Behaviour of pigs with viral and bacterial pneumonia

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Abstract

The behavioural response to infection is well organized and may enhance disease resistance and facilitate recovery, but the behaviour of pigs with an acute respiratory infection has not been assessed. Therefore, the purpose of this study was to evaluate behaviour of pigs inoculated with Mycoplasma hyopneumoniae (Mh) and porcine reproductive and respiratory syndrome virus (PRRSV). Sixty-four pigs were subjected to one of four treatment combinations (2 × 2 factorial) of Mh (inoculated at 4 weeks of age) and PRRSV (inoculated at 6 weeks of age). The four treatments were (1) control, (2) inoculation with Mh, (3) inoculation with PRRSV, and (4) inoculation with both Mh and PRRSV. One-half of the pigs from each treatment were killed 7 days after PRRSV inoculation for purposes unrelated to this study and hence were not used for behavioural analysis. Pigs that were included were video recorded during the 18 h light phase for 13 days beginning the day of PRRSV inoculation. Food intake and time spent feeding, active (standing, including walking, sitting, or feeding) and lying were determined. When pigs were lying a determination was made as to whether they were lying ventrally or laterally, and in contact with a penmate. Body temperature was measured 7 and 14 days after PRRSV inoculation. After inoculation with PRRSV, there was no significant main effect of Mh or interaction between Mh and PRRSV for food intake, body temperature, or any behaviour measured. Thus, the four treatments were pooled to form two treatments designated PRRSV negative (control and Mh; PRRSV−) and PRRSV positive (PRRSV and Mh with PRRSV; PRRSV+) and analyzed. Each day after PRRSV inoculation, PRRSV+ pigs spent less time (P = 0.005) feeding compared to PRRSV− pigs, and the decrease in feeding time was associated with a decrease in food intake (P < 0.001). PRRSV+ pigs decreased (P < 0.001) activity after inoculation with PRRSV compared to PRRSV− pigs and the amount of time spent lying was greater (P < 0.001) in PRRSV+ pigs compared to...
PRRSV— pigs. Furthermore, PRRSV+ pigs spent more of their total lying time in a ventral position ($P = 0.06$) and in contact with a penmate ($P < 0.001$) compared to PRRSV— pigs. Body temperature was increased ($P < 0.001$) in PRRSV+ pigs 7 days after PRRSV inoculation. Since sickness behaviour and fever are adaptive responses to infection, these data indicate that pigs with an acute PRRSV infection evoke a behavioural strategy that may support recovery.

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1. Introduction

In the United States, 99% of the swine herds are infected with *Mycoplasma hyopneumoniae* (Mh) and the prevalence of porcine reproductive and respiratory syndrome virus (PRRSV) in U.S. swine herds is estimated at 60–80% (Zimmerman et al., 1997). These microorganisms are the two most common pathogens isolated from pigs with porcine respiratory disease complex (Thacker, 2001), and infectious disease is arguably the single most important factor affecting the performance and welfare of growing pigs.

Although the growth performance and welfare of pigs with an acute infection is diminished, they can be improved by (1) reducing the severity of infection; (2) treating the symptoms of infection with, e.g., nonsteroidal anti-inflammatory drugs; and/or (3) facilitating recovery to shorten the duration of time spent clinically ill. One strategy to achieve the third aforementioned goal is to facilitate convalescence. It has been suggested that sickness behaviours including inappetence, increased sleep, and lethargy, and are part of an organized host defense strategy (Hart, 1988; Johnson, 2002). The decreased activity may better enable animals to redirect nutrient resources to support immunological defenses; it also facilitates heat conservation, which is critical for producing the beneficial febrile response.

Determining how infection alters behavioural patterns is a prerequisite to assessing how current swine management practices influence convalescence. Rest is presumed to be an important part of the recovery process, but resting behaviour of pigs with an acute respiratory infection has not been assessed. Therefore, the present study reports the behaviour of pigs inoculated with Mh and PRRSV, alone or in combination. This study was part of a larger one that also determined the effects of Mh and PRRSV on growth performance, whole-body composition, and circulating inflammatory cytokines (Escobar et al., 2004).

2. Materials and methods

2.1. Animals and housing

Four-week-old Ausgene pigs that were free of Mh (serology) and PRRSV (virology) were used in each of two separate but identical trials conducted one after the other. Pigs were brought to the University of Illinois at 2 weeks of age and upon arrival were placed in disease-containment chambers. Daily injections of lincomycin (11 mg/kg of body weight; Pharmacia & Upjohn Co., Kalamazoo, MI) were administered for 3 days after arrival, as a precautionary measure against the presence of any mycoplasmal organisms.

At 3 weeks of age, pigs were divided into blocks of four pigs each based on litter of origin, body weight and gender, and randomly assigned from within blocks to one of four treatments. The four treatments were (1) control, (2) inoculation with Mh, (3) inoculation with PRRSV, and (4) inoculation with both Mh and
PRRSV. All experimental treatments were imposed in two suites of disease-containment chambers (8 chambers per suite, 16 chambers in total) that we have described elsewhere (Escobar et al., 2002). Two pigs (one castrated male and one female) were placed in each chamber. After a 1-week acclimation period, treatments were imposed. Pigs were maintained under an 18-h light:6-h dark lighting regimen (lights on at 06:00 h) and had ad libitum access to water and food unless otherwise stated. A commercially available diet (Escobar et al., 2002) provided adequate to superadequate levels of all essential nutrients (National Research Council, 1998). Chamber temperature was maintained at 32 °C for the first 2 weeks after pigs arrived, and was then reduced 2 °C each week until reaching a temperature of 24 °C.

2.2. Experimental procedures

A diagram of the experimental procedures is provided in Fig. 1. For Mh inoculation, 4-week-old pigs were anaesthetized by i.m. injection of TKX solution (telazol:ketamine:xylazine, 2:1:1; 4.4 mg/kg of body weight). Telazol and ketamine were purchased from Fort Dodge Animal Health, Fort Dodge, IA, and xylazine was purchased from Phoenix Pharmaceuticals, Inc., St. Joseph, MO. Pigs in one-half of the chambers were inoculated intratracheally with 3 ml of Mycoplasma hyopneumoniae (strain P5722-3, broth containing 10^7 color-changing units per ml). Pigs in the remaining chambers received 3 ml of sterile Friis medium intratracheally. Two weeks later, one-half of the pigs that received sterile Friis medium were given 5 ml of sterile Dubelco’s modified Eagle’s medium (DMEM, control) intranasally, and the other one-half were inoculated intranasally with 5 ml of a high-virulence strain of PRRSV (American Type Culture Collection [ATCC] VR-2385 isolate P-129 containing 10^5 50% tissue culture infected dose). One-half of the pigs previously inoculated with Mh received 5 ml of sterile DMEM, and the other one-half were inoculated intranasally with 5 ml of PRRSV. The timing of PRRSV inoculation was chosen to coincide with the onset of clinical signs of mycoplasmal pneumonia (Escobar et al., 2002; Thacker et al., 1999). One-half of the pigs (i.e., 32 pigs) were killed 7 days after PRRSV inoculation to obtain data that have been presented elsewhere (Escobar et al., 2004). Pigs killed 7 days after PRRSV inoculation were
used to collect body temperature data but not behavioural data. Thus, behaviour was assessed for 16 pigs (two chambers with two pigs for each of the four treatments) in each of two trials (32 pigs in total) after PRRSV inoculation.

Body weight was measured immediately before Mh inoculation and every 7 days thereafter. To minimize variation in pig body weight due to gut fill, feeders were removed 12 h prior to all weighings. Feeders were weighed weekly after Mh inoculation and daily after PRRSV inoculation between 08:00 and 09:00 h so that group food intake could be determined. Rectal temperature was measured between 08:00 and 09:00 h on days 7 and 14 after PRRSV inoculation.

Biosafety Level 3 procedures (Richmond and McKinney, 1993) were employed at all times. The University of Illinois Institutional Animal Care and Use Committee and the Environmental Health and Safety Committee approved all procedures.

2.3. Behaviours

In both trials, pigs in two chambers per treatment were video recorded using ceiling-mounted cameras (WV-BP103, Panasonic, Osaka, Japan), attached to a quad-split system (Color Quad System WJ-450, Panasonic), and time-lapse video recorder (AG-TL500, Panasonic). Behaviours were recorded during the 18 h light phase (06:00 to 00:00 h) for 13 days beginning the day of PRRSV inoculation, which was 14 days after Mh inoculation. Behaviours were not recorded immediately after Mh inoculation because in this model, clinical signs of mycoplasmal pneumonia are not evident for nearly 2 weeks (Escobar et al., 2002). A trained technician who was blind to treatments reviewed the video records. The amount of time spent lying and feeding, as well as the total time engaged in active behaviours, was estimated from the video records by scan sampling at 15-min intervals. A pig was considered engaged in active behaviour if it was standing (including walking), sitting, or feeding. A pig was presumed to be feeding if its head was in the feeder. Animals may assume postures that conserve heat when sick, therefore, when pigs were lying, a determination was made as to whether they were lying ventrally or laterally and in contact with the other pig. Behavioural data were not collected on days 7 and 14 because pigs were food deprived and subjected to other experimental procedures (e.g., weighing and blood sample collection) necessary to obtain data that have been presented elsewhere (Escobar et al., 2004).

2.4. Statistical analysis

A Shapiro–Wilk statistic and boxplot analysis were used to determine the normality of data. Data were determined to be normally distributed. The general linear model procedure of SAS (SAS Institute Inc., Cary, NC) for a randomized complete-block design with repeated measures was used to analyze behavioural data. For each day and chamber, the number of minutes engaged in each behaviour during the 18-h light phase was estimated by multiplying the frequency the behaviour was observed by the 15 min sampling interval. The chamber was considered the experimental unit for analysis of behavioural data, whereas the pig was the experimental unit for analysis of body temperature. It was assumed that each pig in a chamber contributed equally to behavioural activities and food consumption. Daily food intake, feeding time, and time spent active were subjected to broken-line regression analysis (Robbins et al., 2006) to determine when the change in a variable reached a plateau after PRRSV inoculation. Least squares means were compared using a t-test and Fisher adjustment.

3. Results

There was no significant main effect of Mh or interaction between Mh and PRRSV for food intake, body temperature, or any behaviour measured. Thus, data from control pigs and pigs inoculated with Mh alone were pooled and designated PRRSV— pigs. Similarly, data from pigs
inoculated with PRRSV alone and pigs inoculated with both Mh and PRRSV were pooled and designated PRRSV+ pigs.

Each day after PRRSV inoculation, PRRSV+ pigs spent less time \((P = 0.005)\) feeding compared to PRRSV− pigs (Fig. 2A). Moreover, broken-line regression analysis indicated that feeding time decreased linearly \((P < 0.001)\) in PRRSV+ pigs for 3.5 days after PRRSV inoculation, but not thereafter. The decrease in feeding time was associated with a decrease in food intake \((P < 0.001;\) Fig. 2B) and, as with feeding time, food intake decreased linearly \((P < 0.001)\) in PRRSV+ pigs for 2.6 days after PRRSV inoculation, after which it stabilized at the lower level. Thus, the reduction in food intake reached a plateau 1 day before the amount of time spent at the feeder reached a plateau. In the case of PRRSV− pigs, feeding time \((P = 0.42)\) and food intake \((P = 0.52)\) were unchanged throughout the study. The amount of time spent feeding was positively correlated with food intake \((P < 0.001, r = 0.790)\) and weight gain \((P < 0.001, r = 0.814)\). Thus, pigs that spent more time feeding consumed more food and gained more weight.

![Fig. 2. Average feeding time (A) and average daily food intake (B) of pigs that received sterile DMEM (PRRSV−) or were inoculated with porcine reproductive and respiratory syndrome virus (PRRSV+, VR-2385 isolate P-129) at 6 weeks of age. Each data point represents mean ± pooled S.E.M., repeated measures \(n = 8\). Asterisks (*) indicate that means are different \((P = 0.005)\) within days.](image-url)
The amount of active behaviour per pig during the 18 h light phase is summarized in Fig. 3. In general, PRRSV+ pigs decreased ($P < 0.001$) their activity after PRRSV inoculation compared to PRRSV/C0 pigs. More specifically, broken-line regression analysis indicated that activity of PRRSV+ pigs decreased linearly ($P < 0.001$) for 2.5 days after PRRSV inoculation and then remained unchanged. Thus, PRRSV+ pigs were less active than PRRSV/C0 pigs.

The total time spent lying, the time lying ventrally and the time lying laterally were greater ($P < 0.001$) in PRRSV+ pigs compared to PRRSV/C0 pigs (Fig. 4A). Furthermore, PRRSV+ pigs spent more of their total lying time in a ventral position compared to PRRSV/C0 pigs ($P = 0.06$, Fig. 4A). Pigs infected with PRRSV spent more time ($P < 0.001$) lying in contact with penmates compared to PRRSV/C0 pigs (Fig. 4B). Furthermore, the increased time lying in contact occurred in both the ventral and lateral positions ($P < 0.001$). The PRRSV+ pigs spent more total time lying in contact in the ventral position compared to PRRSV/C0 pigs ($P < 0.001$, Fig. 4B). Body temperature was higher in PRRSV+ pigs 7 days after PRRSV inoculation (40.2 versus 39.4 ± 0.1 °C; $P < 0.001$) but not at 14 days (39.8 versus 39.6 ± 0.1 °C; $P = 0.19$), compared to PRRSV/C0 pigs. Collectively, these data indicate that pigs infected with PRRSV reduced overall activity and assumed postures that conserved body heat.

4. Discussion

We examined herein the effects of Mh and PRRSV, when presented either alone or in combination, on several behavioural traits of growing pigs. These data were collected as part of a larger study that also determined the effects of Mh and PRRSV infection on pulmonary lesions, growth performance, whole-body composition, protein accretion and circulating inflammatory cytokines (Escobar et al., 2004). In brief summary, Mh alone had no effect on growth, and circulating levels of interleukin-1β (IL-1β) and interleukin-6 (IL-6) were undetectable in Mh-infected pigs. In contrast, PRRSV increased circulating levels of IL-1β
and IL-6, and markedly reduced growth. Thus, the observation reported herein that PRRSV-induced inappetence, lethargy, and fever, but that Mh did not, is consistent with the well-regarded notion that sickness behaviour (Hart, 1988) is induced by inflammatory cytokines (Johnson, 2002).

In swine, Mh adheres to the trachea, bronchi, and bronchioles where it causes a localized inflammatory response which contributes to the development of gross pulmonary lesions (Escobar et al., 2002). PRRSV, however, infects alveolar macrophages that migrate to lymphatic tissue and from there can enter circulation and spread to other parts of the body including the brain (Shin and Molitor, 2002). Infected mononuclear myeloid cells secrete inflammatory cytokines (van Reeth et al., 1999) that have been shown in pigs to reduce appetite and cause lethargy (Warren et al., 1997). Therefore, inflammatory cytokines secreted by PRRSV-infected
cells likely orchestrated the sickness behaviour syndrome (Hart, 1988) that was evident in our study.

The behavioural response to infection is well organized and is thought to enhance disease resistance and facilitate recovery. For example, when mice were inoculated with a LD50 dose of Listeria monocytogenes and force-fed the same amount of food consumed by non-infected controls, mortality approached 100% (Murray and Murray, 1979). In young chicks, activating the innate immune system reduced the capacity to accrete whole-body protein so that increasing the dietary concentration of lysine to overcome the reduction in appetite was of no benefit (Webel et al., 1998). In our pigs PRRSV markedly reduced growth and whole body protein accretion (Escobar et al., 2004). In addition, the reduction in food intake preceded the reduction in feeding activity. These data suggest that food intake is a better early indicator of sickness than time engaged in feeding activity. Thus, when caretakers observe reduced feeding activity a disease episode may be in progress, and depression in food intake has likely already occurred. If food intake is inherently linked to the capacity to grow, it is reasonable to view the reduction in feeding activity and food intake (Fig. 2) as an important behavioural adaptation to sickness that may facilitate recovery.

Other studies have reported a beneficial effect of fever. Lizards that must rely on behaviour to thermoregulate, showed a preference for warmer environments when experimentally infected with Aeromonas hydrophila (Vaughn et al., 1974), and when kept in a constant thermal environment that prevented them from increasing body temperature, mortality increased (Kluger et al., 1975). The beneficial aspects of fever were also demonstrated in rabbits (Kluger and Vaughn, 1978) and very recently it has been recommended that febrile humans not at risk of serious complications caused by an extremely high body temperature or experiencing excessive discomfort, be monitored (e.g., temperature, pulse, blood pressure) and informed about the beneficial effects of fever, which is part of a coordinated acute response to infection (Thompson, 2005). Mammals achieve febrile temperatures by increasing heat production and adopting behavioural postures that conserve heat similar to those adopted by pigs during cold sensation (Xin, 1999). In our study, PRRSV pigs were less active, spent more time lying, and a greater proportion of their time lying was in a ventral position, which minimizes their surface area in contact with a cool floor (Grommers et al., 1970). Further suggesting that PRRSV pigs intentionally sought to achieve a febrile temperature was that they spent more time huddling with a penmate. Collectively and in light of other reports on the beneficial nature of the behavioural responses to infection (Hart, 1988; Johnson, 2002; Konsman et al., 2002), it is reasonable to postulate that the behavioural changes evident in PRRSV-infected pigs have recuperative value.

5. Conclusion

Behavioural responses to infection are well organized and thought to enhance disease resistance and facilitate recovery. Therefore, determining how respiratory infections that are common in swine, change behaviour is important to assessing how current management practices affect convalescence. Our results suggest that pigs with an acute PRRSV infection use behavioural responses that may enhance recovery. Therefore, contrary to the proclivity to want to prevent behaviours associated with infection, management practices that facilitate or even enhance the sickness behaviour syndrome should be considered. For example, isolating sick animals from penmates whose normal activities may disrupt resting, or facilitating the febrile response by maintaining sick animals at a higher ambient temperature, may be useful.
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