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Examination of Horizontal Transmission of *Nippostrongylus brasiliensis* in Mice to Assess Biosecurity Risks

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Mice are commonly infected with *Nippostrongylus brasiliensis* (Nb) to study their immune responses. However, biosecurity measures have not been established for housing Nb-infected mice and rats. Transmission reportedly does not occur when infected mice are cohoused with naive mice. To test this, we inoculated female NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/Sz (NSG; *n* = 12) and C57BL/6J (B6; *n* = 12) mice with 750 Nb L_3 larvae. These mice were then cohoused with naïve NSG (*n* = 24) and B6 (*n* = 24) mice (1 infected and 2 naïve mice per cage (24 cages) for 28 d in static microisolation cages that were changed every 14 d. We also did several studies to determine the conditions that favor horizontal transmission. First, we assessed in vitro development to the L_3 stage of Nb egg-containing fecal pellets maintained under 4 environmental conditions (dry, moist, soiled bedding, and control). Second, we assessed infection of naïve NSG mice (*n* = 9) housed in microisolation cages that contained soiled bedding spiked with infective L_3 larvae (10,000/cage). Third, we gavaged NSG mice (*n* = 3) with Nb eggs to model the potential for infection after coprophagy. We found that naïve NSG (9 of 24) and B6 (10 of 24) mice cohoused with an infected cagemate passed Nb eggs in feces as early as 1 d after cohousing and intermittently thereafter for varying periods. This shedding was presumably the result of coprophagy because adult worms were not detected in the shedding mice at euthanasia. Although eggs developed in vitro into L_3 larvae under moist and control environmental conditions, none of the NSG mice housed in cages with L_3-spiked bedding or gavaged with eggs became infected with Nb. These findings indicate that infectious horizontal transmission does not occur when mice are housed with Nb-shedding cage mates in static microisolation cages with a 14-d cage-changing interval. Results from this study can be used to inform biosecurity practices when working with Nb-infected mice.

Abbreviations: dph, days postcohousing; dpi, days postinoculation; epg, eggs per gram of feces; L_3, infective larvae (3rd stage); L_5, adult worms; Nb, *Nippostrongylus brasiliensis*

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Introduction

The mouse model of *Nippostrongylus brasiliensis* (Nb) infection is a well-established model for studying systemic and mucosal immune responses.[3,5,6,18,25,39] An extensive list of publications, dating back to the early 1900 s, describes the parasite's life cycle, biology, and immunophysiology.[3,5,6,18,25] Research using the Nb mouse model has been instrumental in understanding the molecular mechanisms of Th2-type immune responses and the role they play in protective immunity against helminth infections.[2,5,6,16] Nb is commonly propagated by passage through rats because rats generate large quantities of eggs and infective larvae (third stage; L_3) that can easily be collected from fecal pellets or cecal and colonic contents.[6,25] Alternatively, Nb can be propagated through mice, with the Swiss Webster stock most commonly used.[6] Mice, rather than rats, are often used for research studies due to the extensive availability and characterization of both inbred and genetically engineered strains.[25] The mouse strains most commonly used for this purpose are C57BL/6 and BALB/c.[6] Subcutaneous inoculation with 500 (mouse-adapted) or 750 (rat-adapted) L_3 larvae is recommended for studying Nb in mice.[6]

The life cycle of Nb is direct and approximately 2 wk in le ngth.[3,5,6,18,25] Eggs are ellipsoidal, with a thin shell, and measure 50 to 70 × 27 to 40 µm.[6,25] They are excreted in the feces as 16- to 20-cell embryos.[6] L_1 larvae hatch within 18 to 24 h at 18 to 26 °C.[6,25] Over the following 5 to 6 d, larvae undergo 2 additional molts before forming unsheathed, filariform, infective L_3 larvae measuring 620 to 750 µm in length. Infection occurs through direct skin penetration or ingestion of L_3 larvae. Skin penetration occurs within 5 min of contact; larvae migrate through the epidermis into blood vessels to reach the lungs by 11 h after infection. The 3rd molt occurs in the lungs between 19 to 32 h after infection, forming L_4 larvae. L_4 larvae remain in the lungs for up to 50 h before migrating up the trachea, being coughed up and swallowed, proceeding through the esophagus and stomach, and reaching the small intestine, where the final molt occurs to form L_5 immature adults at 90 to 108 h after infection.[2,3,5,6,18,25] There, adult nematodes become sexually mature and reach their final size (male, 3 to 4.5 mm; female, 4 to 6 mm). Gravid females can first be detected at the end of the 5th day after infection, and eggs can be found in the feces approximately 5 to 6 d after infection. In immunocompetent mice and rats, maximum

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egg production occurs at 6 to 9 d after infection, followed by a gradual decline and worm expulsion on day 10 to 15.[2,3,5,6,18,25]

Once Nb infection is cleared, immunocompetent animals have lifelong immunity.[5,6,19] However, susceptibility to infection varies among rodent strains. Th2-skewed strains, such as BALB/c mice, are more resistant than C57BL/6 mice, a Th1-skewed strain that sheds higher numbers of eggs.[44] However, both strains expel the parasite by day 13 after infection.[44] In contrast, immunodeficient mice (e.g., athymic nude) that cannot mount a T-cell response may fail to expel nematodes completely and thus become chronically infected.[1,14,21,22,31]

Infection in humans after skin penetration by L_3 larvae is reported to be self-limiting. Therefore, the use of proper PPE, including a laboratory coat and gloves, is recommended when working with Nb.[6] Consideration must also be given to horizontal transmission to susceptible research animals when using this model. This risk is greatest when immunocompromised mice become chronically infected and shed high numbers of eggs. Given the lack of a definitive published study examining horizontal transmission when housing Nb-infected mice and rats, biosecurity measures vary by institution, with infected rodents maintained according to ABSL1 or ABSL2 practices. One study that provides recommendations for animal facility containment[6] states that horizontal transmission should not occur in mice, even when infected and naïve animals are cohoused, if the cage is changed twice weekly. This cage-change interval is recommended because the infective L_3 stage requires 5 to 6 d to develop under optimal conditions of temperature and humidity.[6] Although twice-weekly cage changing is recommended, even longer periods are reported to be acceptable due to desiccation of larvae in low-humidity, HEPA-filtered air at the cage level.[6] In addition, cage cleaning reportedly prevents transmission, as eggs and larvae are susceptible to hot water and detergent.[6] However, these statements are not supported by references and they presumably refer to unpublished data.

The purpose of the current study was to use multiple strategies to determine the ideal environmental conditions for Nb development, whether horizontal transmission occurs in Nb-infected mice when cohoused with naïve mice, and whether housing conditions (that is, cage change frequency, mouse strain, immune status) influence transmission. In addition, we sought to determine whether L_3 larvae can develop when fecal pellets containing Nb eggs are maintained under optimal environmental conditions and whether transmission occurs when naïve immunocompromised mice are housed in cages spiked with a large number of infective L_3 larvae. Institutions can use the findings from this study to formulate appropriate biosecurity measures to be implemented when using this model.

Materials and Methods

Study design. Three experiments were undertaken to evaluate the likelihood of horizontal Nb transmission.

Intracage transmission. C57BL/6J (B6; n = 12) and NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG; n = 12) mice were inoculated subcutaneously with 750 L_3 larvae suspended in 200 µL of sterile PBS (Gibco PBS; pH 7.4, Gibco, Dublin, Ireland) as described below. Egg shedding was evaluated daily in inoculated mice from day 5 through day 35 post inoculation (dpi), except for B6 mice in which egg counts were reduced to daily determination in 6 mice beginning on 7 dpi. A greater number of NSG mice were evaluated because published historical data is not available regarding egg shedding patterns in this strain, whereas egg shedding in B6 mice is well documented.[6,35,44] On 7 dpi (B6 peak egg shedding), mice that had been inoculated with Nb and were confirmed to be shedding (B6, n = 12; NSG, n = 12) were randomized and cohoused with naïve B6 (n = 24) or NSG (n = 24) mice at a ratio of 1:2 infected:naïve mice per cage to generate 4 experimental groups, as detailed in Table 1. Six cohorts of the 4 groups were generated, yielding a total of 6 Nb-inoculated and 12 naive cage mates per group. Eggs per gram (epg) of feces were determined for the naïve B6 and NSG mice from day 7 until day 28 of cohousing for the first 2 cohorts. Because we unexpectedly found eggs in feces at 7 d post-cohousing (dph), we suspected coprophagy, and we performed egg counts starting at 1 dph for the remaining 4 cohorts (n = 32) to determine whether eggs were present before 7 dph.

Nb-inoculated and naïve mice were euthanized via carbon dioxide on 35 dpi and 28 dph, respectively. Serum was collected for IgE determination in inoculated and naïve B6 mice (n = 24). Gastrointestinal tract, lungs, heart, spleen, and lymph nodes were removed for histopathology and adult L_5 worm burdens were quantified for all naïve and inoculated mice.

Cage inoculation. To determine whether mice can be infected with Nb when exposed to a high burden of infective L_3 larvae in the bedding, naive NSG mice (n = 9) were randomly selected and housed at 3 per cage. Seven days later, each cage, which now contained soiled bedding from the resident mice, was spiked with 10,000 L_3 larvae (approximately 1000 larvae per mL); the larvae were distributed throughout the cage by placing 12 aliquots of 0.84 mL of PBS-suspended larvae onto the bedding in a 3 × 4 evenly-spaced pattern. Seven days after spiking the bedding with larvae, cages were changed, and thereafter 1 to 5 fecal pellets were collected directly from each mouse daily for 21 d and fecal floats performed to determine the presence of eggs. After no eggs were identified in the feces, cages were inoculated a second time with 10,000 L_3 larvae on day 21 as previously above, and fecal pellets were collected for floatation daily until day 35. The mice were then euthanized, tissue samples were collected for histopathology, and worm burdens were quantified.

In vitro larval development. We next tested whether L_3 larvae develop when fecal pellets containing Nb eggs are incubated under environmental conditions that replicate intracage conditions. Approximately 1 g of feces was pooled from 3 Nb-shedding NSG mice and plated on 100-mm Petri plates (Falcon, Corning, Corning, NY) containing either 5 g of autoclaved aspen chip bedding (PWI Industries, Quebec, Canada; dry), 5 g autoclaved aspen chip bedding mixed with 5 mL sterile water (moist), or 5 g of previously autoclaved aspen chip bedding collected from a cage that had housed 5 mice for 7 d (soiled) (n = 3 Petri plates per condition: dry, moist, and soiled). Control plates (n = 3) contained autoclaved activated charcoal and approximately 1 g of Nb-containing fecal pellets softened in sterile water and placed on a piece of moistened 90-mm filter paper (Whatman, Cytiva, Marlborough, MA) as described below.[6,25] The control plates replicated optimal conditions under which the parasite is cultured in vitro.[6,25] All 100-mm plates were placed into larger 150-mm Petri plates (Falcon) containing enough sterile water to completely cover the bottom of plate; the combined plates were incubated at room temperature (approximately 26 °C [79 °F]) for

Table 1. Cohousing configurations

Group	Inoculated	Naïve
1	1 B6	2 B6
2	1 B6	2 NSG
3	1 NSG	2 B6
4	1 NSG	2 NSG

7 d in an incubator (model 100A; Blue M, Blue Island, IL) that was humidified by placing a tray containing 200 mL of sterile water at the bottom of the incubator. Plates were removed from the incubator every 3 d and the lids removed for 1 min to allow aeration. Plates that were intended to remain moist were checked every 3 d, and approximately 2 mL sterile water was added to prevent plates from drying. On day 7 the Baerman technique was used to evaluate each 100-mm plate for L_3 larvae.[6]

Additional studies conducted to investigate unexpected results. ***Nb inoculation of athymic nude stocks.*** We initially used Nb-inoculated, athymic nude mice ($n = 6$; group nude-1) as the immunocompromised mice in the study, because they were reported to shed Nb eggs chronically.[1,21,22,31] However, due to inconsistent infection, low fecal epg, and cessation of egg shedding at 10 dpi, athymic nude mice were replaced with NSGs, as described above and shown in Table 1. An alternate athymic nude mouse stock (group nude-2; $n = 6$) from another vendor (see below) was inoculated with Nb L_3 to test whether the results were attributable to genetic differences between stocks. All mice were inoculated with Nb L_3, daily fecal samples collected, and fecal floats performed until mice were euthanized at 35 dpi, at which point tissues were collected for histopathology and adult worm burdens determined.

Gavage of feces containing Nb eggs. Naïve NSG and B6 mice that were cohoused with Nb-infected NSG mice had eggs in their feces, detected by flotation, as early as 1 dph. To confirm whether coprophagy was the source of the eggs and, if so, whether eggs embryonated and hatched in the gastrointestinal tract, 3 NSG mice were gavaged with a suspension that contained Nb eggs. The inoculate was prepared by mixing 4 or 5 fecal pellets from an Nb egg–shedding NSG mouse with 1 mL of 50% dextrose solution. The resulting slurry was centrifuged (Thermo Scientific TX-400 4 × 400 mL Swinging Bucket Rotor, Waltham, MA) at 317 x *g* for 5 min at room temperature and the supernatant removed. A 100-µL aliquot of the supernatant was used to confirm the presence and number of Nb eggs via flotation. The remainder of the suspension (900 µL) was divided in thirds, each of which was diluted in sterile water (1:1) and administered to an NSG mouse via gavage. Mice were cohoused overnight in a single wire-bottom cage containing a moist paper towel. Approximately 5 g of fecal pellets were collected the next morning and examined via flotation for the presence of eggs. Because these fecal pellets were negative for Nb eggs, the mice were gavaged a second time the following day with a new sample using the method described above. After the second gavage, fecal pellets were collected daily and examined via fecal flotation for 11 d. On the final day, mice were euthanized, and a complete necropsy performed to identify the presence of adult worms.

Mice. These studies used female C57BL/6J (B6; The Jackson Laboratory, Bar Harbor ME), NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ (NSG; The Jackson Laboratory), J:NU (nude 1; The Jackson Laboratory), and Hsd:Athymic Nude-*Foxn1*^{nu} (nude 2; Envigo, Indianapolis, IN) that were 6 to 8 wk old. All mice were free of mouse hepatitis virus, Sendai virus, mouse parvovirus, minute virus of mice, murine norovirus, pneumonia virus of mice, Theiler meningoencephalitis virus, epizootic diarrhea of infant mice (mouse rotavirus), ectromelia virus, reovirus type 3, lymphocytic choriomeningitis virus, K virus, mouse adenovirus 1 and 2, polyoma virus, murine cytomegalovirus, mouse thymic virus, Hantaan virus, mouse kidney parvovirus, murine astrovirus 2, *Mycoplasma pulmonis*, *Citrobacter rodentium*, *Salmonella* spp., *Filobacterium rodentium*, *Clostridium piliforme*, *Corynebacterium bovis*, *Chlamydia muridarum*, fur mites (*Myobia musculi*, *Myocoptes musculinis*, and *Radfordia affinis*), pinworms (*Syphacia* spp. and *Aspiculuris* spp.), and *Encephalitozoon cuniculi* when the studies were initiated, as determined by testing naïve outbred Swiss Webster (Tac:SW) mice that had been repetitively exposed to soiled bedding from cages housing mice in the colony.

Rat. An 8-wk-old male Sprague–Dawley (Crl:SD [SD]; Charles River Laboratories, Wilmington, MA) was used to generate L_3 larvae for these studies. The rat was free of Sendai virus, Hantaan virus, Kilham rat virus, lymphocytic choriomeningitis virus, mouse adenovirus, pneumonia virus of mice, rat minute virus, rat parvovirus, reovirus type 3, Sendai virus, Theiler murine encephalomyelitis virus, Toolan H1 virus, *Filobacterium rodentium*, *Citrobacter rodentium*, *Clostridium piliforme*, *Corynebacterium kutscheri*, *Mycoplasma pulmonis*, *Pasteurella pneumotropica*, *Salmonella* spp., *Streptobacillus moniliformis*, *Encephalitozoon cuniculi*, fur mites, and pinworms (*Syphacia* spp. and *Aspiculuris* spp.) as determined by testing naïve outbred Sprague–Dawley (Crl:SD) rats exposed repetitively to soiled bedding from cages housing rats in the colony.

Husbandry and housing. Mice were housed in polysulfone static microisolation cages with stainless-steel wirebar lids (Allentown Caging, Allentown, NJ). The rat was housed in an individually ventilated polysulfone microisolation cage (model 4; Thoren Caging Systems, Hazelton, PA). Animals were housed on autoclaved corncob bedding (Bed-o'Cobs, Andersons, Maumee, OH), provided HCl-acidified (pH 2.5 to 2.8) reverse osmosis–purified water in polyphenylsulfone bottles with stainless-steel caps and sipper tubes (Techniplast, West Chester, PA), and fed a closed-formula γ-irradiated diet (LabDiet 5053; Purina Mills International, St Louis, MO) ad libitum. Each cage was provided with a Glatfelter paper bag containing 6 g of crinkled paper strips (EnviroPak; WF Fisher and Son, Branchburg, NJ) for enrichment. In addition, the rat received a gnaw toy (Nylabone, Neptune City, NJ). Cages were maintained under ABSL2 conditions in a climate-controlled cubicle at 72 ± 2 °F (22.2 ± 0.5 °C), 30% to 70% relative humidity, and a 12:12-h light:dark cycle (lights on, 0600–1800). Intracage temperature and relative humidity were measured and recorded by placing a data logger (Edstrom Date Logger, Waterford, WI) between the wirebar lid and filter top in 1 cage from each of 3 randomly selected groups during the 28 d of cohousing to confirm that environmental conditions at the cage level were optimal for the development of L_3 larvae.

All animal use was approved by Weill Cornell Medicine's IACUC and maintained in accordance with *the Guide for the Care and Use of Laboratory Animals, 8th Edition*.[20] All animal use was conducted in agreement with AALAS' position statements on the Humane Care and Use of Laboratory Animals and Alleviating Pain and Distress in Laboratory Animals. Weill Cornell Medicine's animal care and use program is AAALAC-accredited.

Generation of *N. brasiliensis* L_3 larvae. Approximately 130,000 Nb L_3 larvae generated by serial passage through Sprague–Dawley rats were kindly donated by the Rudensky Laboratory at the Memorial Sloan Kettering Cancer Center. Additional L_3 larvae were generated by inoculating a Sprague–Dawley rat with approximately 5000 L_3 suspended in 500 µL of sterile PBS (10× PBS, pH 7.4, Gibco) subcutaneously in the interscapular area by using a 23-gauge needle (BD PrecisionGlide Single-use Needle; Becton Dickinson, Franklin Lakes, NJ). At 6 dpi, the rat was placed in a barren cage containing a wire-grid floor and lined with water-moistened paper towels (Wypalls; Kimberly-Clark, Irving, TX).[6] Water-moistened paper towels were replaced daily. The presence of Nb eggs in the feces was confirmed on 6 dpi by flotation. Approximately

50 fecal pellets were collected during 7 to 11 dpi, placed in a 50-mL sterile conical tube (Falcon, Corning).[6] An equal volume of autoclaved activated granulated charcoal (Ebonex, Melvindale, MI) was added to form a coarse paste. The fecal and charcoal paste was evenly distributed over a moistened piece of 90-mm filter paper (Whatman; Cytiva, Marlborough, MA) placed in a 100-mm Petri plate (Falcon). Prepared Petri plates were placed into larger 150-mm Petri plates (Falcon) filled with approximately 20 mL of sterile water and covered to provide elevated humidity and were then incubated at room temperature for approximately 7 d.[6] Dishes were checked every 3 d to ensure they were sufficiently moist and to provide aeration by removing the lid for 1 min. Additional sterile water was added to maintain moisture as needed. L_3 larvae were harvested beginning on day 7 by adding approximately 1 mL of water to the periphery of the smaller dish plate using a sterile transfer pipette and gently swirling the dishes, followed by aspiration of the added water into a 50-mL conical tube (Falcon) as previously described.[6] L_3 larvae were washed by adding 20 mL of sterile water to the tube, the larvae allowed to settle, and the added water was gently aspirated. The larvae were washed 2 additional times with 40 mL of sterile water followed by 3 washes with 40 mL of sterile PBS (Gibco). Larvae were counted under a dissecting microscope by using a McMaster slide (FEC Source, Grand Ronde, OR) and were stored at 2,000 L_3 per mL, at room temperature, in foil-wrapped, vented, U-shaped 275-mL tissue culture flasks (Polystyrene tissue culture flasks with vented caps, Corning).

Inoculation of B6 and NSG mice with Nb larvae. A 5-mL aliquot of L_3 larvae was removed from a tissue culture flask, placed in a 50-mL conical tube (Falcon, Corning), and allowed to settle to form a pellet. The supernatant was then removed and 20 mL of sterile PBS was added. This procedure was repeated 3 times using the same tube; the final pellet was resuspended in approximately 3 mL of sterile PBS.[6] Larval counts in a 100-µL aliquot were determined by using a McMaster counting slide. The larval concentration was adjusted by using PBS to achieve 3750 L_3 larvae per milliliter.[6] The concentration determination was repeated 3 times to ensure accuracy. Approximately 750 L_3 suspended in 200 µL sterile PBS were injected subcutaneously into the interscapular area of a mouse by using a 23-gauge needle (BD PrecisionGlide Single-use Needle) as previously described.[6]

Detection and enumeration of Nb eggs. Individual mice were lightly restrained over a disposable towel (Wypall®) and 1 to 5 fecal pellets collected. Pellets were placed in 5-mL microcentrifuge tubes (Eppendorf, Hamburg, Germany) and their weight determined to calculate the number of eggs per gram of feces (epg). Fecal pellets were then soaked in 2 mL of a sodium nitrate solution with a specific gravity of 1.200 (Fecasol; Vetoquinol, Fort Worth, TX) for 15 to 20 min to soften. Then an additional 2 mL of the sodium nitrate solution was added. The contents of the tube were mixed again to form a homogenous slurry. An aliquot was immediately pipetted (Blood Bank Pipets; Copan Diagnostics, Murrieta, CA) into a McMaster counting chamber slide (FEC Source) until the chamber was filled. After 5 min, the slide was examined under a compound microscope (Eclipse E200; Nikon, Feasterville, PA) at 10× for the presence of Nb eggs. Egg counts were performed 3 times using different sample aliquots. The mean of the 3 counts was used to calculate the epg value.[6] When eggs were absent from all 3 of the slides examined, the sample was identified as negative. When eggs were only found outside of the counting chamber on any of the replicates, the sample was considered positive and recorded as less than 25 epg, which was the sensitivity of the McMaster counting slide reported by the manufacturer.

Collection and quantification of adult Nb. After euthanasia, the proximal half of the small intestine was excised and placed in a 100-mm Petri plate (Falcon, Corning) containing 1 mL PBS. The intestine was sliced longitudinally to reveal the mucosa and then cut into approximately 3- to 5-mm lengths, placed in cheese cloth (Natural Ultra Fine Cheesecloth; Regency Wraps, Dallas, TX), submerged in a 50-mL conical tube (Falcon) containing 45 mL PBS, and incubated in a water bath for 2 h at 37 °C (98.6 °F). After incubation, tubes were examined visually for any adult worms that had settled to the bottom, and the small intestines were examined with a dissecting microscope for any worms left attached to the mucosa. Worms were collected by using a transfer pipette (Blood Bank Pipets; Copan Diagnostics, Murrieta, CA), and the total number collected was counted under a dissecting microscope based on reported methods.[6,25]

Isolation and counting of L_3 larvae using the Baerman technique. A Baerman apparatus consisting of a funnel, plastic tubing, and a clamp was used.[6] The contents from each culture dish maintained under different environmental conditions were placed in cheese cloth (Natural Ultra Fine Cheesecloth; Regency Wraps) and cut into an 8 × 8-cm square that was tied with a piece of string to form a pouch attached to a wooden applicator. Warm (37 °C) PBS was added until it reached approximately 1 cm below the rim of the funnel. Pouches were placed into funnels such that they were suspended by the wooden applicator and their contents were completely submerged. Additional PBS was added as needed until the pouches were completely submerged.[6] After 24 h, a 5-mL sample was collected into a 15-mL conical tube (Falcon, Corning) by slowly releasing the clamp placed at the bottom of the tube. The samples were left alone for 5 min to allow L_3 to settle. The L_3 larvae were aspirated and counted under a dissecting microscope by using a McMaster counting slide as previously desctibed.[6]

Histopathology. After mice were euthanized by CO_2 asphyxiation, the distal half of the small intestine (including jejunum and ileum), sections of the cecum, the proximal, middle, and distal colon, the mesenteric lymph nodes, all lung lobes, the tracheobronchial lymph nodes, heart, and spleen were removed and fixed in 10% neutral buffered formalin. Tissues were processed in ethanol and xylene and then embedded in paraffin by using a tissue processor (ASP6025; Leica Biosystems, Richmond, IL). Paraffin blocks were sectioned at a thickness of 5 µm, stained with hematoxylin and eosin, and examined by a board-certified veterinary pathologist (SEC).

Determination of serum IgE concentrations. Immediately after euthanasia, 0.5 to 0.7 mL of blood was collected via cardiocentesis or from the caudal vena cava from 8 Nb-inoculated B6 mice and 16 naïve B6 mice and placed in serum separator tubes (BD Microtainers, Becton Dickinson). Clotted blood samples were centrifuged at 171 × *g* for 10 min at room temperature, and serum was collected and stored at −80 °C until analyzed. Total IgE concentrations in these samples were determined by using a commercially available IgE ELISA kit (IgE Mouse uncoated ELISA Kit, Invitrogen, Thermo Fisher Scientific) according to the manufacturer's instructions. In brief, microliter wells were coated with capture antibody (pretitrated, purified antimouse antibody) in coating buffer, sealed, and incubated at 4 °C (39 °F) overnight. The wells were aspirated and washed (1× PBS, 0.05% Tween 20), blocked (1:10 dilution with deionized water of 20× PBS containing 1% Tween 20 and 10% BSA), and incubated for 2 h at 4 °C. Wells were aspirated and washed, and serum

samples diluted 1:50 in sample diluent were added in duplicate. Samples then were aspirated, and wells washed prior to the addition of pretitrated, biotin-conjugated antimouse IgE monoclonal antibody. The plate was sealed with parafilm and incubated for 1 h at room temperature on a microplate shaker at 400 rpm. Detection antibody was removed, the wells were washed, and pretitrated streptavidin was added. Detection enzyme was removed, and the wells were washed prior to addition of tetramethylbenzidine. The reaction was stopped by adding 1 M H_3PO_4, and plates were read at 450 nm (ELx808 Ultra; Bio-Tek Instruments, Winooski, VT). The mean of duplicate samples of total IgE concentrations was determined by comparison against a standard curve.

Statistical analysis. Cumulative mean epg values for Nb-inoculated NSG and B6, nude-1 and nude-2 mice were compared by using the Mann–Whitney *U* test with the Holm–Šídák method to compare egg shedding on individual days. Total IgE serum concentrations in Nb-inoculated and naïve B6 mice were compared by using the Mann–Whitney *U* test. One-way ANOVA was used to compare the cumulative mean epg values of naïve and Nb-inoculated mice and the 4 different environmental conditions under which eggs were incubated, with the Tukey multiple-comparison test performed to compare individual variables. A *P* value of less than or equal to 0.05 was considered statistically significant. All statistical analyses were performed by using Prism version 9.4.1 (GraphPad Software, La Jolla, CA).

Results

Intracage transmission. ***Egg shedding kinetics in Nb inoculated mice.*** All Nb-inoculated NSG ($n = 12$) and B6 ($n = 6$) mice developed patent infections (Figure 1). Eggs were first detected in the feces of most NSG (8 of 12; 67%) and B6 (10 of 12; 83%) mice on 6 dpi. In the remaining mice, eggs were first observed on 7 dpi, except for one NSG mouse for which eggs were first detected on 9 dpi. Cumulative epg values over 30 d (mean ± SEM) were significantly higher ($P < 0.05$) in NSG mice (14,936 ± 2686) as compared with B6 mice (1647 ± 1223). However, epg values at 7 and 8 dpi were not significantly different in B6 mice and NSG mice. In B6 mice, epg values peaked at 7 dpi, and no eggs were detected after 9 dpi. In NSG mice, peak shedding occurred at 9 dpi and they continued to shed lower numbers of eggs through the endpoint of the study at 35 dpi.

Horizontal transmission of Nb to naïve B6 and NSG mice. Eggs were detected intermittently in varying numbers in 10 of the 12 (83%) naïve B6 and 9 of the 12 (75%) naïve NSG mice cohoused with Nb-inoculated NSG mice during the 28-d evaluation period. Eggs were detected as early as 1 dph in 2 of 12 B6 and 2 dph in 2 of 12 B6 mice (Figure 2). The percentage of naïve B6 and NSG mice shedding eggs varied daily throughout the period of cohousing with Nb-inoculated NSG mice (Figure 3). The number of days during cohousing in which eggs were detected in the feces of individual naïve mice ranged from 0 to 17. No eggs were identified in fecal pellets of naïve NSG ($n = 12$) or B6 ($n = 12$) mice cohoused with Nb-inoculated B6 mice. Mean cumulative epg values were not significantly different in naïve B6 and NSG mice. The naïve mice with the highest epg values had been cohoused with the Nb-inoculated NSG mice that had shed the highest number of eggs. None of the naïve mice had lesions suggestive of Nb infection, larvae in the lungs or adult worms in the gastrointestinal tract.

Adult (L_3) worm counts. No adult worms were detected in Nb-inoculated B6 or in naïve NSG and B6 mice. However, all 12 Nb-inoculated NSG mice had adult worms (mean ± SEM, 16.7 ± 5.6) in the small intestine.

Serum IgE concentration. Mean serum IgE levels on 28 dph were significantly higher ($P < 0.05$) in Nb-inoculated B6 mice than in naïve B6 mice (Figure 4). Although one naïve B6 mouse had an elevated IgE concentration (2993 ng/mL) as compared with other naïve B6 mice ($n = 15$; mean ± SEM, 429 ± 90), no eggs or adult worms were detected in this mouse, nor were lesions suggestive of an Nb infection seen on histopathology.

Histopathology. Complete necropsies were performed on Nb-inoculated (B6, $n = 11$; NSG, $n = 12$) and naïve (B6, $n = 24$; NSG, $n = 24$) mice at 35 dpi. On gross necropsy, the lungs from the majority (20 of 23; 87%) of Nb-inoculated mice failed to collapse, were mottled, and had variable degrees of pulmonary emphysema characterized by multifocal, gas-filled, cystic-like structures on the pulmonary surface. Due to tissue damage during storage, histopathology was unavailable for a single Nb-inoculated B6 mouse. Microscopically, the lungs in 10 of 11 (91%) Nb-inoculated B6 and all 12 (100%) Nb-inoculated NSG mice showed multifocal to coalescing regions of pulmonary

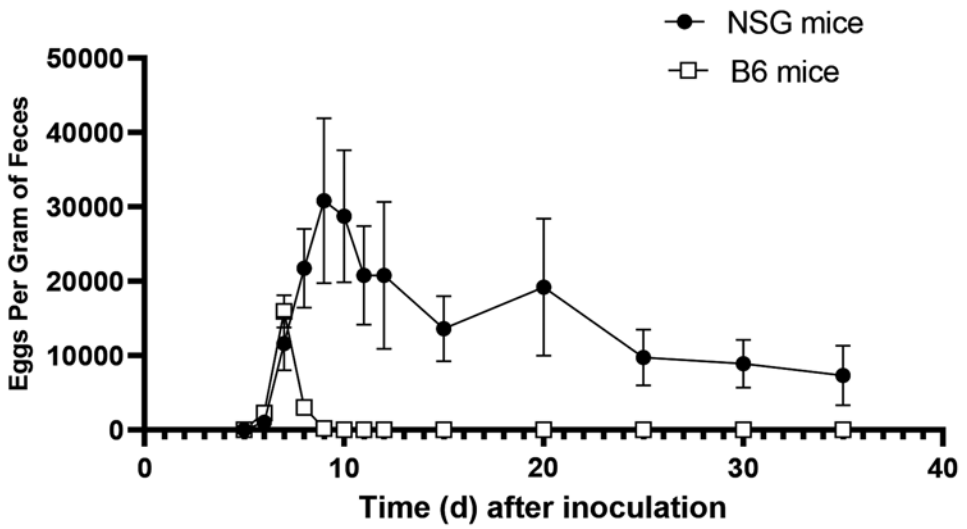

Figure 1. Number of eggs per gram of feces (mean ± SEM) for B6 ($n = 6$) and NSG ($n = 12$) mice inoculated with Nb on various days after inoculation for the study duration of 35 d.

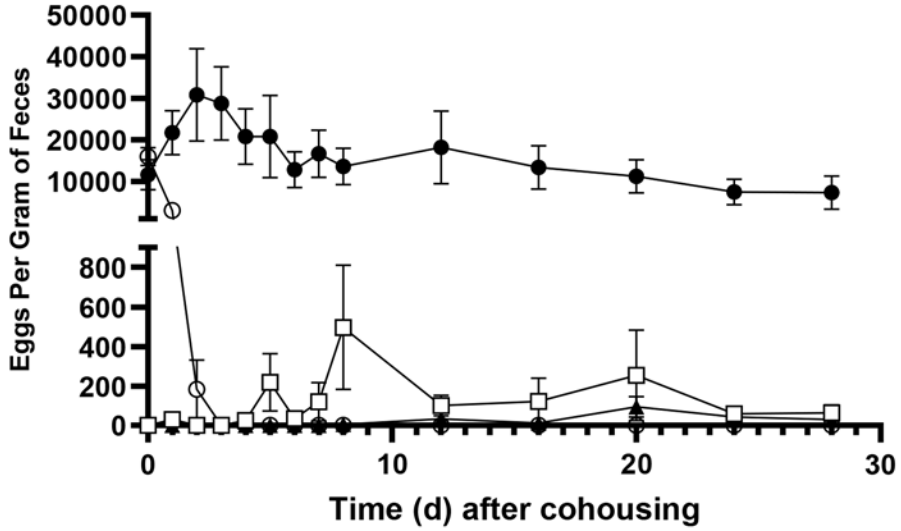

Figure 2. Number of eggs per gram of feces (mean ± SEM) for naïve B6 (n = 12) and naïve NSG mice (n = 12) cohoused with Nb-inoculated NSG (n = 12) or Nb-inoculated B6 (n = 6) mice on days 1 through 28 after cohousing. Open circles, Nb-inoculated B6 mice; solid circles, Nb-inoculated NSG mice; open squares, naïve B6 cohoused with Nb-inoculated NSG mice; solid triangles, naïve NSG cohoused with Nb-inoculated NSG mice.

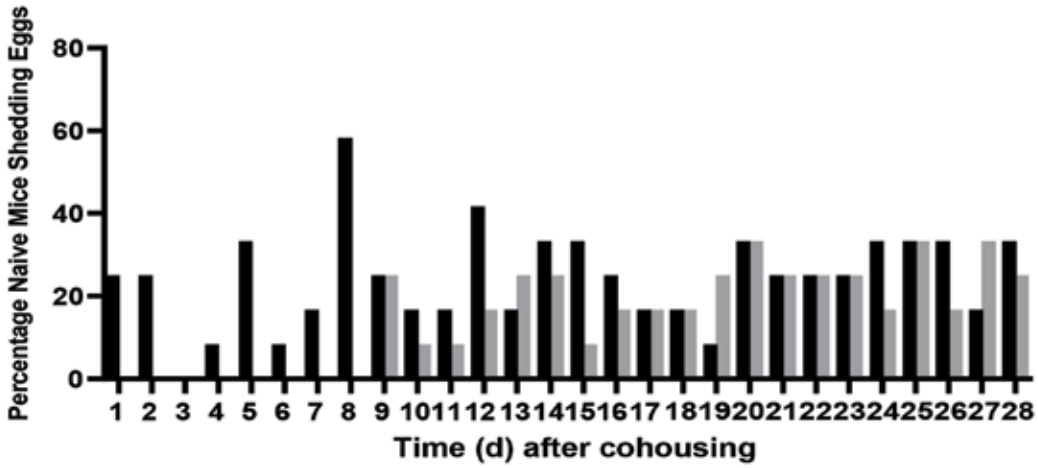

Figure 3. Percentage of naïve NSG (n = 8; gray bars) and B6 (n = 8; black bars) mice from 1 until 6 d after cohousing and for all naïve NSG (n = 12) and B6 (n = 12) mice from 7 until 28 d after cohousing that had eggs detected in feces while being cohoused with Nb-inoculated NSG mice.

emphysema characterized by alveolar septal wall loss and dilatation (Figure 5). Alveolar histiocytosis, hemorrhage, and hemosiderin-laden macrophages accompanied the lesions in most Nb-inoculated B6 (10 of 11; 91%) and NSG (12 of 12; 100%) mice (Figure 5 F). Although Nb larvae rarely were seen in the airways of either mouse strain, the lesions are consistent with alveolar damage induced by migrating nematode larvae. In addition, the lungs of Nb-inoculated B6 mice (8 of 12; 67%) often had focal to multifocal areas of perivascular and peribronchiolar inflammation (Figure 5 C), whereas this lesion was seen only occasionally in the lungs of Nb-inoculated NSG mice (2 of 12; 17%). The terminal bronchioles of a subset of Nb-inoculated NSG cases (6 of 12; 50%) had individual or small clusters of epithelioid macrophages and pyknotic cells containing intracytoplasmic coarsely granular dark-brown pigment (Figure 5 E), which reflects prior bronchiolar epithelial damage and hemorrhage after L_3 migration. In contrast, none of the naïve B6 and NSG mice cohoused with Nb-inoculated mice had gross abnormalities, alveolar emphysema, pulmonary hemorrhage, or hemosiderin-laden macrophages at euthanasia on 28 dph (Figure 5). Alveolar histiocytosis was seen in a small number of naïve B6 (4 of 24; 17%) and NSG (8 of 24; 33%) mice, and perivascular or peribronchiolar inflammation was identified in 7 of 24 (29%) naïve B6 mice.

Consistent with Nb histopathology, the small intestinal lumens in fewer than half of the Nb-inoculated NSG mice (5 of 12; 42%) contained individual adult nematodes (Figure 6 C), whereas no adult nematodes were seen microscopically in the small intestine of Nb-inoculated B6 mice. Nb eggs occasionally were observed under brightfield microscopy in the cecal and/or colonic content of (3 of 12; 25%) Nb-inoculated NSG mice euthanized on 35 dpi (Figure 6 F). The small intestine, cecum, colon, heart, lymph nodes, and spleen were histologically normal in all Nb-inoculated and naïve mice.

Intracage temperature and humidity levels. Temperature and humidity (mean ± SEM) measured in a single cage from each of 3 randomly selected groups in different cohort repetitions during the 28 d of cohousing were 22.8 ± 0.02 °C and 83.9% ± 0.22%.

Cage inoculation. No Nb eggs were detected in fecal samples collected for 21 consecutive days from any of the 9 NSG mice housed in cages spiked twice with 10,000 infective Nb L_3.

Neither adult worms nor lesions suggestive of Nb infection were identified at necropsy or on histopathology at 35 dph.

In vitro larval development. Nb eggs developed into infective L_3 larva under 2 different environmental conditions (moist and control). Nb L_3 larval counts were not significantly different (P = 0.13) in moist bedding plates (mean ± SEM, 1095 ± 205 larvae) as compared with control plates (710 ± 136 larvae). No L_3 larvae were found under dry or soiled environmental conditions.

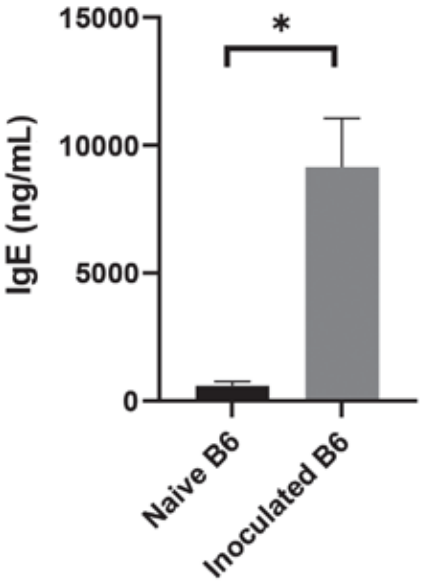

Figure 4. Serum IgE concentrations (mean ± SEM) for naïve B6 mice (n = 16) cohoused with Nb-inoculated B6 or NSG mice at 28 d after housing and Nb-inoculated B6 mice (n = 8) at 35 d postinfection. *, Value differed significantly (P < 0.05) between Nb-inoculated B6 mice and naïve B6 mice.

Additional studies conducted to investigate unexpected results.

Nb inoculation of athymic nude stocks. Eggs were detected after Nb-inoculation in only 2 of 6 (33%) mice of both nude stocks (Figure 7) and were first detected on 7 dpi. Peak egg shedding occurred at 7 dpi in nude-1 mice and at 8 dpi in nude-2 mice. Nude-1 mice ceased to shed eggs after 9 dpi, whereas nude-2 mice ceased shedding on 10 dpi. The cumulative mean epg value was not significantly different between stocks.

Grossly, the lungs from the majority (11 of 12; 92%) of Nb-inoculated nude mice failed to collapse, were mottled, and had variable degrees of pulmonary emphysema characterized by multifocal gas-filled cystic-like structures on the pulmonary surface. Microscopically, the lungs of Nb-inoculated nude mice (11 of 12; 92%) had lesions consistent with Nb larva migration, characterized by multifocal to coalescing regions of pulmonary emphysema with alveolar histiocytosis with or without perivascular and peribronchiolar inflammation, hemosiderin-laden macrophages, and hemorrhage. No histopathologic lesions were seen in the small intestines, cecum, colon, heart, lymph nodes, or spleen, and no adult nematodes or eggs were detected in the intestines at necropsy on 35 dpi.

Gavage of feces containing Nb eggs. To determine whether naïve mice that unexpectedly shed eggs had a patent Nb infection or whether the eggs detected reflected passage of eggs ingested during coprophagy, 3 NSG mice were gavaged twice with a sugar solution containing approximately 200 Nb eggs. The initial pooled fecal sample from the gavaged mice was negative; 6 eggs were observed in the pooled fecal sample from the

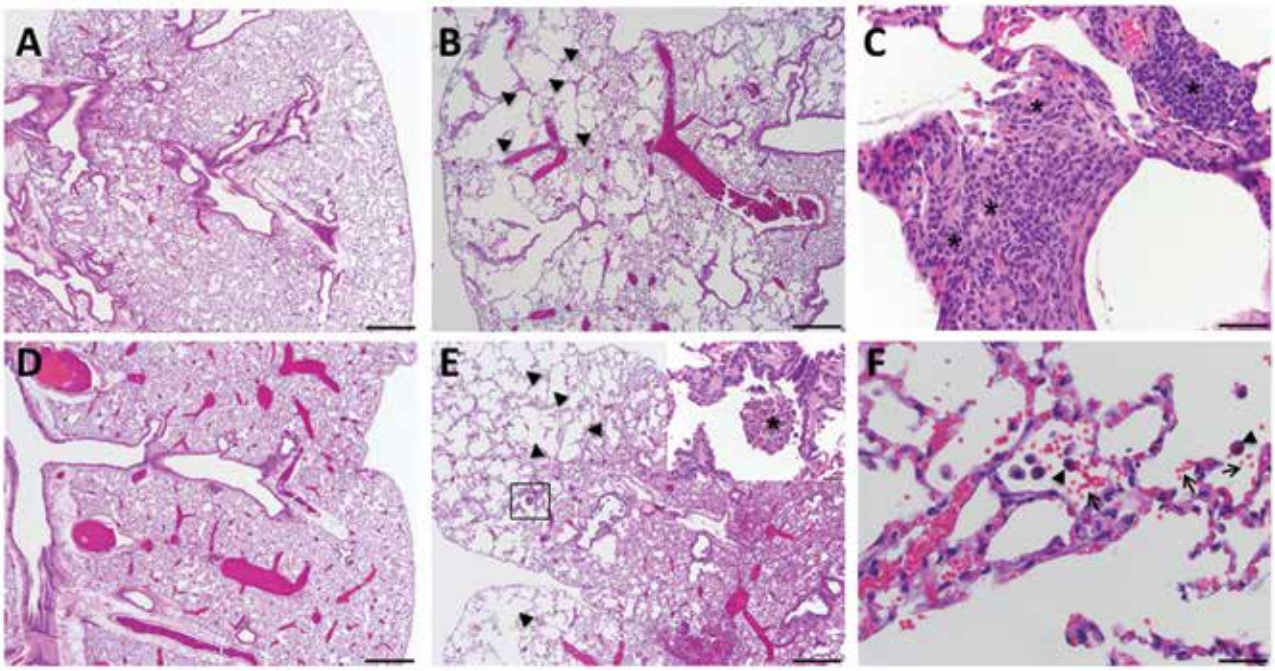

Figure 5. Pulmonary histology of (A and D) naïve and (B, C, E, and F) Nb-inoculated (A–C) B6 and (D–F) NSG mice. (A) Normal bronchi, bronchioles, and alveoli from a naïve B6 mouse (magnification, 4×; scale bar, 500 µm). (B) Lung from a Nb-inoculated B6 mouse demonstrated marked multifocal to coalescing regions of alveolar emphysema (arrowheads; magnification, 4×; scale bar, 500 µm) with (C) foci of perivascular and interstitial inflammation composed of mixed histiocytic, lymphocytic, and neutrophilic infiltrates (asterisk, magnification, 40×; scale bar, 50 µm). (D) Normal bronchi, bronchioles, and alveoli from a naïve NSG mouse (magnification, 4×; scale bar, 500 µm). (E) Lung from a Nb-inoculated NSG mouse with marked multifocal areas of alveolar emphysema (arrowheads; magnification, 4×; scale bar, 500 µm) and clusters of epithelioid macrophages and pyknotic cells containing intracytoplasmic, coarsely granular dark-brown pigment (asterisks) within the bronchiolar lumens (inset; magnification, 40×; scale bar, 50 µm). (F) The emphysematous alveolar spaces often contained individual hemosiderin-laden macrophages (arrowhead) associated with extravasated erythrocytes (arrows; magnification, 40×; scale bar, 50 µm).

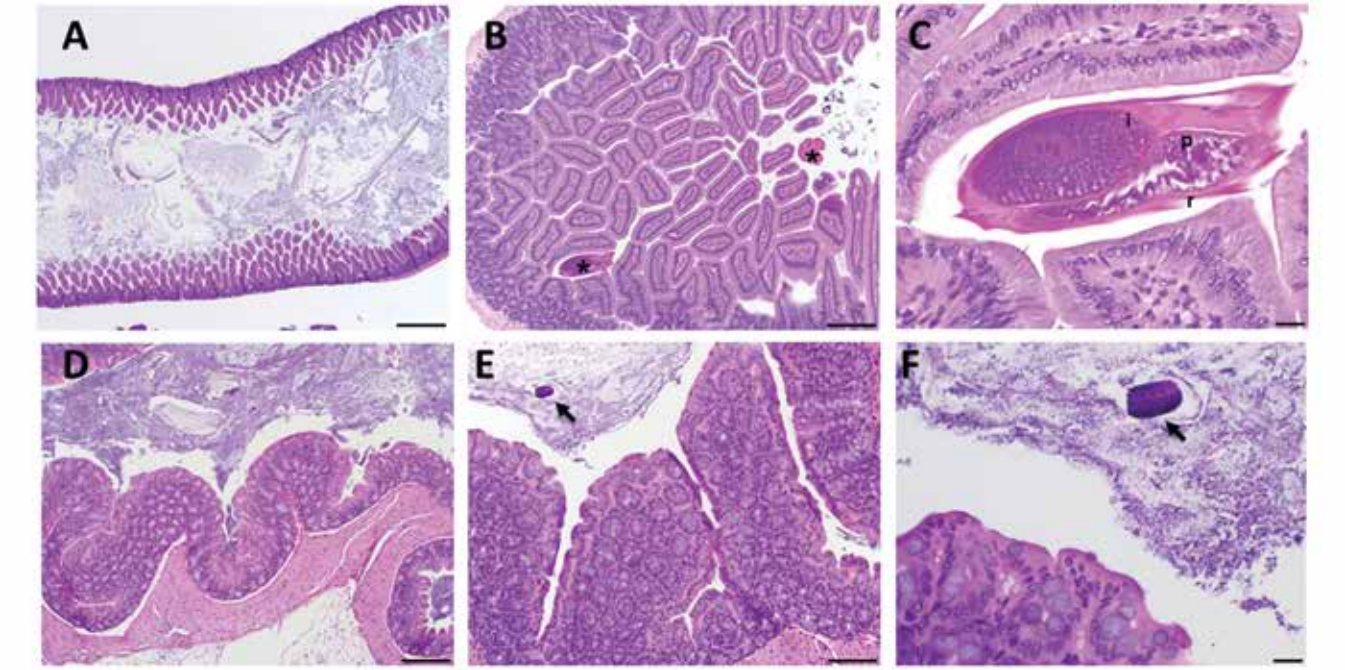

Figure 6. Histology of the (A–C) small and (D–F) large intestine of (A and D) naïve and (B, C, E, and F) Nb-inoculated NSG mice. (A) Small intestine from a naïve NSG mouse (magnification, 4×; scale bar, 500 µm). (B) Small intestine from a Nb-inoculated NSG mouse, demonstrating 2 Nb adult nematodes (asterisks) associated with normal villous epithelium at day 35 postinoculation (magnification, 10×; scale bar, 200 µm). (C) Nb adult (L_5) nematode with unevenly spaced external cuticular ridges (c) and digestive tract (i) in the pseudocoelom (p; magnification, 60×; scale bar, 20 µm). (D) Colon from a naïve NSG mouse (magnification, 10×; scale bar, 200 µm). (E) Colon from a Nb-inoculated NSG mouse, showing a morulated nematode egg (arrow) in the intestinal lumen (magnification, 20×; scale bar, 100 µm). (F) High-power image of the section in panel E, with nematode egg (arrow; magnification, 60×; scale bar, 20 µm).

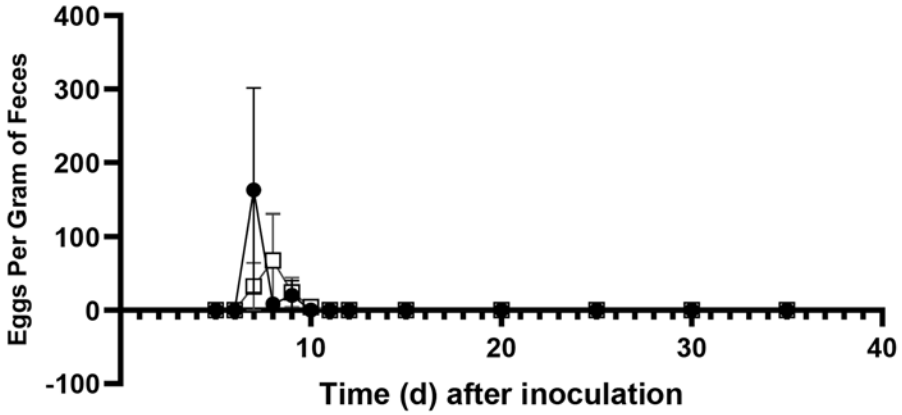

Figure 7. Number of eggs per gram of feces (mean ± SEM) for nude-1 (n = 6; solid circles) and nude-2 (n = 6; open squares) mice inoculated with Nb L_3 larvae on day 0. Egg shedding was assessed on various days until 35 d after inoculation.

NSG mice at approximately 16 h after the second gavage. No additional eggs were detected in feces collected for the next 10 consecutive days, no adult worms were observed at necropsy, and no lesions consistent with Nb infection were identified on histopathology.

Discussion

Our data demonstrate that horizontal transmission of Nb did not occur, even when experimentally infected, chronically shedding mice were cohoused with naïve highly immunocompromised cagemates under ideal intracage conditions (temperature and humidity) for L_3 development in static isolation cages changed every 14 d.[6,25] Furthermore, highly susceptible NSG mice did not become infected when housed in cages spiked with large numbers of L_3 larvae. Although eggs were able to develop into L_3 larvae under ideal artificial environmental conditions (moist and control), they did not develop under conditions used to replicate the intracage environment (dry and soiled).[6,25]

Although eggs were detected in the feces of naïve mice cohoused with Nb-inoculated, egg-shedding NSG mice, we provide data to support our conclusion that these eggs were ingested during coprophagy, given that they were detected too early to reflect a patent infection, and mice gavaged with Nb eggs did not become infected. Eggs were detected only in naïve NSG and B6 mice that were cohoused with Nb-inoculated NSG mice, which shed large numbers of eggs for the entire period of cohousing. No eggs were identified in naïve NSG and B6 mice when they were cohoused with Nb-inoculated B6 mice, which shed considerably fewer eggs for a short period.

When eggs were detected in naïve mice, the number shed was low and shedding was sporadic, and even though eggs were detected in the feces, no adult worms nor lesions consistent with Nb larval migration infection were found at necropsy. In contrast, all Nb-inoculated NSG mice were still shedding eggs at necropsy, L_5 nematodes were identified in all mice, and almost all Nb-inoculated B6 mice had histologic evidence of larval migration in their lungs at 35 dpi. Our observations of egg shedding in naïve B6 mice cohoused with Nb-inoculated NSG mice are contrary to what has been described in the literature, and what we observed in the Nb-inoculated B6 mice; B6 mice typically shed high numbers of eggs for a few days and then stop shedding by 10 dpi.[6,35,44] In addition, once infected B6 mice expel the parasite, they have lifelong immunity.[5,6,19]

During our study, eggs initially were detected in the feces of naive B6 mice on day 7 dph and were detected intermittently thereafter until 28 dph. Nb eggs embryonate and hatch approximately 5 to 6 d after fertilization.[5,6,18,25] Because we had not expected to find eggs on 7 dph, we began fecal egg counts at 1 dph in subsequent cohorts; eggs were still detected at this time point, providing further evidence that the eggs were passing through the gastrointestinal tract after the ingestion of egg-containing feces during coprophagy. Rats experimentally infected with low doses of L_3 larvae daily over weeks, mimicking natural exposure, shed eggs persistently and also had L_5 Nb in their intestines at necropsy.[23,24] To confirm our hypothesis, NSG mice were gavaged with eggs collected from fresh fecal pellets obtained from Nb-inoculated NSG mice. The mice were gavaged twice because eggs were not detected after the first administration. A few eggs were identified in fecal pellets collected from mice on the day after the second gavage. No additional eggs were found in fecal samples collected over the next 10 consecutive days, and no adult worms or microscopic lesions suggestive of Nb infection were found at necropsy. We speculate that the negative result we found on the day after the initial administration was due to egg degradation in the gastrointestinal tract and the small sample size.

Nb infection is known to induce production of polyclonal and specific IgE.[5,6,32,43] Total serum IgE levels are elevated in Nb-inoculated BALB/c mice within 3 to 5 d after infection, peak at 10 to 17 d, and remain elevated for more than 60 d.[43] One study demonstrated that total IgE serum concentrations in Nb-inoculated BALB/c mice reached a maximum of 200 µg/mL at 15 dpi, but levels declined to 40 µg/mL by day 25 dpi.[27] In B6 mice, mean total serum IgE levels measured 14 d after inoculation were 2060 ± 753 ng/mL.[28] In our study, total IgE concentrations in Nb-inoculated B6 mice differed from those previous reports; however, mouse strain, collection time point, and possibly assay differences likely account for the variations. The B6 strain is well known to mount a Th1-dominant response, whereas the response of BALB/c mice is Th2-skewed.[36] Rats given low doses of Nb L_3 larvae meant to simulate natural infection develop significant and sustained IgE responses.[41] Therefore, if horizontal transmission occurred, we would have expected to detect markedly elevated IgE levels in naïve B6 mice. Although a single naïve B6 mouse had an elevated serum IgE concentration, no eggs were detected during 28 d of cohousing, and neither lesions consistent with Nb infection nor nematodes were detected at necropsy. IgE concentrations can be elevated for a variety of reasons, including biologic variation and exposure to allergens. In addition, the finding that IgE levels of B6 naïve mice with Nb eggs in their feces were significantly lower than those of Nb-inoculated mice provides further support that these eggs were ingested and not indicative of patent infections.

Although Nb infections have been studied in immunocompromised mouse strains, our current study reflects the first time that the infection has been characterized in the severely immunocompromised NSG strain.[1,4,10,19,21,22,31,35,37] NSG mice reached peak egg shedding at 9 dpi and shed markedly more eggs than B6 mice during the study period. Although the quantity of eggs shed did decrease over time, NSG mice continued to shed high numbers of eggs throughout the study, suggesting that these mice likely would remain persistently infected.

We originally used athymic nude mice as the immunocompromised strain for this study because of their reported ability to shed high numbers of eggs for a protracted period due to chronic infection.[1,14,21,22,31] However, we found eggs in only 2 of the 6 nude-1 mice, the number of eggs shed was low, and shedding was sporadic and ceased at 9 dpi. To determine whether these findings were a result of genetic differences, a different nude stock from an alternate vendor (nude-2) was inoculated with the same number of L_3 larvae and yielded similar results. Pulmonary lesions, consistent with larval migration, were identified in 92% of the athymic nude mice with no discernable differences in lesion severity between the 2 stocks. Although patent infection did develop, no L_5 Nb were detected at 35 dpi. Resistance to Nb infection in FVB/N mice and IL5-transgenic mice has been reported, but has not been described in athymic nude mice.[11,12,26] Pulmonary lesions consistent with Nb infection and low to absent egg counts suggest that damage to larvae likely occurred during early stages of infection, implicating the innate immune system as a potential source of resistance.[11,12,26] Athymic nude mice are resistant to some pathogens, including experimental cutaneous infection with *Bacillus anthracis*, *Candida albicans*, *Listeria monocytogenes*, and *Brucella abortus*.[7,13,17,40] Proposed mechanisms for resistance include potent neutrophilic responses, augmented effects of prior microbial exposure in stimulating the innate immune system due to lack of T-cell regulatory responses, and enhanced activated macrophage functions.[7,13,17,40] The differences between our observations and earlier studies using athymic nude mice could be attributed to the use of inbred compared with outbred nude mice, the number of larvae administered, and the route of inoculation.[1,14,21,22,31] In addition, parasite infectivity and worm burden can be affected by the strain of nematode used, and the number of passages and time since hatching can contribute to changes in parasite viability and variations in worm burden.[5] Finally, serial passage of Nb through rats rather than mice might have altered infection kinetics in athymic nude mice.[40]

The pulmonary changes seen in all Nb-inoculated mice were consistent with the chronic phase of Nb infection, which is characterized by pulmonary emphysema with alveolar hemorrhage and hemosiderin-laden macrophages in the absence of an overt inflammatory leukocytic cell infiltrate.[5,29,33] Damage to lung tissue occurs as the L_3 larvae migrate from blood vessels into alveolar spaces during early stages of Nb infection.[5,8,9,29] Mechanical tissue destruction during larval migration causes leakage of erythrocytes into alveolar spaces, which is resolved by the recruitment of alveolar macrophages and erythrophagocytosis.[5,8] In mice, Nb-induced emphysema typically is studied at around 30 dpi.[5] In our study, we wanted to compare histopathologic lesions in Nb-inoculated mice with those of cohoused naïve mice to determine whether horizontal transmission occurred. No lesions consistent with chronic Nb infection were identified in the lungs of any naïve mice. Alveolar histiocytosis was identified in a few naïve B6 and NSG mice, and perivascular and peribronchiolar inflammation was identified in about a third of the naïve B6 mice. These lesions

are known incidental background lesions in these strains and are not suggestive of Nb infection.[34]

Small intestinal inflammation, villous atrophy, flattening and fusion, and crypt hyperplasia have been described in heavy Nb infections.[3,25] However, small intestinal lesions were not noted in Nb-infected or naïve mice. In our study, the proximal half of the small intestine was removed to perform nematode counts because L_5 Nb typically inhabit the proximal portion of the small intestine and thus was not examined microscopically. This omission may explain why small intestinal lesions were not observed and why only a few adult worms were found in the small intestines of Nb-infected NSG mice on histopathology. Another reason that lesions may have been absent in the gastrointestinal tract is that necropsies were performed in Nb-inoculated B6 mice at 35 dpi rather than during the period of active infection (6 to 10 dpi).[5,6,35,44]

NSG mice were housed in cages spiked with 10,000 infective L_3 larvae on days 7 and 21 to model a 'worst case' scenario with a high likelihood that larvae would penetrate the skin, resulting in infection. Larvae initially were added to the cage at 7 d after housing to ensure that the bedding was moist enough to support larval survival by promoting skin penetration. Fecal flotation performed daily after the cages were initially spiked remained egg-free. In addition, no adult L_5 Nb or lesions suggestive of infection were identified at necropsy.

Our data showed that although eggs could develop, hatch, and release L_3 larvae under extremely moist environmental conditions, larvae did not develop under environmental conditions likely to be found in mouse cages, despite the use of the optimal temperature and humidity for Nb development.[5,6,25] The data suggests that moisture is crucial to the ability of eggs to hatch and develop into L_3 larvae. Although soiled plates did contain moisture, urine has an adverse effect on eggs, perhaps explaining why L_3 larvae did not develop under these conditions.[6]

An unexpected challenge in this study was the inability to use fecal flotation to confirm horizontal transmission. Typically egg counts are used to confirm and quantify infection in Nb-inoculated mice, but this method was ineffective in our study due to coprophagy.[6] Another challenge was that because mice were cohoused for 28 d to allow sufficient time for horizontal transmission to occur, a definitive day after exposure has not been identified for detecting larvae in the skin or lungs or nematodes in the small intestine.[2,5,6,18,25] A potential limitation of our study was that we limited assessment of immune response to total serum IgE in B6 mice. We chose to measure IgE because it remains elevated for an extended period after infection.[15,27,43] Natural and experimental infection of immunocompetent mice with Nb induces a profound $CD4^+$, Th2-dependent immune response characterized by the production of IL4, IgE, IL13, IL10, alternatively activated macrophages, type 2 innate lymphoid cells, Th2 thymocyte expansion, IL5-dependent eosinophil recruitment, goblet cell hyperplasia, and mastocytosis.[5,6,37,39] Further analysis of the lungs and gastrointestinal tract could have been performed to examine cytokine levels and changes in T-cell, eosinophil, and type 2 innate lymphoid cell populations.[15,30] In addition, we used a rat-adapted Nb strain, which might have altered infectivity kinetics. However, many laboratories that use the parasite to interrogate the mouse immune system use rat-adapted Nb, and we inoculated our mice with the recommended and commonly used larval dose.[6,41]

In summary, our current study provides compelling objective evidence that horizontal transmission of Nb in mice under research conditions does not occur under various scenarios expected to provide optimal conditions for its transmission. The scope of this study did not include horizontal transmission of Nb in rats used for Nb propagation or as an experimental model of Nb infection. These results led our institution to allow the use of ABSL1 practices for studies of murine Nb infection, rather than the ABSL2 standards that were previously required. Our findings may inform decisions of other institutions regarding the appropriate biosecurity measures for using Nb-infected mice.

Acknowledgments

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