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Experimental inoculation of house flies *Musca domestica* with *Corynebacterium pseudotuberculosis* biovar *equi*

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Abstract

Corynebacterium pseudotuberculosis (Actinomycetales Corynebacteriaceae) infection in horses causes three different disease syndromes: external abscesses, infection of internal organs and ulcerative lymphangitis. The exact mechanism of infection in horses remains undetermined, but transmission by insect vectors is suspected. The present study first determined the optimal culture media for inoculation of house flies (*Musca domestica* L.) (Diptera Muscidae), with *C. pseudotuberculosis* biovar *equi* and the time required for fly inoculation. A second experiment determined the duration of bacterial survival on flies. Exposure of house flies to 3 different preparations of blood agar supplemented with dextrose and colonized with *C. pseudotuberculosis* determined that a 10 minute exposure to the bacteria was enough to inoculate the flies. *C. pseudotuberculosis* could be recovered for up to 24 hours after house flies were exposed for 30 minutes to a blood agar plate colonized with the bacteria and moistened with 10% dextrose. These findings support the hypothesis that the house fly is a potential vector of pigeon fever and aid in establishing a protocol for a future experimental model to demonstrate the role of house flies as mechanical vectors in *C. pseudotuberculosis* infection.

Key words: Horse, pigeon fever, vector, equine pathogen, arthropod, transmission.

Introduction

Corynebacterium pseudotuberculosis (Actinomycetales Corynebacteriaceae) is a pleomorphic, facultative intracellular, Gram-positive rod with a worldwide distribution (Quinn *et al*., 2011). Two biotypes of *C. pseudotuberculosis* are described and are distinguished by genetic characteristics, including restriction fragment length polymorphisms and the ability of cultured organisms to reduce nitrate to nitrite. Nitrate-positive biovar *equi* is commonly isolated from horses and cattle but nitrate-negative biovar *ovis* is typically recovered from small ruminants and cattle (Biberstein *et al*., 1971; Sutherland *et al.*, 1996). In ruminants, *C. pseudotuberculosis* causes caseous lymphadenitis, abscesses, and occasionally mastitis (Yeruham *et al.*, 1996). In horses, *C. pseudotuberculosis* biovar *equi* causes three clinical forms of disease (Aleman *et al.*, 1996). The most common form, known as "pigeon fever" or "dryland distemper", is characterized by subcutaneous abscessation of the pectoral region; the second form causes abscesses of internal organs; and the third causes ulcerative lymphangitis (Aleman *et al.*, 1996). Natural cross-species infection by specific biotypes is not known to occur (Foley *et al*., 2004). Human infection, although rare, is considered an occupational zoonosis (Dorella *et al*., 2006).

C. pseudotuberculosis biovar *equi* infection is prevalent in the western United States (California, Utah, Colorado and Texas) and Brazil, but a recent increase in case numbers and spread to regions previously considered non-endemic have been reported (Foley *et al.*, 2004; Pratt *et al*., 2005; Kilyone *et al*., 2014).

The routes of *C. pseudotuberculosis* infection in horses remain undetermined (Pratt *et al*., 2005; Dorella *et al*., 2006). Transmission by insects is suspected because of the seasonal occurrence patterns during fall and early winter in the western United States (Miers *et al.*, 1980). The highest annual incidences in horses have been observed during dry months of the year following winters with above average rainfall, which provides optimal breeding conditions for insects in the subsequent summer and fall (Miers *et al.*, 1980; Aleman *et al.*, 1996; Kilyone *et al*., 2014; Szonyi *et al*., 2014). 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 by PCR detection of the *C. pseudotuberculosis* biovar *equi* phospholipase D (PLD) exotoxin gene in field samples of fly homogenates (Spier *et al*., 2002). In house flies contaminated with *C. pseudotuberculosis* biovar *ovis* from cattle, bacteria were isolated from the flies' intestine and feces, and saliva for 1-4 and 1-3 h, respectively, post exposure (Yeruham *et al*., 1996; Braverman *et al*., 1999).

The objectives of the present study were to determine the optimal inoculation time of *C. pseudotuberculosis* biovar *equi*, the most appropriate inoculation media, the duration of bacterial retention in house flies after exposure and the survival of flies after inoculation. The overall goal of this project was to develop a house fly inoculation system which could be used subsequently in a controlled experimental model to evaluate the role of house flies as mechanical vectors of *C. pseudotuberculosis* in horses.

Materials and methods

Bacterial identification

A field strain of *C. pseudotuberculosis* biovar *equi* isolated from an abscess on a horse from California was grown aerobically on 5% bovine blood agar for 24 h at 37 °C in 10% carbon dioxide and confirmed as *C. pseudotuberculosis* biovar *equi* by morphology, culture characterization, and conventional biochemical testing: the bacterium was beta-hemolytic, catalase and nitrate-positive, and fermented glucose without gas production.

Fly rearing

Naïve laboratory-bred *M. domestica* pupae (n = 500) from the USDA (Gainesville, FL) *M. domestica* colony were placed in cages of nylon netting 1.50 × 1.50 mm mesh (24.5 × 24.5 × 24.5 cm) (BugDorm, MegaView Science Co., Ltd., Taiwan), and were maintained at 37 °C and 30% RH until emerging. Adult flies emerged in 1 to 3 days, were supplied with a mix of 2 ml of water, 5 g of powdered milk and 5 g of sugar in a small Petri dish, and were maintained under laboratory conditions (27 °C, 40% RH). Flies were starved for 24 h before being used in experiments.

Experiment one: optimization of bacterial inoculation of flies (n = 180)

Bacterial preparations

Three different media were used to propagate *C. pseudotuberculosis* to determine if different media preparations would affect feeding and thus recovery of the bacterium. Preparation A consisted of overnight cultures incubated at 37 °C of *C. pseudotuberculosis* grown to confluence on 5% bovine blood agar. Preparation B consisted of overnight cultures of *C. pseudotuberculosis* grown to confluence on 5% bovine blood agar containing 10% dextrose. The preparation A and B plates were inoculated with a standardized suspension of *C. pseudotuberculosis* in saline at a high concentration of 3×10^8 CFU/ml. The saline suspension was prepared from a culture of *C. pseudotuberculosis* grown in 5% CO_2 for 18-24 h on 10% bovine blood agar. Prior to exposing the flies to preparations A and B, the entire agar surface of each plate was moistened with a sterile swab impregnated with sterile 10% dextrose solution. Preparation C consisted of 5% bovine blood agar in which the surface was swabbed with a 10% dextrose solution containing a suspension of *C. pseudotuberculosis* (3×10^8 organisms per ml) immediately before exposure to flies. There were 10 Petri dishes (60 mm × 15 mm height) for each of the three preparations.

Fly feeding on the bacterial preparations

To determine the exposure times necessary for inoculation of the flies with the bacterium, an inverted plastic cup (1 oz: 45 mm × 40 mm height) (Bio-Serv, Flemington, NJ) containing 6 naïve flies (3-5 days old, mixed sexes) was placed over each of 10 agar plates of the three preparations (n = 30 plates) (figure 1). The flies were allowed to feed for 10 or 30 min or 1, 2, 4, 6, 8, 12, 24 or 48 h. The flies were observed for 1 min 3 times during the first 10 min of the experiment, and the number of individuals feeding at every time point until termination was recorded to assess how often they fed on the preparation. Following the period of exposure, the number of surviving flies was recorded. Then, the plastic cup containing the flies over the agar plate was inverted and placed in the freezer at −20 °C for 3 min. The cold immobilized flies fell into the cup, were removed using forceps, and placed in a 2-ml sterile microcentrifuge tube. Four flies out of each group of six flies were manually homogenized in the microcentrifuge tubes with a sterile stick and 100 µl of phosphate buffered saline (PBS). An aliquot of the homogenate was inoculated onto a 5% bovine blood agar plate for culture. After 36 h of incubation at 37 °C, the density of bacterial growth was semi-quantitatively scored using standard methods. Bacterial growth was recorded based on each quadrant to delineate numbers since the method of plating dilutes the specimen from quadrant to quadrant (Tille, 2013). Heavy growth corresponded to the presence of *C. pseudotuberculosis* colonies in the fourth quadrant of the agar plate, moderate growth corresponded to isolated colonies in the third and/or second quadrant, whereas light growth was equivalent to colony formation limited to the first quadrant and no growth was the total absence of *C. pseudotuberculosis* growth.

Culture controls

Bacterial viability in each of the three preparations was evaluated by sampling C. *pseudotuberculosis* from each culture at 24 and 48 h and spreading on 5% bovine blood agar plates as described above.

Figure 1. Clear plastic cups were used to contain house flies over the preparations.

Experiment two: bacterial survival in/on flies (n = 60)

Six naïve flies (9-11 days of age, mixed sexes) were contained in inverted plastic cups (described above) over each of 10 agar plates containing preparation A (5% bovine blood agar with *C. pseudotuberculosis* colonies moistened with sterile 10% dextrose solution). Preparation A was selected for use in this experiment, based on the result from experiment one. After 30 min, the number of surviving flies was recorded and then the flies were cold immobilized at −20 °C for 3 min and the agar plate was removed. The flies were transferred to a sterile Petri dish at room temperature without addition of water and food. Shortened fly survival was expected due to starvation, but this method decreased the risk of escape of flies carrying *C. pseudotuberculosis* and minimized the addition of confounding factors due to fly interaction and contamination with other bacteria. The retention of *C. pseudotuberculosis* in flies was examined at T = 10 and 30 min and 1, 2, 4, 6, 8, 12, 24 and 48 h using the same protocol as experiment one.

Negative fly controls (n = 48)

Prior to the start of experiments one and two, six additional naïve flies were collected and homogenized as described above for bacterial culture at T = 0 to verify the absence of contamination with *C. pseudotuberculosis*. In addition, six naïve flies were contained by an inverted plastic cup over a Petri dish with food (milk, sugar and water) for the duration of the experiment (48 h). The number of surviving flies was recorded and then the flies were cold immobilized and homogenized for culture to confirm absence of contamination with *C. pseudotuberculosis* due to physical manipulation.

Statistical analysis

Descriptive statistics was used to summarize the bacterial growth on the different media, fly survival and fly feeding time. Fly survival was analyzed using logistic regression techniques as implemented in SAS® PROC GLIMMIX. Time after treatment was used as a covariate and fly survival modeled as Growth Medium + Time (Growth Medium). Estimates for intercept and slope were compared using linear contrasts. Least squares means were calculated for the two experiments at time points 0 and 48 h; the means for the media were then compared to the negative control using Dunnett's test.

Results

Experiment one: optimization of bacterial inoculation of flies

C. pseudotuberculosis was readily transmitted to house flies by feeding (table 1). Heavy growth of *C. pseudotuberculosis* recovered from fly homogenates indicated that house flies could become highly inoculated after an exposure of 10 min to 24 h to preparations A and B (figure 2). After 48 h of exposure, heavy growth of *C. pseudotuberculosis* was observed from preparation A, while moderate growth was observed from preparation B. Preparation C showed more variable bacterial growths, with moderate growth observed after 6 h and 24 h of exposure and no growth after 48 h of exposure. These results indicated that exposure for 10 min to the bacterium was sufficient to inoculate house flies.

Table 1. Number of flies alive at the end of each experiment out of 6 initial flies and culture scores of *C. pseudotuberculosis* recovered from homogenates of flies after 10 different exposure times to three experimental preparations. Negative controls were evaluated before (T = 0) and after the experiment (T = 48). Viability cultures were evaluated after 24 and 48 h. Bacterial preparation: A) overnight cultures of *C. pseudotuberculosis* grown to confluence on 5% bovine blood agar; B) overnight cultures of *C. pseudotuberculosis* grown to confluence on 5% bovine blood agar containing 10% dextrose; C) 5% bovine blood agar in which the surface was swabbed with a 10% dextrose solution containing a suspension of *C. pseudotuberculosis.*

Exposure times	Bacterial preparations					
	A	Flies alive	B	Flies alive	C	Flies alive
10 min	Heavy growth	6	Heavy growth	6	Heavy growth	5
30 min	Heavy growth	6	Heavy growth	6	Heavy growth	5
1 h	Heavy growth	6	Heavy growth	6	Heavy growth	4
2 h	Heavy growth	6	Heavy growth	6	Heavy growth	5
4 h	Heavy growth	6	Heavy growth	6	Heavy growth	5
6 h	Heavy growth	5	Heavy growth	6	Moderate growth	6
8 h	Heavy growth	6	Heavy growth	6	Heavy growth	4
12 h	Heavy growth	6	Heavy growth	6	Heavy growth	6
24 h	Heavy growth	5	Heavy growth	4	Moderate growth	6
48 h	Heavy growth	4	Moderate growth	6	No growth	4
Neg control T = 0	No growth	6	No growth	6	No growth	6
Neg control T = 48	No growth	6	No growth	6	No growth	4
Viability culture 24 h	Heavy growth	6	Heavy growth	6	Moderate growth	6
Viability culture 48 h	Heavy growth	6	Heavy growth	6	No growth	4

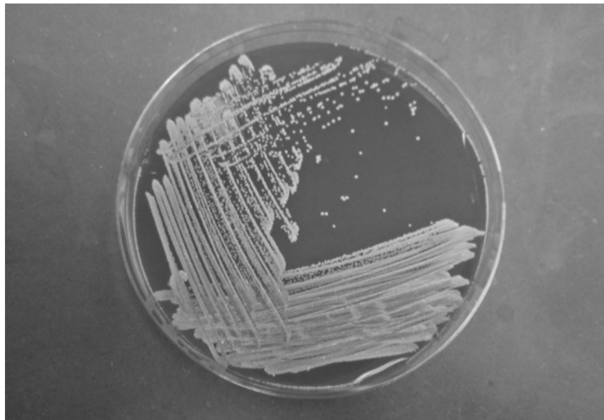

Figure 2. Heavy growth of *C. pseudotuberculosis* pure culture observed in a blood-agar plate recovered from fly homogenates after a 10-min exposure to preparation A.

Table 2. Number of flies alive at the end of each experiment out of 6 initial flies and culture scores of *C. pseudotuberculosis* growth at 10 post-exposure intervals from homogenates of flies exposed for 30 minutes to preparation A and from a negative control.

Post-exposure times	Culture scores	Flies alive
0 min	Heavy growth	6
10 min	Heavy growth	5
30 min	Heavy growth	6
1 h	Heavy growth	6
2 h	Heavy growth	5
4 h	Heavy growth	4
6 h	Heavy growth	4
8 h	Moderate growth	5
12 h	Moderate growth	4
24 h	Moderate growth	4
48 h	No growth	2
Negative control T0	No growth	6
Negative control T48h	No growth	2

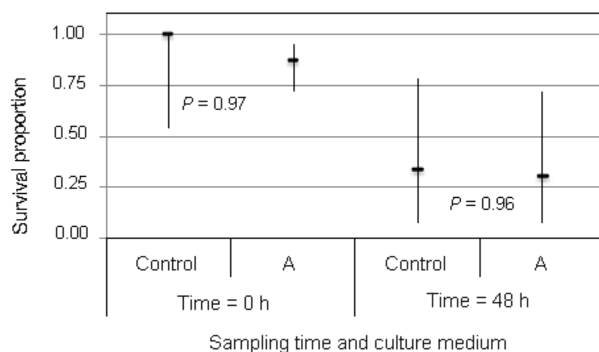

Figure 3. Fly survival estimates, 95% CI(p), and contrast *P*-values reflect the contrast of the *C. pseudotuberculosis* medium A versus the negative control at time T = 0 and T = 48 h in experiment 2. No difference in survival was detected between the exposed and the control groups. However a decrease in survival was observed at the end of the experiment, which was attributed to the long period of starvation.

The flies were assessed by subjective observation to feed more often on preparations A and C than on preparation B, in which they expended more time resting on the wall of the cup.

The negative controls evaluated at T = 0 and T = 48 h were culture-negative for *C. pseudotuberculosis*. The positive culture controls yielded heavy growth of *C. pseudotuberculosis* confirming bacterial viability.

Preparation A was selected as the ideal medium for the remainder of experiments because of its simplicity of preparation, the consistency of bacterial growths, and the subjective observation of higher feeding frequency of the flies. Overnight bacterial culture on 5% blood agar is also a standard culture method.

One or two out of the six flies exposed to the preparations died during the experiment in some of the groups due to manipulation or contact of the wings with the preparation. These flies were not included in the four flies sampled per group. The mortality of flies during the experiment is shown in table 1. The survival of flies was not statistically different among groups whether exposed or non-exposed to the bacteria at the beginning or end of the experiment, indicating that exposure to the bacteria did not increase fly mortality.

Experiment two: bacterial survival in/on flies

Inoculated flies continued to harbor viable *C. pseudotuberculosis* for at least 24 h following exposure (table 2). *C. pseudotuberculosis* was not detected in samples from negative controls. The growth scores of *C. pseudotuberculosis* recovered from homogenates of exposed flies were heavy growths from T = 0 to T = 6 h and moderate growths from T = 8 to 24 h. No growth of *C. pseudotuberculosis* was obtained from flies sampled at T = 48 h.

The mortality of flies during the experiment is shown in table 2. Fly survival was not statistically different between group A and control at the beginning or end of the experiment (figure 3).

Discussion

The results of this study show that adults of *M. domestica* can be experimentally inoculated with *C. pseudotuberculosis* in an exposure as short as 10 min and under the conditions of this experiment the flies harbored the bacteria for up to 24 h post-exposure. Of the three preparations used to investigate the optimal technique for inoculation of adult house flies, two preparations (A and B), were overnight incubated cultures of *C. pseudotuberculosis* and one (preparation C), was freshly swabbed with a suspension of *C. pseudotuberculosis*. Blood agar was used in all three preparations and a 10% dextrose solution was applied to the surface of all three prior to their exposure to the flies to increase the attractiveness for the flies and to moisten the surface of the agar thus providing a water source to the flies during the experiment. All 3 preparations were successful in allowing inoculation of the flies, and therefore any of these preparations could be used in future experiments. However greater growth was observed when flies were exposed to the overnight incubated cultures of *C. pseudotuberculo-*

sis, and flies appeared to feed more readily on preparation A despite the fact that the blood agar in preparation B also contained dextrose. Because of the consistent heavy growth on preparation A at all exposure times, the maintenance of a heavy growth in the viability controls performed from the media, the simplicity of preparation and the subjective feeding time of the flies, preparation A was selected for use in experiment 2.

In the present study, *C. pseudotuberculosis* was recovered from the flies for up to 24 h following 30 min of exposure to the bacteria. Thirty minutes of exposure was chosen as the feeding time for this second experiment due to operational convenience. These results contrast with previous studies. Braverman *et al*. (1999) recovered *C. pseudotuberculosis* from the intestine of house flies only between 1-4 h after feeding for 2-4 h on contaminated milk. Similar results were obtained in another study, in which *C. pseudotuberculosis* was recovered from house flies for up to 4 h after they fed on contaminated milk (Yeruham *et al.*, 1996). The strain used by Braverman *et al*. (1999) and Yeruham *et al*. (1996) was nitrate-negative, which is commonly isolated from ruminants (Biberstein *et al.*, 1971). In contrast, a *C. pseudotuberculosis* nitrate-positive strain was investigated in this study, which has been previously isolated from house flies recovered from horses with ulcerative lymphangitis (Addo, 1983); this strain may have characteristics different from the ruminant strain that allow it to survive longer in mechanical vectors or our experimental methods may have been more successful at fly inoculation and /or microbiological culture. In addition, the inoculation method of these studies included contaminated milk or sugar cubes, which differed from the method used in the present study. Our results showed that *M. domestica* could be more easily inoculated with *C. pseudotuberculosis* and that the length of bacterial survival was 6 times as long as previously reported (Braverman *et al*., 1999; Yeruham *et al*., 1996).

Exposure to *C. pseudotuberculosis* did not affect mortality of the flies, however a higher mortality of flies was observed at the end of experiment 2 in both the exposed and the control groups. This could be attributed to the starvation time of 48h at the end of that experiment.

There are several important limitations to this study. Firstly, ten minutes of exposure to *C. pseudotuberculosis* was chosen as the shortest feeding time due to operational management reasons. The homogenates of flies exposed for only 10 min to the inoculated blood agar preparations produced heavy growth scores when plated on sterile blood agar. The shortest time of exposure to the *C. pseudotuberculosis* investigated in previous experiments was 1 hour (Braverman *et al*., 1999). In our experiments the house flies were confined in plastic cups over the inoculated preparations, and it is unlikely that flies fed or walked continuously on the blood agar during the entire exposure time, however due to the previous starvation the flies fed on the blood agar to some degree. Thus inoculation was likely achieved in less than the specified time intervals. House flies in nature will rarely have the opportunity to feed undisturbed on infected abscesses for 10 min, thus additional research is needed to determine the minimum exposure period required to enable a house fly to become contaminated. Our results indicated how easily house flies became inoculated in the laboratory with *C. pseudotuberculosis*, however replication of the experiments would have been beneficial to demonstrate the reproducibility of the inoculation protocol.

Second, the variability of the individual flies feeding behavior was not evaluated in this study. Each fly could have ingested a variable quantity of *C. pseudotuberculosis*. The ability or need to feed can be related to several factors such as the age, sex of the fly, or the egg development stage for female flies. Another confounding factor would be the potential cross-contamination among the 6 flies kept in the same cup. Differentiation of individual fly feeding behaviors was beyond the scope of this study. Additional research would be required to determine how much *C. pseudotuberculosis* growth could be attributable from an individual fly of a particular sex or life stage. By homogenizing 4 flies some of these individual variations were averaged out, which is more reflective of a field situation where a large number of flies are likely to feed on an abscess or wound on a horse.

Third, the experimental conditions of this study differ substantially from the natural conditions that are present during the potential transmission of *C. pseudotuberculosis* between horses. Our results show *M. domestica* was inoculated under the laboratory conditions discussed above, however further research is needed to investigate inoculation under natural conditions from a horse abscess.

A fourth limitation of this study is that the mechanism of transmission was not investigated. Yeruham *et al.* (1996) demonstrated excretion of *C. pseudotuberculosis* biovar *ovis* in *M. domestica* feces for up to 4 h and in saliva for up to 3 h post infection, but the bacteria survived on the external organs of house flies for no longer than 10 min post exposure. Several factors including the time of exposure, environmental conditions, competition with other bacteria in the fly digestive tract flora or the immune response of the house fly may affect the survival time of pathogenic bacteria (Fleming, 2012). Further research is needed to determine the potential mechanism of transmission of *C. pseudotuberculosis* and the survival of the bacterium within the fly.

Flies and other mechanical vectors may play an important role in the transmission of *C. pseudotuberculosis* among horses, as indicated by the recent emergence and spread of the disease in previously non-endemic areas. Although three main muscoid fly pests have been suspected as mechanical vectors of *C. pseudotuberculosis*, the house fly likely has the highest potential because of its tendency to readily move between locations and feed on numerous materials (Hogsette *et al*., 2000). House flies can fly 8 km/h (Hogsette *et al*., 1989) and disperse 20 km or more in one day (Bishopp *et al*., 1921). Our experiments indicate that *C. pseudotuberculosis* can survive in/on house flies for at least 24 h. Therefore, flies could potentially spread the disease over a wide geographic area, making the house fly a plausible mechanical vector for dissemination of *C. pseudotuberculosis* and transmission among horses. More research is needed to better define the role of *M. domestica* in *C.*

pseudotuberculosis transmission to emphasize the need for effective fly management as a means of limiting host-to-host *C. pseudotuberculosis* transmission.

Conclusions

The results obtained from this study will be essential in developing a house fly inoculation system for further investigating the role of *M. domestica* as a mechanical vector of *C. pseudotuberculosis* biovar *equi* in horses. Results show that house flies can become contaminated within 10 min and continue to harbor live bacteria up to 24 h following a 30 min exposure. Although not demonstrated experimentally, this is indicative of the possible duration of the mechanical transmission following a single exposure. Flies have been implicated as vectors of *C. pseudotuberculosis* biovar *equi* (Spier *et al*., 2002), however, this is the first experiment to demonstrate actual contamination of *M. domestica*. Transmission studies are needed to demonstrate a causative relationship between *M. domestica* inoculated with *C. pseudotuberculosis* biovar *equi* and abscess development in horses.

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References

ADDO P. B., 1983.- Role of the common house fly (*Musca domestica*) in the spread of ulcerative lymphnagitis.- *Veterinary Record*, 113: 496-497.

ALEMAN M., SPIER S., WILSON W. D., DOHERR M., 1996.- *Corynebacterium pseudotuberculosis* infection in horses: 538 cases (1982-1993).- *Journal of the American Veterinary Medical Association*, 209: 804-809.

BIBERSTEIN E. L., KNIGHT H. D., JANG S., 1971.- Two biotypes of *Corynebacterium pseudotuberculosis*.- *Veterinary Record*, 89: 691-692.

BISHOPP C., LAAKE E. W., 1921.- Dispersion of flies by flight.- *Journal of Agricultural Research*, 10: 729-766.

BRAVERMAN Y., CHIZOV-GINZBURG A., SARAN A., WINKLER M., 1999.- The role of houseflies (*Musca domestica*) in harbouring *Corynebacterium pseudotuerculosis* in dairy herds in Israel.- *Revue Scientifique et Techinque - Office International des Epizooties*, 18: 681-690.

DORELLA F. A., PACHECO L. G. C., OLIVEIRA S. C., MIYOSHI A., AZEVEDO V., 2006.- *Corynebacterium pseudotuberculosis* : microbiology , biochemical properties , pathogenesis and molecular studies of virulence.- *Veterinary Research*, 37: 201-218.

FLEMING A., 2012.- Spatial and temporal immune response in house flies in response to ingestion of *Bacillus cereus* and *Escherichia coli* 0157-H7.- *Electronic Theses and Dissertations, Georgia Southern University*, paper 21. [online] URL: http://digitalcommons.georgiasouthern.edu/etd/21

FOLEY J. E., SPIER S. J., MIHALYI J., DRAZENOVICH N., LEUTENEGGER C. M., 2004.- Molecular epidemiologic features of *Corynebacterium pseudotuberculosis* isolated from horses.- *American Journal of Veterinary Research*, 65: 1734-1737.

HOGSETTE J. A., 1992.- Autoseparation method for harvesting house fly (Diptera: Muscidae) pupae of known age.- *Journal of Economic Entomology*, 85: 2295-2297.

HOGSETTE J. A., FARKAS R., 2000.- Secretophagous and hematophagous higher Diptera.- *Contributions to a Manual of Palaeactic Diptera*, 1: 669-792.

HOGSETTE J. A., RUFF J. P., JONES C.J., 1989.- Dispersal behavior of stable flies (Diptera: Muscidae).- *Current Status of Stable Fly (Diptera: Muscidae) Research*, 74: 23-32.

KILCOYNE I, SPIER S. J., CARTER C. N., SMITH J. L., SWINFORD A. K., COHEN N. D., 2014.- Frequency of *Corynebacterium pseudotuberculosis* infection in horses across the United States during a 10-year period.- *Journal of the American Veterinary Medical Association*, 245: 309-314.

MIERS K. C., LEY W. B., 1980.- *Corynebacterium pseudotuberculosis* infection in the horse: study of 117 clinical cases and consideration of etiopathogenesis.- *Journal of the American Veterinary Medical Association*, 177: 250-253.

PRATT S. M., SPIER S. J., CARROLL S. P., VAUGHAN B., WHITCOMB M. B., WILSON W. D., 2005.- Evaluation of clinical characteristics, diagnostic test results, and outcome in horses with internal infection caused by *Corynebacterium pseudotuberculosis*: 30 cases (1995-2003).- *Journal of the American Veterinary Medical Association*, 227: 441-448.

QUINN P, MARKEY B., LEONARD F., FITZPATRICK E. S., FANNING S., HARTIGAN P. J., 2011.- *Veterinary microbiology and microbial disease*. 2nd ed.- Wiley-Blackwell, Oxford, UK.

SPIER S. J., LEUTENEGGER C. M., VET M., CARROLL S. P., LOYE J. E., PUSTERLA J. B., CARPENTER T. E., MIHALYI J. E., MADIGAN J. E., 2002.- Use of a real-time polymerase chain reaction-based fluorogenic 5’ nuclease assay to evaluate insect vectors of *Corynebacterium pseudotuberculosis* infections in horses.- *American Journal of Veterinary Research*, 65: 829-834.

SUTHERLAND S. S., HART R., BULLER N. B., 1996.- Genetic differences between nitrate-negative and nitrate-positive *Corynebacterium pseudotuberculosis* strains using restriction fragment length polymorphisms.- *Veterinary Microbiology*, 49: 1-9.

SZONYI B., SWINFORD A., CLAVIJO A., IVANEK R., 2014.- Re-emergence of pigeon fever (*Corynebacterium pseudotuberculosis*) infection in Texas horses: epidemiologic investigation of laboratory-diagnosed cases.- *Journal of Equine Veterinary Sciencie*, 34: 281-287.

TILLE P., 2013.- Traditional cultivation and identification, pp. 81-105. In: *Bailey and Scott’s diagnostic microbiology*.- Elsevier Mosby, St. Louis, USA.

YERUHAM I., BRAVERMAN Y., SHPIGEL N. Y., CHIZOV-GINZBURG A., SARAN A., WINKLER M., 1996.- Mastitis in dairy cattle caused by *Corynebacterium Pseudotuberculos*is and the feasibility of transmission by houseflies.- *Veternary Quarterly*, 18: 87-89.

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