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Experimental Transmission of *Corynebacterium pseudotuberculosis* Biovar *equi* in Horses by House Flies

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Background: The route of *Corynebacterium pseudotuberculosis* infection in horses remains undetermined, but transmission by insects is suspected.

Objectives: To investigate house flies (*Musca domestica* L.) as vectors of *C. pseudotuberculosis* transmission in horses.

Animals: Eight healthy, adult ponies.

Methods: Randomized, controlled, blinded prospective study. Ten wounds were created in the pectoral region where cages for flies were attached. Three ponies were directly inoculated with *C. pseudotuberculosis*. Four ponies were exposed for 24 hours to 20 hours *C. pseudotuberculosis*-inoculated flies. One negative control pony was exposed to noninoculated flies. Ponies were examined daily for swelling, heat, pain, and drainage at the inoculation site. Blood was collected weekly for CBC and biochemical analysis, and twice weekly for synergistic hemolysis inhibition titers.

Results: Clinical signs of local infection and positive cultures were observed in 7/7 exposed ponies and were absent in the negative control. In exposed ponies, peak serologic titers (1 : 512 to 1 : 2,048) were obtained between days 17 and 21. Seroconversion was not observed in the negative control. Neutrophil counts were higher in the positive and fly-exposed groups than in the negative control ($P = .002$ and $P = .005$) on day 3 postinoculation. Serum amyloid A concentrations were higher in the positive control than in the negative control and fly-exposed ponies on days 3 ($P < .0001$) and 7 ($P = .0004$ and $P = .0001$). No differences were detected for other biochemical variables.

Conclusions and Clinical Importance: House flies can serve as mechanical vectors of *C. pseudotuberculosis* and can transmit the bacterium to ponies.

Key words: Equine; *Musca domestica*; Pigeon fever; Vector.

Corynebacterium pseudotuberculosis (Actinomycetales: Corynebacteriaceae) is a pleomorphic, facultatively intracellular, Gram-positive rod, with a worldwide distribution.[1] The 2 described biotypes of *C. pseudotuberculosis* are distinguished by genetic characteristics, including restriction fragment length polymorphisms and the ability of cultured organisms to reduce nitrate to nitrite.[2] The nitrate-positive biovar *equi* is commonly isolated from horses and cattle but the nitrate-negative biovar *ovis* typically is recovered from small ruminants and cattle.[3] In ruminants, *C. pseudotuberculosis* causes caseous lymphadenitis, abscesses, and occasionally mastitis.[4] In horses, *C. pseudotuberculosis* infection manifests itself in 3 clinical forms of disease.[5] The most common form, known as "pigeon fever" or "dryland distemper", causes external SC abscessation, especially in the pectoral region; a second form causes abscesses of internal organs including liver, lung, and kidneys; and, a third form causes ulcerative lymphangitis in the limbs.[5] Natural infection in horses caused by the ovine biovar and vice versa is not known to occur.[6] Infection of humans, although rare, is considered an occupational zoonosis.[7]

Abbreviations:

SAA	serum amyloid A
SHI	synergistic hemolysis inhibition

Very little is known about *C. pseudotuberculosis* biovar *equi* infection, except that it is unpredictable and contagious. Previously confined to the arid, western United States (California, Utah, Colorado and Texas) and Brazil, *C. pseudotuberculosis* cases have been recently increasing in regions where the disease previously was unknown or considered nonendemic (Wyoming, Kentucky, Tennessee, Oklahoma, Louisiana, Florida, and South Carolina).[6,8,9]

The routes of *C. pseudotuberculosis* transmission in horses remain undetermined.[7,10] Speculated routes include horse-to-horse contact, contact with pathogen-infested soil, and via insect vectors. *Corynebacterium*

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The study was performed at the Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL.

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pseudotuberculosis can penetrate the skin through abrasions. Transmission by insects is suspected because of the seasonal occurrence of clinical cases during fall and early winter in the western United States.[11] The highest annual incidence in horses has been documented during dry months of the year after winters with above average rainfall, which provides optimal breeding conditions for insects in the subsequent summer and fall.[5,11–13] Three fly species, including the horn fly (*Haematobia irritans* L.), the stable fly (*Stomoxys calcitrans* L.), and the house fly (*Musca domestica* L.) were reported as potential vectors after PCR detection of *C. pseudotuberculosis* biovar *equi* phospholipase D (PLD) exotoxin gene in field samples of fly homogenates.[14] In house flies inoculated with *C. pseudotuberculosis* biovar *ovis* from cattle, bacteria were isolated from the flies' intestines and feces for 1–4 hours and from saliva for 1–3 hours.[4,15]

In a previous study[a] a fly inoculation system developed with *C. pseudotuberculosis* was used to demonstrate that house flies can become contaminated within 10 minutes and continue to harbor live bacteria for up to 24 hours after 30 minutes of exposure. This observation suggests that mechanical transmission after a single exposure is possible. Experimental transmission studies with horses are needed to investigate the capability of house flies as vectors.

Using a challenge model in ponies, the primary hypothesis of this study was to evaluate the role of house flies as mechanical vectors of *C. pseudotuberculosis* in horses. We hypothesized that ponies directly inoculated with *C. pseudotuberculosis* would be confirmed to be infected by presence of clinical signs, bacterial isolation from external or internal abscesses, and seroconversion as indicated by reciprocal serum synergistic hemolysin inhibition (SHI) titers. The second hypothesis was that ponies inoculated with *C. pseudotuberculosis* using house flies as mechanical vectors would become infected, resulting in clinicopathologic, bacteriologic, and serologic responses similar to those of the directly inoculated ponies.

Materials and Methods

Bacterial Identification

A field strain of *C. pseudotuberculosis* biovar *equi* isolated from an abscess on a horse from Salinas, CA was cultivated aerobically on 5% bovine blood agar for 24 hours at 37°C in 10% carbon dioxide and confirmed to be *C. pseudotuberculosis* by cellular and colony morphology and conventional biochemical testing. The bacterium was beta-hemolytic, catalase, and nitrate positive, and fermented glucose without gas production.

Fly Rearing

Naïve laboratory-reared house fly pupae (n = 500) from the USDA house fly colony[b] were placed in cages (24.5 × 24.5 × 24.5 cm) of nylon netting (1.50 × 1.50 mm mesh)[c] supplied with a mix of 2 mL of water, 5 g of powdered milk, and 5 g of sugar in a small petri dish under laboratory conditions (temperature, 37°C; relative humidity, 30%). Adult flies emerged in 1–3 days and were maintained according to established protocols.[16] Flies were starved for 24 hours before being used in experiments.

Bacterial Inoculation of Flies

Ninety naïve adult flies (2–4 days of age) were inoculated for 30 minutes while contained in inverted plastic cups (6 flies per cup; 1 oz: 45 mm × 40 mm height) over 15 agar plates (1 cup per agar plate; 60 mm × 15 mm height) containing 5% bovine blood agar with *C. pseudotuberculosis* colonies moistened with sterile 10% dextrose solution for 30 minutes as previously described.[a] After inoculation, each plastic cup and agar plate combination was inverted and placed in a freezer at −20°C for 3 minutes. The cold-immobilized flies dropped into the cups, were removed using forceps and then transferred to cylindrical fly cages (9 cm diameter × 1.5 cm height, covered with nylon screen with 64 holes per cm^2). Twenty flies were placed inside each cage at the start of an experiment. Flies recovered mobility 5 minutes after immobilization.

To confirm successful inoculation, 6 additional inoculated flies were homogenized in 100 μL of phosphate buffered saline and an aliquot was inoculated onto a 5% bovine blood agar plate for culture. To verify the absence of *C. pseudotuberculosis* in the naïve flies, 6 flies were selected and homogenized as described above for bacterial culture.

Animals

This research was performed under approval of the Institutional Animal Care and Use Committee of Auburn University. Eight healthy, adult ponies owned by Auburn University were used in a randomized, controlled, blinded prospective study. Before the study began, each animal was subjected to a complete physical examination, thoracic and abdominal ultrasound examinations, and SHI titers, CBC, serum biochemical profile, and plasma fibrinogen concentration were determined. Inclusion criteria included SHI titers ≤1 : 32.

Experimental Design

The ponies were randomly assigned to fly-exposed (n = 4), positive control (n = 3), or negative control (n = 1) groups by random drawing of numbers from a hat and were housed individually in isolation stalls for 5 weeks after inoculation. After aseptic preparation and local anesthesia with 2% lidocaine of the left pectoral region of each pony, 10 wounds (each with a diameter of 6 mm) were created over an area of approximately 63 cm^2 using a skin biopsy punch. Wounds were clustered so they would all fit underneath a fly cage. In fly-exposed ponies, fly cages containing 20 *C. pseudotuberculosis*-inoculated house flies were sutured over the wounds using nonabsorbable suture material. The nylon screen, which was in contact with the wounds, allowed flies to access and feed on the wounds, but prevented the flies from escaping (Fig 1). The flies and cages were removed after 24 hours, and fly homogenates were tested for *C. pseudotuberculosis* by culture as described above.

The negative control pony was exposed to 20 noninoculated flies contained in cages sutured over the wounds and attached to the pony as previously described. Positive control ponies were inoculated by swabbing the skin wounds with a solution containing 6×10^8 viable *C. pseudotuberculosis* bacteria suspended in 2 mL saline. An empty fly cage also was sutured over the wounds as described above. Fly cages were removed after 24 hours. The investigators handling the ponies (MB, AS, TP, AW, MC) were blinded as to which cage contained the noninoculated flies, therefore, they remained blinded to which ponies were the fly-exposed ponies and which was the negative control pony. A physical examination was performed daily on all ponies, and attitude, appetite, body temperature, heart rate, respiratory rate, mucous membrane

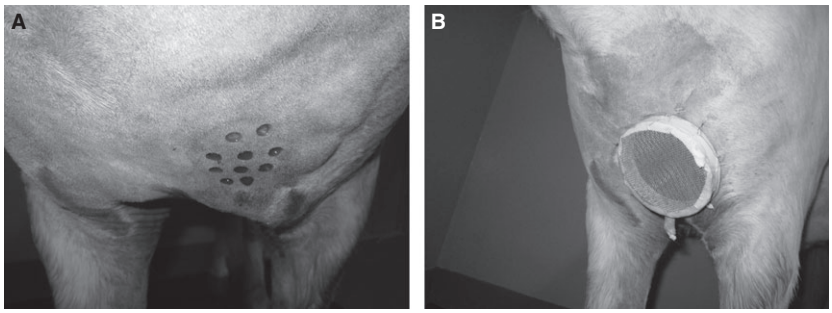

Fig 1. **(A)** Image of the pectoral region of 1 of the ponies after creation of the skin wounds to allow the nonbloodsucking flies to feed. **(B)** Fly cage attached to the pectoral region.

color, and capillary refill time were recorded. Presence of heat, pain, swelling, and discharge over the inoculation site was recorded as present or absent. Heat was subjectively assessed by palpation of the skin surrounding the inoculation site. Pain was subjectively assessed by applying digital pressure around the inoculation site. Blood was collected on days 0, 3, 7, 14, 28, and 35 for CBC and measurement of serum concentrations of fibrinogen, albumin, globulin, iron, and serum amyloid A (SAA). Ultrasound examination of the inoculation site and thoracic and abdominal cavities was performed weekly, and findings were recorded. Swabs from the inoculation site were submitted for culture before exposure. Samples of wound discharge and aspirates of any detectable abscesses were collected and submitted daily for the first 3 days and then twice weekly until the discharge abated. Serum samples were collected at 0, 7, 10, 14, 18, 21, 24, 28, 31, 35, and 38 days after inoculation and frozen for batched *C. pseudotuberculosis* SHI antibody titers.[d]

Five weeks after inoculation, all ponies were humanely euthanized. A complete necropsy examination was performed. Samples from any abscess and aspirates from the inoculation site were submitted for microbiological culture. Histopathology was performed on tissue samples from the inoculation sites.

Statistical Analysis

Normality of the data was assessed using analysis of residuals (Plots = StudentPanel' option in the procedure call[e]). All responses, except SHI titers, fulfilled the normality assumption and therefore were analyzed using a generalized linear mixed model (PROC GLIMMIX[f]), and factors included in the model were pony, treatment, time, and interactions of those variables. Based on the limitations of study design and availability of isolation facilities, the negative control was not replicated. The analysis thus assumed homogeneity of variances among treatment groups, but there was no variance estimate for the negative control. The residual variances for the control treatment therefore were estimated from the other treatments, which was possible because the ponies were assigned randomly to the treatment.[17] A parametric analysis with PROC GLIMMIX was chosen based on 2 criteria: the higher power of parametric tests (versus nonparametric) and the ability of this procedure to analyze non-Gaussian distributed data and the correlated data typical of repeated measures experiments.[e] Differences in rectal temperature, heart rate, respiratory rate, duration in days of clinical signs (heat, pain, swelling, and discharge), and clinicopathological variables over time within groups or between groups at each evaluation time were analyzed. Random effects because of repeated measures were accounted for in the GLIMMIX procedure using R-side modeling. The SLICEDIFF option was utilized to calculate pairwise comparisons among treatment means for each time point at a standard Type I error rate of $\alpha = 0.05$.

Because serum SHI titers did not fulfill the normality assumption, they were subjected to $\log^2$ transformation and were analyzed with SAS PROC NLMIXED using a 3-variable logistic growth model. Nondetectable samples were set to $\log^2 = 1$. Pairwise contrasts for each time point were constructed from the regression equation. Synergistic hemolysis inhibition data are shown as median and range, which were calculated using Microsoft Excel.[g] Because the fly-exposed group consisted of 4 ponies, the median was calculated as the average of the second and third highest values. Values of $P < .05$ were considered significant.

Results

Clinical Signs

Inoculation site reactions, including moderate swelling, increased heat, sensitivity, and purulent discharge were observed in all 7 *C. pseudotuberculosis*-exposed ponies (Fig 2). A mild reaction was seen in the negative control, which lasted 2 days and did not develop purulent discharge. The mean duration of local heat, pain, swelling, and discharge in the exposed groups was 13, 12, 16, and 12 days, respectively. Differences between exposed groups and the negative control were detected for the duration of pain ($P = .0139$), heat ($P = .0085$), swelling ($P = .0045$), and discharge ($P = .0001$; Fig 3). There were no statistically significant differences between the fly-exposed and positive control groups.

Signs of local inflammation resolved without treatment in all ponies. Pyrexia (rectal temperature >38.6°C) was not observed in any pony at any time point. However, mean rectal temperatures in the positive control and fly-exposed groups were significantly higher ($P = .034$ and $P = .048$, respectively) on day 1 postinoculation than that in the negative control. Mean rectal temperature was significantly higher in the positive control group compared with the negative control pony on day 19 ($P = .049$). No statistically significant differences were detected for respiratory and heart rates.

Clinical Pathology

Neutrophil counts were significantly higher in the positive and fly-exposed group than in the negative control ($P = .002$ and $P = .005$, respectively) on day 3 postinoculation. SAA concentrations were significantly higher in the positive control group than in the negative control on days 3 ($P < .0001$) and 7 ($P = .0004$), and

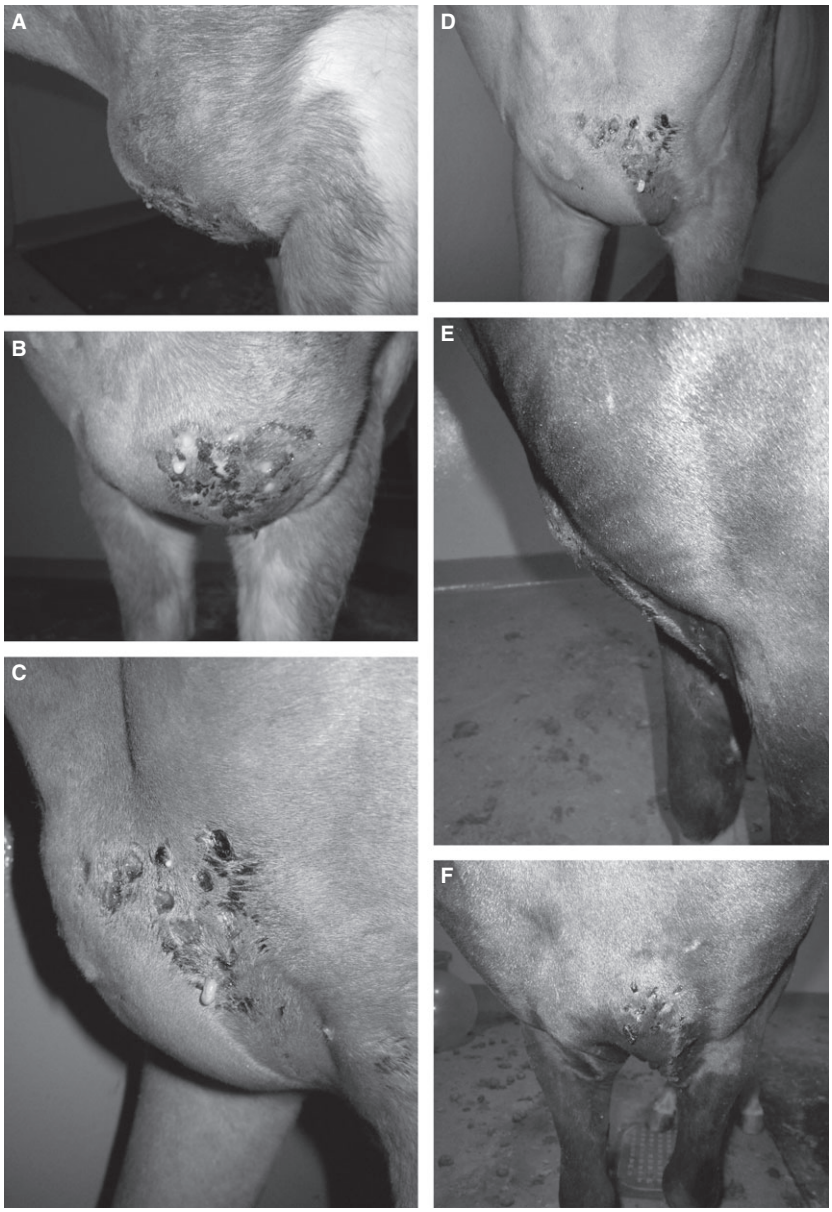

Fig 2. Images from ponies 36 hours after exposure to *Corynebacterium pseudotuberculosis* biovar *equi*. **(A)** Positive control pony, lateral view. **(B)** Positive control pony, frontal view. **(C)** Fly-exposed pony, lateral view. **(D)** Fly-exposed pony, frontal view. **(E)** Negative control pony, lateral view. **(F)** Negative control pony, frontal view.

significantly higher in the positive control group than in the fly-exposed group on days 3 ($P < .0001$) and 7 ($P = .0001$; Fig 4). No statistically significant differences were detected among groups for the rest of the clinical pathology variables (Fig 4).

Ultrasound Examination

Although superficial abscesses were detected externally in all exposed ponies for the time that purulent discharge was present, no deep abscesses were detected by ultrasound examination. Marked swelling associated with hypoechogenic areas within the SC tissue was observed, compatible with cellulitis. Thoracic and abdominal ultrasound examinations remained normal in all ponies during the experiment.

Necropsy

Several epidermal crusts with minimal superficial dermal thickening were observed at the inoculation site in all ponies. No gross abnormalities of the regional lymph nodes were observed. In 1 fly-exposed pony, a 2 mm pustular epidermal-dermal wound was observed at the inoculation site, and the left axillary lymph node was enlarged. Heavy growth of *C. pseudotuberculosis* was obtained from the purulent material, but the lymph node was negative.

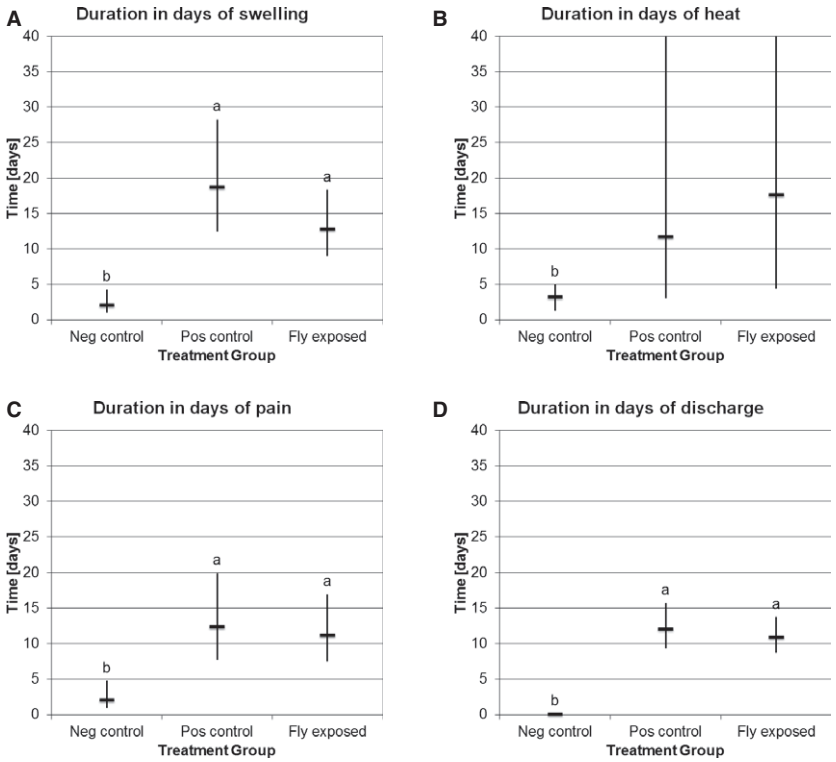

Fig 3. Mean duration (in days) and 95% confidence interval of local swelling **(A)**, heat **(B)**, pain **(C)** and discharge **(D)** from the inoculation site for the 3 groups of ponies after exposure to *Corynebacterium pseudotuberculosis* biovar *equi*. Groups with different letter superscripts are significantly different ($P < .05$).

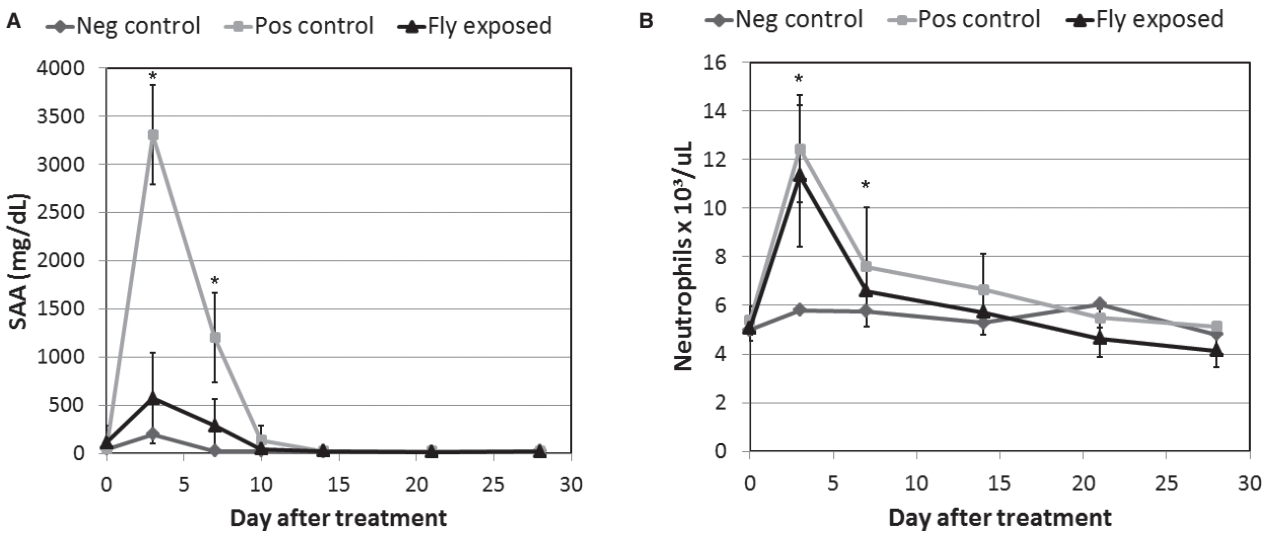

Fig 4. Mean ± SD serum amyloid A (SAA) concentration **(A)** and neutrophil count **(B)** compared over time after exposure to *Corynebacterium pseudotuberculosis* biovar *equi* for the three groups of ponies. Days with asterisk (*) are significantly different between positive control and negative control ($P < .05$).

Multiple hepatic white parenchymal foci, capsular foci, or both (2–4 mm in diameter) were observed in all ponies, and were considered incidental findings and not related to *C. pseudotuberculosis* exposure. Cultures of these samples were negative. The remainder of the examination was normal. Histopathology of the inoculation site tissue disclosed minimal to mild intra-epidermal edema, infiltration of leukocytes and orthokeratosis. Mononuclear cell infiltrate was present perivascularly in the superficial and deep dermis in addition to focal scarring characterized by fibrovascular tissue formation and replacement of adnexal structures.

Bacteriological Cultures from Ponies

All samples collected from the pectoral region before inoculation were negative for growth of *C. pseudotuberculosis*. Twenty-four hours after removal of the fly cage, heavy growth of *C. pseudotuberculosis* biovar *equi* was obtained from the samples taken at the inoculation site from all 7 exposed ponies; a sample from the negative control produced no growth. Positive cultures were obtained from the purulent discharges collected from the 7 exposed ponies, daily for the first 3 days and then twice weekly until the discharge abated (between 7 and 13 days postexposure). Daily negative cultures for the first 3 days postinoculation were obtained in the negative control from the inoculation site. After this time, absence of discharge and presence of normal crusting as a result of normal wound healing precluded further collection of culture samples from the negative control. Almost all samples collected during necropsy examinations, including inoculation site, regional lymph nodes, lung, spleen, and liver were culture-negative. The exception was the fly-exposed pony with the small superficial closed abscess in the pectoral region, which yielded heavy growth of *C. pseudotuberculosis* biovar *equi*.

Serology

Initial serologic titers before exposure all were ≤1 : 32. Peak serologic titers were obtained between 17 and 21 days after infection in the directly inoculated and fly-exposed groups, respectively (Fig 5). The maximum peak titer in the directly inoculated group was 1 : 2,048 and in the contaminated fly-exposed group was 1 : 512. No seroconversion was observed in the negative control. A statistical difference was detected between negative and positive control and between the fly-exposed group and negative control for the increase in titers, respectively ($P = .05$).

Fly Survival and Culture

Subjective evaluation 12 hours after the fly cage placement indicated that the majority of flies were alive in all of the cages. All flies were dead by the time of cage removal 24 hours after exposure to the ponies, with the exception of 1 cage, where 2 out of 20 flies were still alive. Cultures from homogenates of noninoculated flies were negative. Cultures from flies after experimental exposure to the bacterium and after removal of the fly cages 24 hours postinoculation of the ponies yielded heavy *C. pseudotuberculosis* growth in all samples.

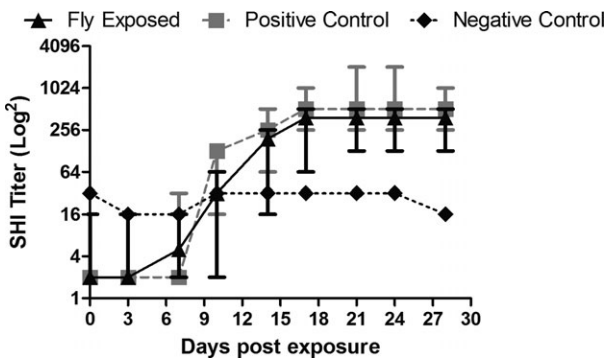

Fig 5. Median and range of synergistic hemolysis inhibition (SHI) titers compared over time for the three groups of ponies exposed to *Corynebacterium pseudotuberculosis* biovar *equi*.

Discussion

This study has shown that *C. pseudotuberculosis* can be transmitted to superficial skin wounds in horses by direct inoculation and by using a mechanical insect vector. Clinical signs were consistent with those expected from *C. pseudotuberculosis* infection with all exposed ponies developing pectoral edema, heat, pain, and swelling and superficial abscess development with positive *C. pseudotuberculosis* culture. Laboratory findings also were consistent with *C. pseudotuberculosis* infection with increased SHI titers, neutrophil counts, and SAA concentrations.

Clinical signs of infection in ponies in this study included marked local swelling, mild heat and pain, and moderate purulent discharge from the inoculation site for 7–22 days. A more marked response was observed in the positive control group, which is likely because of the higher bacterial dose used in this group by direct swabbing of the inoculation sites with a highly concentrated bacterial solution. Variability introduced during inoculation of flies and differences in feeding behavior of individual flies while maintained on the wounds precluded determination of the precise inoculation dose in the fly-exposed group. Although the bacterial dose transmitted by flies was suspected to be lower than in positive controls, the dose was sufficient for disease transmission to all fly-exposed ponies. Using this transmission model, the classic pectoral swelling so called "pigeon breast" was induced, but only superficial abscesses were detected by ultrasound examination. The marked local swelling that resulted from exposure to *C. pseudotuberculosis* was a result of severe cellulitis and not large deep tissue abscesses. The severe inflammation likely was induced by bacterial exotoxins, including phospholipase D.[5] Phospholipase D damages the vascular endothelium increasing its permeability and resulting in edema.[5,18] Results of this study explain why field veterinarians reportedly have difficulty identifying abscesses by ultrasound examination in the swollen brisket area of acutely infected horses. The percentage of field cases that develop the initial pectoral cellulitis and go on to develop the classic large draining abscesses is unknown. A proportion of field cases also may have complete resolution of initial cellulitis without abscess development. Such lesions may occur, but usually are not differentiated from trauma. Although an exact clinical representation of naturally occurring disease was not induced, we propose that the model developed in this study will be particularly important for evaluation of early stages of *C. pseudotuberculosis* infection.

Compared to the frequency of development of external abscesses, internal infection is thought to represent <10% of *C. pseudotuberculosis* biovar *equi* infections in the field.[5] No cases of internal abscesses were detected in the 7 exposed ponies in our study. An approximately 2-month delay in the detection of internal infections was observed in a large retrospective study.[8] This study was terminated after 5 weeks, but because clinicopathological signs of external inflammation had resolved, the risk of internal abscesses was considered to be low. Other factors including variable virulence among bacterial strains and variable immunological responses of animals likely play a role in development of internal abscessation.[19,20]

Neutrophil counts and SAA concentrations were significantly increased in both exposed groups. The same findings were observed in of sheep with experimentally induced caseous lymphadenitis, where serum concentrations of SAA and haptoglobin peaked 7 days postinfection, and then declined to concentrations equal to those of control sheep by day 18.[21] Based on results of this study, neutrophil count and SAA are the best indicators of acute infection compared to fibrinogen, iron, or globulin concentrations. This observation may be useful for veterinarians monitoring transmission of *C. pseudotuberculosis* within a herd or in a horse with suspected exposure.

Obtaining seronegative animals for the study was difficult even if ponies came from nonendemic regions, and 1 pony included in the study had a titer of 1 : 32. Rapid increases in serological titers was observed in both exposed groups, with an increase in titers between 5- to 10-fold in the positive control and 4- to 9-fold in the fly-exposed groups in 17–22 days. A higher serologic response was observed in the positive control group which could be attributed to the higher bacterial inoculation dose. Results of serologic testing in this study indicated that the clinical signs observed in the exposed ponies were attributable to the bacteria. The variability in the titers can be related to individual response or to the infectious dose. A 1-fold decrease in titer was detected in 1 pony after 29 days, but further continuation of the study would have been necessary to determine the length of time ponies would remain seropositive after exposure. The SHI titers in the positive control (median, 1 : 512; maximum, 1 : 2,048) and inoculation group (median, 1 : 384; maximum, 1 : 512) were comparable to titers obtained from horses with naturally occurring external abscesses which can be between <1 : 8 and 1 : 10,240.[5] Horses with naturally occurring internal abscesses can have titers between 1 : 256 and 1 : 10,240,[5,22] but no internal abscesses were found at necropsy. Higher titers and more severe disease have been reported in many naturally occurring cases than that induced in our experimental model.[5,8]

Natural transmission might occur via insect vectors, and biting flies such as the horn fly (*Haematobia irritans* L.), and the stable fly (*Stomoxys calcitrans* L.), and the nonbiting house fly (*Musca domestica* L.), have been implicated.[14] Because house flies are not bloodsucking flies, but may transmit the bacterium through excoriated skin via mouthparts and tarsal exoskeleton, superficial skin wounds were created on the pectoral region of each pony. This method provided consistent and reproducible experimental induction of infection in both exposed groups. This approach was advantageous, particularly considering the small sample size. The fact that the majority of flies were dead 24 hours after exposure was attributed to lack of water consumption because the blood and discharge from the wounds had dried at that time. Further investigation is warranted to determine if house flies act as mechanical vectors or amplify the bacteria, and to determine if bacteria are carried on the external or in the internal organs. House flies were chosen for this study, but further experiments could be performed using biting flies and the established fly cage system without creation of skin wounds. Similar studies were performed using the same fly cage system using biting horn flies to evaluate transmission of Bovine Viral Diarrhea virus in cattle.[23]

Limitations of this study included the small sample size and presence of only 1 pony in the negative control group. However, it was decided to terminate the study after successful inoculation of 7/7 ponies because proof of concept of induction of disease by house flies had been achieved. Additional animals would have made statistical analysis easier, and additional inflammatory variable data may have been useful but sacrifice of additional animals was not considered warranted. The differences between the positive and fly-inoculated groups compared to the negative control were significant, but quantitative aspects of the results could have been influenced by biological variability in the individual horses and differences in the inoculum between these 2 groups.

The methods utilized in this study did not allow for quantification of the bacterial load transmitted by flies, which may have been different from that used for inoculation of positive controls. All flies were exposed to bacterial culture plates in which *C. pseudotuberculosis* had been grown to confluence, but the bacterial load carried by individual flies could have been affected by time of feeding on the culture, survival of bacteria on individual flies, location of bacteria on flies, and bacterial growth on the flies. Furthermore, the number of bacteria transmitted to each horse could have been affected by the mechanism of transmission, time spent on the wounds, and survival of flies. Therefore, future research should evaluate the mode of transmission by flies (eg, contact with mouth parts or other external parts, by feces, saliva, or regurgitation), and number of bacteria that individual flies can transmit.

In conclusion, we have demonstrated that house flies can serve as mechanical vectors of *C. pseudotuberculosis* biovar *equi* in horses demonstrated by development of clinical signs of local infection with positive culture and increases in neutrophil count, SAA concentration, and SHI titers. To our knowledge, this is the first time that *C. pseudotuberculosis* biovar *equi* has been transmitted experimentally to horses. The disease induced experimentally resolved spontaneously without formation of large abscesses that required draining, which is different

from most reported cases of naturally occurring disease. This model potentially can be used in future epidemiological investigations or to test the effectiveness of vaccination or other treatment.

Footnotes

[a] Barba M, Xing PH, Hathcock T, Hogsette JA, Wooldridge AA, Passler T, Chamorro MF, Cattley R and Stewart AJ. Experimental inoculation of house flies, *Musca domestica* L., with *Corynebacterium pseudotuberculosis* biovar *equi*. Abstract presented at the Entomological Society of America annual meeting November 2013, Austin, TX

[b] USDA/ARS/CMAVE, Gainesville, FL

[c] MegaView Science Co, Ltd. Taichung 40762, Taiwan

[d] California Animal Health and Food Safety (CAHFS-Davis), University of California, Davis, W. Health Sciences Drive, Davis, CA

[e] http://support.sas.com/documentation/cdl/en/statug/63347/HTML/default/viewer.htm#glimmix_toc.htm

[f] SAS 9.2, SAS Institute Inc, Cary, NC

[g] Microsoft Excel 2010, Redmond, WA

Acknowledgments

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Conflict of Interest Declaration: The authors disclose no conflict of interest.

Off-label Antimicrobial Declaration: The authors declare no off-label use of antimicrobials.

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