Table 1**). The IHC evaluation of ileum and colon tissues revealed a significantly ($P < 0.001$) higher number of *Lawsonia*-specific lesions in the challenge-control group (mean score for ileum, 2.4; mean score for colon, 1.5), compared with the vaccinates (mean score for ileum, 0.4; mean score for colon, 0.0). Mean score for microscopic lesions of the ileum and colon in vaccinates was similar to the mean score of those tissues in the unvaccinated, unchallenged, negative-control group.

Experiment 2—Analysis of clinical observations did not reveal significant differences among vaccinates (orally administered via drench or delivery in water supply) and challenge-control pigs during the experiment. Mean initial weight for vaccinates via drench, vaccinates via water supply, challenge-control pigs, and negative-control pigs was 5.5, 5.5, 5.4, and 5.4 kg/pig, whereas mean final weight for each group was 26.7, 26.0, 24.9, and 27.3 kg/pig. Daily weight gains were numerically higher for pigs vaccinated via drench but not significantly different from values for the challenge-control pigs during the experiment. However, during days 21 to 42, average daily weight gain of the challenge-control group (0.49 kg/pig) was significantly lower than that of the water-delivery vaccinates (0.55 kg/pig).

Fecal shedding of *L intracellularis* became detectable by use of the PCR assay on day 28 (7 days after challenge exposure) in 4 of 20 (20%) challenge-control pigs. On day 35 of the experiment, significantly more PCR-positive pigs were evident in the challenge-control group (14/20, 70%) than in the water-delivery and drench vaccinates (2/20 [10%] and 6/20 [30%], respectively). On day 42, significantly more PCR-positive pigs were again evident in the challenge-control group (15/20, 75%) than in the water-delivery and drench vaccinates (2/20 [10%] and 4/20 [20%], respectively).

All pigs were seronegative on the basis of analysis of serum samples obtained during the 3 weeks before and at the time of challenge exposure. Seroconversion first became apparent on day 28 of the experiment. At that time, 4/20 (20%) pigs in the drench-vaccinate group were seropositive, whereas anti-*Lawsonia* IgG antibodies were not detected in the serum samples obtained from any other group, including the challenge-control group. On day 35, the proportion of seropositive pigs was 3 of 20 (15%) for water-delivery

Table 1—Mean ± SE scores for macroscopic and microscopic lesions in samples of ileum and colon obtained from 3 groups* of pigs in experiment 1

		Macroscopic lesions†		Microscopic lesions‡	
Group	**n**	**Ileum**	**Colon**	**Ileum**	**Colon**
Vaccinates	15	1.5 ± 0.19[a]	1.0 ± 0.00[a]	0.4 ± 0.19[a]	0.0 ± 0.00[a]
Challenge-control pigs	10	3.6 ± 0.22[b]	2.0 ± 0.39[b]	2.4 ± 0.34[b]	1.5 ± 0.43[b]
Negative-control pigs	10	1.1 ± 0.10[a]	1.0 ± 0.00[a]	0.0 ± 0.00[a]	0.0 ± 0.00[a]

*Groups consisted of pigs vaccinated by oral administration of an avirulent live vaccine of *Lawsonia intracellularis* on day 0 and challenge exposed on day 21 with virulent heterologous *L intracellularis* (vaccinates), pigs that were orally administered culture medium on day 0 and challenge exposed on day 21 with virulent *L intracellularis* (challenge-control pigs), and pigs that were orally administered culture medium on day 0 and were not challenge exposed (negative-control pigs). †Macroscopic lesions were scored on a scale from 1 (normal) to 4 (severe thickening). ‡Microscopic lesions were scored on a scale from 0 (normal) to 3 (severe-diffuse).

[b]Within a column, values with different superscript letters differ significantly ($P < 0.05$).

Table 2—Mean ± SE scores for microscopic lesions and percentage of tissues with positive results for immunohistochemical (IHC) analysis by use of *Lawsonia*-specific monoclonal antibodies in samples of ileum and colon obtained from 4 groups* of pigs in experiment 2

		Ileum		Colon	
Group	**n**	**Lesion score†**	**IHC (%)**	**Lesion score†**	**IHC (%)**
Vaccinates-water delivery	20	0.30 ± 0.18^{a}	15^{a}	0.15 ± 0.11^{a}	$10^{a,b}$
Vaccinates-drench	20	0.65 ± 0.25^{a}	30^{a}	$0.25 \pm 0.18^{a,b}$	$10^{a,b}$
Challenge-control pigs	19‡	2.32 ± 0.29^{b}	79^{b}	0.70 ± 0.29^{b}	35^{b}
Strict-control pigs	13	0.0 ± 0.00^{c}	0^{ϕ}	0.0 ± 0.00^{a}	0^{ϕ}

*Groups consisted of pigs vaccinated by oral administration of an avirulent live vaccine of *L intracellularis* on day 0 via addition to the water supply and challenge exposed on day 21 with virulent heterologous *L intracellularis* (vaccinates-water delivery), pigs vaccinated by direct oral drench administration of a 2-mL volume to each pig and challenge exposed on day 21 with virulent *L intracellularis* (vaccinates-drench), pigs administered culture medium on day 0 and challenge exposed on day 21 with virulent *L intracellularis* (challenge-control pigs), and pigs that were not administered vaccine or culture medium and were not challenge exposed (strict-control pigs). †Microscopic lesions were scored on a scale from 0 (normal) to 3 (severe-diffuse). ‡One ileum sample had inflammation and fibrosis indicative of proliferative enteropathy and as a result, mucosal crypts had been shed and could not be evaluated. [a,b]Within a column, values with different superscript letters differ significantly ($P < 0.05$).

[φ]Group not included in the statistical analysis of percentage IHC positive samples.

vaccinates, 9 of 20 (45%) for drench vaccinates, 4 of 20 (20%) for challenge-control pigs, and 0 of 13 (0%) for unvaccinated, unchallenged, negative-control pigs. On day 42, the proportion of seropositive pigs was 4 of 20 (20%) for water-delivery vaccinates, 10 of 20 (50%) for drench vaccinates, 12 of 20 (60%) for challenge-control pigs, and 0 of 13 (0%) for unvaccinated, unexposed, negative-control pigs. On days 35 and 42, there were no significant differences among both groups of vaccinates and challenge-control pigs with regard to the proportion of seropositive pigs.

Lawsonia-specific PCR testing for tissue colonization yielded negative results for the tonsils and mesenteric lymph nodes. On day 42, the proportion of ileum samples with positive results when tested by use of the *Lawsonia*-specific PCR assay was 0 of 20 (0%) for water-delivery vaccinates, 2 of 20 (10%) for drench vaccinates, 4 of 20 (20%) for challenge-control pigs, and 0 of 13 (0%) for unvaccinated, unchallenged, negative-control pigs. On day 42, the proportion of colon tissues with positive results when tested by use of the *Lawsonia*-specific PCR assay was 1 of 20 (5%) for water-delivery vaccinates, 4 of 20 (20%) for drench vaccinates, 4 of 20 (20%) for challenge-control pigs, and 0 of 13 (0%) for unvaccinated, unchallenged, negative-control pigs. No significant differences among groups were detected for *L intracellularis* tissue colonization by use of the PCR assay.

Evaluation of the primary variables used to determine vaccine efficacy revealed that in the challenge-control group, mean score for macroscopic lesions was significantly higher (ie, lesions were more severe) in the ileum (2.55) and colon (0.75) than in those tissues for either the water-delivery (0.2 and 0.2, respectively) or drench (0.6 and 0.2, respectively) vaccinates. Similarly, the proportion of samples with positive results for the *Lawsonia*-specific IHC analysis and the mean scores for microscopic lesions in the ileum was significantly lower in both vaccinate groups, compared with values for the challenge-control pigs (**Table 2**). Mean scores for microscopic lesions in the colon were significantly reduced in both vaccinate groups (water delivery, 0.15; drench, 0.25), compared with the mean score for the challenge-control group (0.7).

Discussion

Results of these experiments document that oral administration of an avirulent live vaccine against *L intracellularis* by direct drench or via drinking water to young pigs provides substantial amounts of protection against subsequent challenge exposure with a virulent *L intracellularis* isolate of differing origin from the vaccine's parent isolate. This was confirmed by a significant reduction in development of lesions as indicated by the prevalence and severity of macroscopic and microscopic lesions in the ileum of vaccinated pigs. Significant higher average daily weight gains (experiment 2) and reduced fecal shedding of *L intracellularis* (experiments 1 and 2) were also detected in vaccinated pigs after challenge exposure.

Vaccinates did not shed or seroconvert during the period prior to challenge exposure; however, we did not begin to collect samples until 7 days after vaccination. It is possible that collection of samples prior to day 7 or improved PCR assays may reveal shedding in vaccinates prior to day 7 after vaccination. However, the lack of detectable *L intracellularis* in the feces of all vaccinated pigs during the 3-week period after vaccination suggests that the isolate used in the live avirulent orally administered vaccine is not likely to have a great potential to spread to other pigs via the feces. It is common for avirulent bacterial isolates to have a reduced capacity for replication in the host and, hence, a reduced ability to spread to penmates.[21] This compares to virulent isolates, including those of *L intracellularis*, which typically are adapted to maximize their animal-to-animal spread and can attain high amounts of organisms in the feces.[17,21,23] Analysis of results of these studies on shedding of vaccine isolates of *L intracellularis* suggests that this organism also fits the pattern of bacterial adaptation and virulence. Therefore, feces from vaccinates should not be considered a reliable source of useful material from which to obtain vaccine isolates. The lack of detectable seroconversion following vaccination in both of the experiments reported here was consistent with our experiences involving this vaccine isolate and was not an unexpected result for oral versus parenteral administration of vaccine. In these experiments, seroconversion was

not necessary for protecting vaccinated pigs from disease, suggesting that nonhumoral factors stimulated by vaccination are perhaps more important for immunity to *L intracellularis*.

A general advantage of the use of an avirulent live vaccine is that the efficacy and duration of immunity are usually superior to those for inactivated vaccines because the host's immune system is exposed to all of the antigenic properties of the organism in its native state. Specifically for intracellular bacterial agents such as *L intracellularis*, a live avirulent vaccine offers excellent protection for vaccinated animals because of a complete T-cell-based immune response, possibly in conjunction with a complete humoral or mucosal immune response.[17] This response develops even with administration of small doses of avirulent agent and is in contrast with the variable or poor immunity associated with the use of subunit or killed vaccine types for intracellular bacteria.[14,15] This is also true for obligate intracellular bacteria (*Chlamydia* spp) that cause pathogenic infections within mucosa.[12,13] Challenge exposure of pigs via oral administration of live *L intracellularis* consistently leads to intracellular infection in the crypt epithelial cells of the intestinal mucosa.[2,4] Many studies[10,11,22] have also revealed that mucosal macrophages immediately beneath this epithelium regularly have intracellular infections attributable to *Lawsonia* sp during the active infection phases. This infection of macrophages implies a clear exposure of the host's immune system to *L intracellularis* and presentation of its antigens to other components of the immune system in the intestinal mucosa and beyond, probably via a Th1-based immune response typical of that to intracellular organisms.[13] In a clinical study[17] of pigs orally exposed to wild-type *Lawsonia* sp or to another live avirulent vaccine isolate, investigators determined that pigs developed intestinal infections and cell-mediated T-cell responses in a similar manner. The B cell or humoral and mucosal antibody responses of pigs to *L intracellularis* infection have been characterized in several studies.[9,11,18, 26,27] Similar to most bacterial infections, an initial IgM antibody response is followed by a peak of IgG antibody concentrations with a subsequent decrease during the next few weeks. In most cases of acute hemorrhagic proliferative enteropathy, the IgG peak is much higher and its subsequent decrease is much slower than in animals with the chronic form of the disease.[24,27] Mucosal infection is associated with a high amount of IgA mucosal antibody.[11] Therefore, both T- and B-cell responses are associated with the development of protective immunity in pigs infected with *L intracellularis*. A study[13] that investigated immunity developed against other intracellular bacteria revealed that the T-cell response is the more active response for inducing actual immune protection, although local antibody responses are also likely to be important in the target mucosa.

Possible immunogenic sites on *L intracellularis* have not been fully explored. A wide range of outer membrane proteins and glycoproteins have been identified in initial studies.[1,4] Some of these were found on bacteria located in the intestines in pigs with natural infections.[28,29] One of these surface antigens was characterized and cloned in a subsequent study, but its exact role in the immune process in natural infection is not clear.[28,29] Detailed studies[14,15,30,31] of several other intracellular bacteria have established that single antigen peptides used in vaccines are highly unlikely to produce a meaningful or protective immune response in animals, compared with the excellent protection afforded by use of whole live-avirulent bacteria.

The vaccine isolate of *L intracellularis* used in the experiments reported here (B3903) originated from an acutely infected pig in Denmark, whereas the challenge isolate (N101494) originated from a pig born in the United States that was afflicted with the same form of disease (hemorrhagic proliferative enteropathy). Therefore, the respective *L intracellularis* isolates used in our experiments were separated by a time frame of 3 years and a distance of thousands of miles. Despite these differences, genomic and proteomic analysis of *L intracellularis* indicated that it is a single strain or monotypic organism with no variant strains yet detected.[1,3] This is also consistent with the suggestion that *L intracellularis* is a recently evolved intracellular organism that has had a large genetic shift from its free-living Desulfovibrio family ancestors.[1,3] The difficulty of culturing virulent *L intracellularis* precluded the use of challenge exposures with a wide range of heterologous organisms. However, we consider that heterologous protection was achieved in these initial controlled experiments.

Some of the reasons to investigate vaccination via a water-delivery system are concerns about stress and injury to pigs, amount of human-animal contact time, costs, and labor (including possible difficulties with full compliance and injury to humans administering the vaccine). These factors are frequently seen as a result of numerous vaccinations to each pig in a herd, compared with the ease and consistency of administration of vaccine via the drinking water. Additional advantages of oral administration of vaccine via drenching of each animal or for a herd via the water supply include elimination of the possible transmission of major blood-borne infections (eg, porcine reproductive and respiratory syndrome virus) via multiple-use needles.[32] Oral administration of vaccine would reduce concern among meat retailers about injection-site reactions or broken needles retained in carcasses after attempted injections.[33] Methods for mass vaccination have been widely and effectively used on poultry farms for many years, and we speculate that they will become more widely used on swine farms as the number of pigs per farm increases. An additional possible benefit of an effective vaccine against *L intracellularis* is that the total antibiotic usage for prevention and treatment of proliferative enteropathy on swine farms may be reduced. It is possible that antibiotic use could be reduced considerably if this endemic disease could be controlled through use of vaccines alone or vaccines used in combination with strategic, periodically administered feed-additive antibiotics rather than constant use of feed-additive antibiotics.

[a]Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
[b]Q-one Biotech Ltd, Glasgow, Scotland.
[c]Center for Veterinary Biologics Laboratory, Ames, Iowa.

[d]DMEM F12, JRH Biosciences, Lenexa, Kan.
[e]Newborn Bovine Serum, JRH Biosciences, Lenexa, Kan.
[f]Enterisol Ileitis, Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo.
[g]IsoQuick DNA extraction kit, Orca Research Inc, Bothell, Wash.

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