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Exposure to hog barn dust alters airway epithelial ciliary beating

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Abstract

Swine confinement workers are at increased risk for airway diseases, including mucus membrane irritation syndrome, chronic rhinosinusitis, and chronic bronchitis. Dust extracts from swine confinement facilities stimulate the production of proinflammatory cytokines in bronchial epithelial cells, including interleukin-8 (IL-8). Because IL-8 is capable of blocking beta-agonist-stimulated increases in cilia beating, which impacts mucociliary clearance, we hypothesized that hog barn dust exposure might alter cilia responses to stimulation.

To test this hypothesis, ciliated bovine bronchial epithelial cell cultures were exposed to hog barn dust extract (HDE) and cilia beat frequency (CBF) assayed. An elevation in baseline CBF was observed. This effect appeared to be independent of endotoxin, but dependent upon nitric oxide. HDE also stimulated nitric oxide production in bronchial epithelial cells; however, stimulation of cilia beating by a beta-agonist did not occur in cells pre-exposed to HDE.

These data demonstrate that hog barn dust can alter normal stimulation of cilia, suggesting a mechanism for the abrogation of stimulated increases in mucociliary clearance in response to inhaled dust exposure.

Keywords

airway epithelial cells; cilia; hog barn dust; swine confinement

Introduction

Modern swine operations raise hundreds to thousands of animals in closed confinement buildings, raising concern over the occupational, environmental, and community hazards posed by these large concentrated animal feeding operations [1]. This method of raising swine has become the major workplace activity for a number of agricultural workers. The air in swine confinement barns often has high levels of ammonia and dust that is rich in endotoxin [2]. Endotoxin, ammonia, and total dust levels are associated with the presence of airway disease in these workers [2,3]. Swine confinement workers commonly report increased nasal and sinus symptoms, cough, chest tightness, wheezing and shortness of breath with exertion [4]. These symptoms are associated with the presence of airflow obstruction on pulmonary function tests and evidence of lower respiratory tract inflammation [2,3,5]. Significant upper respiratory tract inflammation also occurs and is marked by increased neutrophils and inflammatory cytokines

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in the nasal lavage of normal volunteers exposed to swine confinement facilities [6,7]. Furthermore, exposed workers commonly experience nasal congestion, chronic sinonasal disease, and reduced sense of smell [8]. However, little is known about the effects of work in swine confinement barns on mucociliary clearance.

Mucociliary clearance is a regulable innate host defense that consists of a complex regulation of secreted mucus, airway surface liquid, and ciliary beating [9]. The stimulation of mammalian ciliary beating involves a nitric oxide-mediated process [10] that regulates cyclic nucleotide-dependent pathways [11]. Beta agonists have been widely used to stimulate an increase in cilia beat for *in vitro* studies [12]. We have identified that the beta-agonist stimulation of cilia beat can be desensitized by exposure to alcohol [13] or blocked by the presence of interleukin-8 [14]. Previously, our *in vitro* studies have shown that hog barn dust stimulates the production of interleukin (IL)-6 and IL-8 in human bronchial epithelial cells [15]. This response was independent of the endotoxin concentrations present in the dust and required the activation of protein kinase C.

Because swine confinement workers are at an increased risk of developing upper and lower respiratory tract airway diseases, we hypothesized that one innate defense mechanism, mucociliary clearance, may be altered in ciliated epithelial cells exposed to swine confinement facility dust. Because exposure of airway epithelium to hog barn dust results in the production of interleukin-8, we further hypothesized that such exposure would block the stimulatory effect of beta-agonists on cilia beating. These mechanistic studies of *in vitro* cilia beating will provide insight into the effects of working in a swine confinement environment on the respiratory tract.

Materials and Methods

Cell Preparation

To model particle stimulation of cilia *in vivo*, bovine ciliated epithelial cells were stimulated with beta-agonists to increase cilia beating *in vitro*. Primary bovine bronchial epithelial cells were prepared from bovine lung as described [16]. Bronchi were necropsied from the lung and digested overnight at 4°C in 0.1% bacterial protease type IV in minimum essential media (M199 with Earl's salts (Invitrogen, Carlsbad, CA). The following day, the bronchi were repeatedly rinsed in M199 containing 10% fetal bovine serum (FBS, Invitrogen) to collect the epithelial cells lining the lumen. Ciliated clump cells were collected by a 40 μm mesh filter which has been shown to effectively isolate >95% viable ciliated cells in the flow through. These cells were then washed in M199 media, counted with a hemacytometer and 3×10^6 cells were plated on 60 mm tissue culture dishes coated with 1% type I collagen (Vitrogen; Cohesion, Palo Alto, CA). Cells were grown in M199 complete media containing 10% FBS, 50 U/ml penicillin and streptomycin (Invitrogen, Carlsbad, CA), 2 μg/ml fungizone, (Invitrogen) in a humidified 95% air/5% CO_2 incubator at 37°C. Confluent monolayers of primary ciliated cells were obtained in 3 days and treated as described below.

Preparation of Hog Barn Dust Extract

Settled dust was collected approximately 1–2 m above the floor from swine confinement buildings with approximately 500–700 swine weighing around 100 kg. [17] The dust of each facility was processed separately and experiments repeated using different sources of dust, including dust obtained fresh and dust that was stored at −80°C. This extract was prepared in a similar fashion as to grain dust extracts utilized in previously published work [18] and to hog barn dust extract used in animal model studies [19]. Hog barn dust extract (HDE) was prepared by placing one gram of dust in 10 ml of HBSS (Biosource International, Camarillo, CA) without calcium. The mixture was vortexed and allowed to stand at room temperature for 1 hour. The mixture was centrifuged for 20 minutes at 2000 × g, supernatant recovered and centrifuged

again for 20 minutes at 2000 × g. The final supernatant was filter sterilized and used immediately. This supernatant is considered as 100% HDE and diluted into culture medium to achieve the various HDE concentrations used in the experiments. Others have reported 0.3 $\times 10^4$ to 3.3 $\times 10^8$ CFU/ m^3 culturable bacteria in workers' breathing zones [20]. We cultured approximately 5 $\times 10^6$ CFU bacteria/gram of hog barn dust used to produce 100% HDE. This suggests the concentration of culturable bacteria in the HDE used in the experiments has similarity to concentration of bacteria in breathing zone of workers.

The extract made with hog feed from a matching swine confinement facility, or grain feed dust extract, was prepared in an identical fashion to the HDE. The amount of endotoxin in HDE was quantitated using limulus amebocyte lysate gel clot LAL assay commercially available and recorded on each extract prepared [21]. Endotoxin-reduced HDE (40 EU/ml pre- vs 0.06 EU/ml post-polymyxin B) was prepared and characterized as previously described [15].

Ciliary Beat Frequency Assay

Cells were maintained at a constant temperature (25°C ± 0.5°C) during all experimental procedures with the use of a thermostatically controlled heated stage. The motion of cilia was processed using the Sisson-Ammons Video Analysis (SAVA) system as described [22]. Images of ciliated cells were visualized on an Olympus IMT-2 inverted phase-contrast microscope using a 20× objective lens with a 1.5× tube multiplier and images captured using a Kodak 310 analog/digital video camera (Eastman Kodak Motion Analysis System Division, San Diego, CA). The sampling rate was set at 85 frames per second for all experimental conditions. Captured digital video was transmitted from the camera directly into an IMACQ OCI/PXI-1422 digital acquisition board (National Instruments, Austin, TX) within a Dell Precision 420 Workstation. The entire captured image of 640×480 pixels was automatically analyzed for motion by SAVA using a process known as Whole Field Analysis. The Whole Field Analysis technique has been validated against specific region-of-interest analysis as described [22]. The SAVA software analyzed each image containing 19,200 possible motile zones to determine the average frequency and the standard error of the mean for each field captured. For each experimental condition, a minimum of 6 separate fields were captured, analyzed and expressed as a data point. ANOVA was run on each data point and was considered significant with a P value ≤ 0.05.

Nitric Oxide Analysis

Bronchial epithelial cell nitric oxide production was monitored via the detection of nitric oxide by a gas-phase chemiluminescent reaction between nitric oxide and ozone (Sievers Instruments Model 280i, GE Analytical Instruments, Boulder, CO). Monolayers of airway epithelial cells were treated with various concentrations of HDE for various times. The cell medium was separated from the cells and both fractions flash frozen to halt metabolic reactions. The proteins were precipitated in equal volumes of 0.5 N NaOH and 10% $ZnSO_4$ for 15 minutes prior to being centrifuged at 14,000*g* for 5 minutes at 4°C. Supernatants (10 μl) were injected into a reflux column containing 0.1 *M* VCl_3 in 1 *M* HCl at 80°C to reduce any nitrates and nitrites into nitric oxide (NO). NO then combines with O_3 produced by the nitric oxide analyzer to form NO_2. The resulting emission from the excited NO_2 is detected by a photomultiplier tube and recorded digitally (mV). The values were then interpolated to a standard curve of $NaNO_2$ concentrations concurrently determined. Sample measurements were made in triplicate for each cell treatment with each sample injected a minimum of three times for a total of nine readings per data point. Significance was determined by ANOVA.

Cell Viability Assay

Media supernatant (50 μl) from cell monolayers treated with HDE or media alone were sampled for cell viability assays. In addition, confluent monolayers of cells were lysed as a positive

control for lactate dehydrogenase (LDH) release. Cell viability was determined by a commercially available kit (Sigma, St. Louis, MO) to measure LDH release, according to the manufacturer's instructions.

Western blotting

The solubilized HDE proteins or grain feed dust proteins were separated on SDS-PAGE under reducing conditions and transferred to nitrocellulose as detailed [23]. Nitrocellulose membranes were blocked overnight at 4°C in a buffer consisting of 5% milk, 50 m*M* of Tris-HCl, 90 m*M* of NaCl, 2 m*M* of $CaCl_2$ (Blotto). The detection of IL-8 was visualized by incubating the blots with a solution of Blotto containing 0.2 mg/ml rabbit anti-porcine interleukin-8 (R&D Systems, Minneapolis, MN) and 0.5 mg/mL peroxidase-conjugated goat anti-rabbit secondary antibody (Rockland, Gilbertsville, PA). Blots were washed extensively with phosphate buffered saline (PBS) and Tween-20, washed once with PBS and developed using enhanced chemiluminescence (SuperSignal West Pico, Pierce, Rockford, IL). Controls consisted of probing the blot with a non-specific rabbit IgG in place of the rabbit anti-porcine IL-8.

ELISA for IL-8

The concentration of IL-8 in the HDE samples was determined by ELISA as previously reported [15].

Statistical analysis

All quantitative experiments were performed at least three times with each data point assayed in triplicate (n=9). Data were analyzed using Graphpad Prizm (San Diego, CA) and represented as mean ± standard error. Data were analyzed for statistical significance using one-way ANOVA for nitric oxide assays and paired Student's t-test for the CBF assays. Significance was accepted at the 95% confidence interval.

Results

HDE Elevates Baseline CBF

Because acute exposure to inhaled particles typically stimulates mucociliary clearance, we hypothesized that brief exposure of naïve ciliated cells to HDE would stimulate ciliary motility. To test this hypothesis, we determined the short-term effect of HDE on mucociliary clearance *in vitro*, by exposing ciliated bovine bronchial epithelial cells to HDE and examining cilia motility. Confluent monolayers of ciliated cells were maintained as a submerged culture in M-199 media. Cells were treated in the presence or absence of 5% HDE and CBF assayed for up to 5 hours at room temperature using SAVA [22]. Exposure to culture media demonstrated a consistent baseline CBF of approximately 9 Hz (Figure 1). An elevation in baseline CBF was observed by 30 minutes exposure to HDE with the maximal increase (~ 1 Hz) observed by 1 hour treatment with HDE. The effect of HDE on CBF was concentration- and time-dependent. At 30 minutes exposure, increasing concentrations of HDE from 5–25% elevated baseline CBF vs. media control exposure (Figure 2A). While early exposure (30– 90 min) to 5–25% HDE results in the elevation of CBF, higher concentrations (10–25%) of HDE do not increase CBF after 2–3 hour (Figure 2B). In fact, higher concentrations (25%) of HDE produced a sustained slowing of the cilia after 3 hours. At longer exposure times, the 25% HDE is associated with decreased cell viability as determined by LDH release (data not shown). These data show that HDE elevates baseline CBF in bronchial epithelial cells.

Because endotoxin is a known mediator of inflammation due to dust inhalation, we hypothesized that the HDE-induced elevation of CBF was independent of endotoxin. To test

this hypothesis, a 5% dilution of HDE was eluted from a polymyxin B column to reduce the level of endotoxin contained in the sample. This detoxified HDE solution was then exposed to ciliated cells and CBF measured. No differences in CBF were observed between 5% HDE exposure vs. polymyxin treated HDE exposure (Figure 3A). Control media eluted from a polymyxin B column had no effect on baseline CBF levels vs. media control (data not shown). The amount of endotoxin contained in a 5% dilution of HDE was determined by Limulus test [21] to be approximately 40 EU/ml. When 40 EU/ml of lipopolysaccharide (LPS) was added directly to ciliated cells, there was no increase in CBF baseline beating (Figure 3B). The addition of 40 EU/ml LPS to 5% HDE did not alter the elevated CBF observed with 5% HDE (data not shown). These data provide evidence that endotoxin is not the component of HDE responsible for elevations in baseline CBF.

HDE Stimulates Nitric Oxide Production

Because exposure to dusts from swine confinement facilities has been associated with elevated exhaled nitric oxide [24] and nitric oxide production is a known stimulator of CBF [10], we hypothesized that the effect of HDE on ciliated airway epithelial cells was regulated by NO production. Confluent monolayers of media-submerged ciliated cells were treated in the presence or absence of HDE and media release of nitric oxide assayed for up to 2 hours at room temperature using a chemiluminescence detector of nitrite and nitrate accumulation. A significant accumulation of nitric oxide into the cell media was observed beginning at 30 min exposure to 5% HDE (Figure 4). Elevations in nitric oxide continued up to 2 hours beyond which no further accumulation was observed. Higher concentrations of HDE (10–25%) also elevated nitric oxide, but lower HDE concentrations (1%) did not result in significant elevations (data not shown). Pre-incubation of the cells for 1 hour with 1μ*M* of the nitric oxide synthase inhibitor, L-NMMA, blocked HDE stimulated production of nitric oxide (data not shown). Likewise, the HDE-stimulated elevations in baseline CBF were blocked when the cells were pre-incubated with L-NMMA (Figure 5). These data reveal that HDE increases the production of nitric oxide, a stimulatory regulator of CBF in ciliated cells.

HDE Exposure Blocks Isoproterenol-Stimulated CBF

Because nitric oxide-dependent regulation is important in the response of ciliated cells to beta agonist stimulated increases in CBF [25], we examined the *in vitro* effect of HDE exposure on isoproterenol-stimulation of cilia beating. Similar to previous findings [26], isoproterenol (100 μ*M*) rapidly stimulated a 3–4 Hz increase in CBF from 30–90 minutes followed by a subsequent return to or decrease in baseline beating after 2 hours exposure (Figure 6A). This stimulation in response to isoproterenol was blocked when the cells were pretreated with 5% HDE for 1 hour. Because our previous studies have shown that interleukin-8 exposure blocks beta-agonist stimulated increases in CBF [14] and HDE stimulates the release of interleukin-8 from bronchial epithelial cells [15], we investigated the role of interleukin-8 in this HDE cilia inhibitory response. Pre-incubation of undiluted HDE with 20 μg rabbit anti-porcine interleukin-8 for 24 hours reversed the inhibitory effect of 5% HDE on isoproterenol-stimulated increases in CBF (Figure 6B). The presence of anti-porcine interleukin-8 antibody in HDE produced no differences in CBF vs. HDE alone (data not shown). Isotype control IgG antibody did not restore the ability of isoproterenol to stimulate CBF in the presence of HDE (data not shown). These data demonstrate that the presence of interleukin-8 in HDE is responsible for the lost responsiveness to isoproterenol-stimulated CBF in ciliated bronchial epithelium.

HDE contains IL-8

To identify the presence of interleukin-8 in hog barn dust, Western blot analysis was performed on samples of HDE. Six different samples of hog barn dust each from a different farm were prepared in aqueous solutions as described in Methods. Samples were resolved by

polyacrylamide gel electrophoresis and Western blotted using rabbit anti-porcine interleukin-8 antibodies. An 8-kilodalton protein band was observed in all samples assayed (Figure 7). As a negative control, extracts from grain feed dust demonstrated no porcine interleukin-8 (data not shown). Using antibodies against porcine IL-8 which recognize bovine IL-8, each of the six different HDE samples contained an average of 258.3 pg/ml of IL-8 as determined by ELISA. These data demonstrate that porcine interleukin-8 is present in hog barn dust.

Discussion

In this study, we found that extracts of hog barn dust increase baseline cilia beating in an *in vitro* bovine cell model. However, it was also observed that exposure to hog barn dust blocked the ability of a beta-agonist, isoproterenol, to stimulate increased cilia beating. The stimulation of the ciliary “fight or flight” response as mimicked by beta-agonists may be more important in mucociliary clearance than maintenance of baseline beating.

These observations also underscore that ciliary stimulatory pathways may involve different mechanisms of regulation than the cellular machinery that maintains baseline continual cilia beating.

These observations have potential implications for the effectiveness of beta-agonists in persons working in hog confinement facilities. Poole, et al have reported that the majority of swine veterinarians with airflow obstruction did not demonstrate reversibility with beta-agonists [27]. Although the mechanisms by which beta-agonists mediate bronchodilation and mucociliary clearance may not be completely identical, the lack of responsiveness to beta-agonists on spirometry suggests the possibility that exposure to hog barn dust interferes with the ability of beta-agonists to regulate airway function and is consistent with ability of HDE to block isoproterenol-stimulated ciliary beat frequency.

Our data revealed that bronchial epithelial cells generated nitric oxide in response to HDE exposure *in vitro*. Similarly, elevated exhaled nitric oxide has been reported in normal subjects [24] and workers [28] exposed to swine confinement facility dust. We found that the increased nitric oxide levels were coincident with the elevation of baseline cilia beating in HDE exposed cells, and HDE effects on beating cilia were blocked by the nitric oxide synthase inhibitor, L-NMMA. Nitric oxide is an established regulator of cilia first reported to control stimulated increases in beating by Sisson et al. [10]. The functional significance of this nitric oxide-elevated beating in response to HDE is not clear. Our *in vitro* studies represent an acute exposure of naïve cells. In fact, longer exposure times to HDE exhibited a return to the same baseline beating as control media-exposed cells. This contrasts with the rapid effect of HDE on beta-agonist-stimulated cilia beating. However, it would be interesting to explore the effect of swine confinement dust on nitric oxide and ciliary beating in naïve subjects. Naïve subjects have a more pronounced inflammatory response of the respiratory tract than do swine confinement workers, suggesting that an adaptation to the environment likely develops in workers over time [29].

A one-hour exposure to HDE completely blocked isoproterenol HDE-stimulation of cilia beating. In exploring the mechanism of this inhibition, we found that neutralizing interleukin-8 levels in the HDE restored the isoproterenol cilia stimulation response. This observation agrees with our previous finding that interleukin-8 desensitizes the beta agonist response [14]. In that study, we identified the mechanism of the interleukin-8 inhibitory response to exist at the level of adenylyl cyclase and not due to alterations in beta-adrenergic receptor. Studies have shown that particles themselves do not alter the beta-agonist receptor, as human subjects respond to beta agonist after particle instillation [30]. While we have shown that HDE stimulates a significant production and release of interleukin-8 between 6–48 hours exposure [15], the cilia

desensitization effect occurred after 1 hour HDE exposure. This rapid effect of desensitization suggested the presence of interleukin-8 in dust and this was confirmed by Western blot. However, it is more likely that a sustained beta-agonist desensitization response from HDE would also involve airway epithelium stimulation of IL-8 over a longer period of time.

It is also possible that the short-term organic dust stimulation may be analogous to the beta-agonist stimulation in that it requires a “flight response” for clearance. This may represent a distinct difference from the maintenance of baseline beating as altered by nitric oxide. HDE may therefore desensitize this flight or stimulation response. Alternatively, HDE activates bronchial epithelial cell protein kinase C [15], which is consistent with the published observations that PKC-activating agents cause cilia slowing [31]. The HDE-mediated activation of a PKC isoform may result in a ciliary axonemal protein phosphorylation event that leads to the uncoupling of the cilia flight response.

Although we have demonstrated that HDE contains IL-8 that contributes to the regulation of ciliary beat frequency, it is possible that other constituents of the dust are also involved. Studies to characterize components of HDE responsible for stimulation of airway epithelial cell IL-8 release are ongoing in our laboratories. The component(s) of HDE mediating IL-8 release appears to be large molecule(s) that are stable to boiling, acid and base treatment (data not shown). Further work is required to determine the specific nature of these large molecules that induce IL-8 production and whether these molecules also directly regulate baseline or isoproterenol-stimulated ciliary beating.

In conclusion, dysfunction in mucociliary clearance occurs in workers chronically exposed to swine confinement operation environments. The mechanism of this dysfunction is complex. Dust extracts from these facilities alter ciliary beat at baseline and significantly diminish the beta agonist-enhanced beat frequency. In part, nitric oxide mediates this process. In addition, we found a novel component, IL-8, in the dust extract that may explain the rapid changes in mucociliary clearance that is observed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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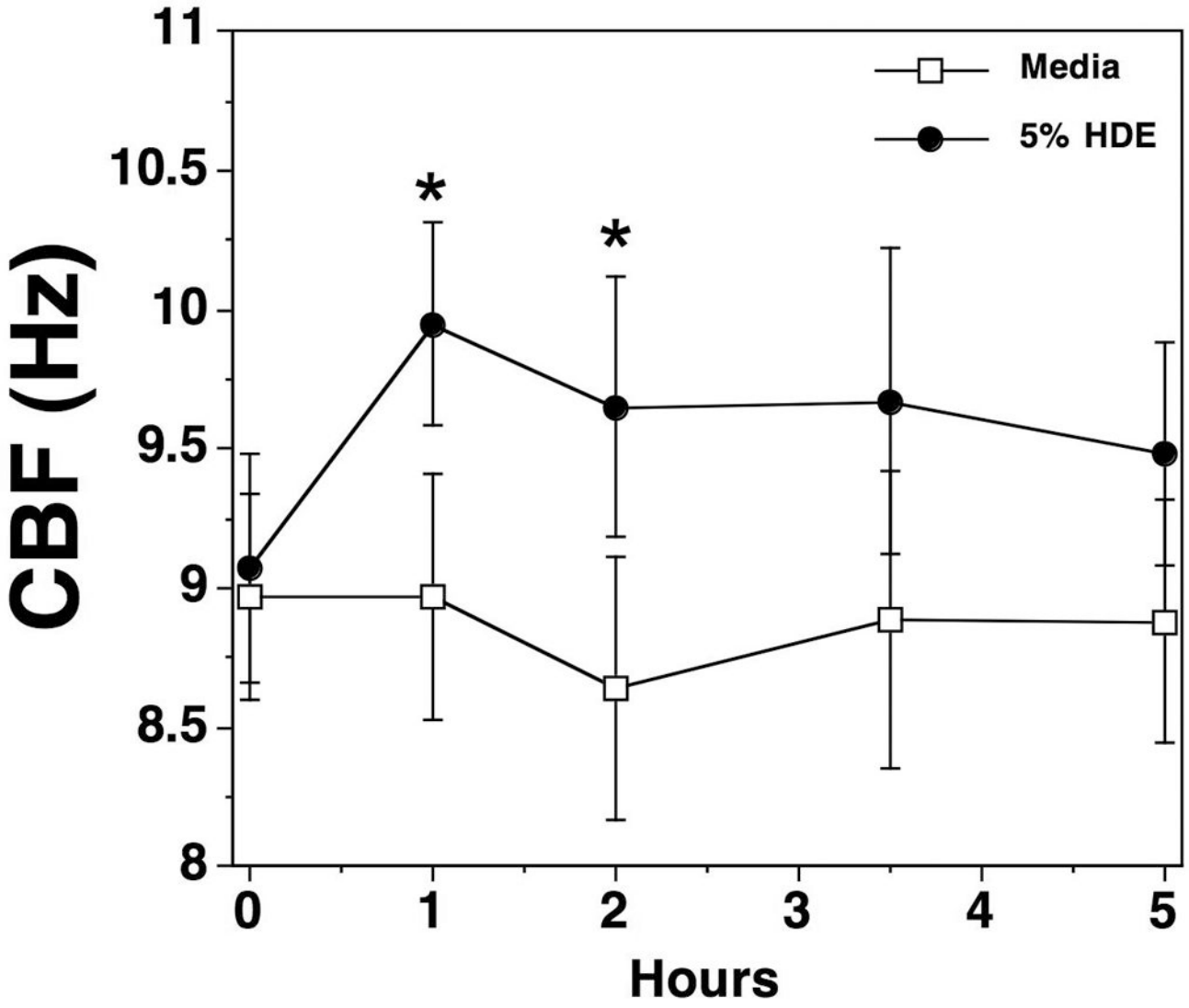

Figure 1. Baseline ciliary beat frequency (CBF) is elevated in airway epithelial cells exposed to hog barn dust extract (HDE)

Monolayers of confluent ciliated bovine bronchial epithelial cells were treated with (5% HDE) or without (media) a 5% dilution of HDE in M-199 media in liquid submerged cultures for up to 5 hrs and CBF was measured. Exposure to 5% HDE increased CBF by approximately 1 Hz vs. media control at 1–2 hr (*P<0.05).

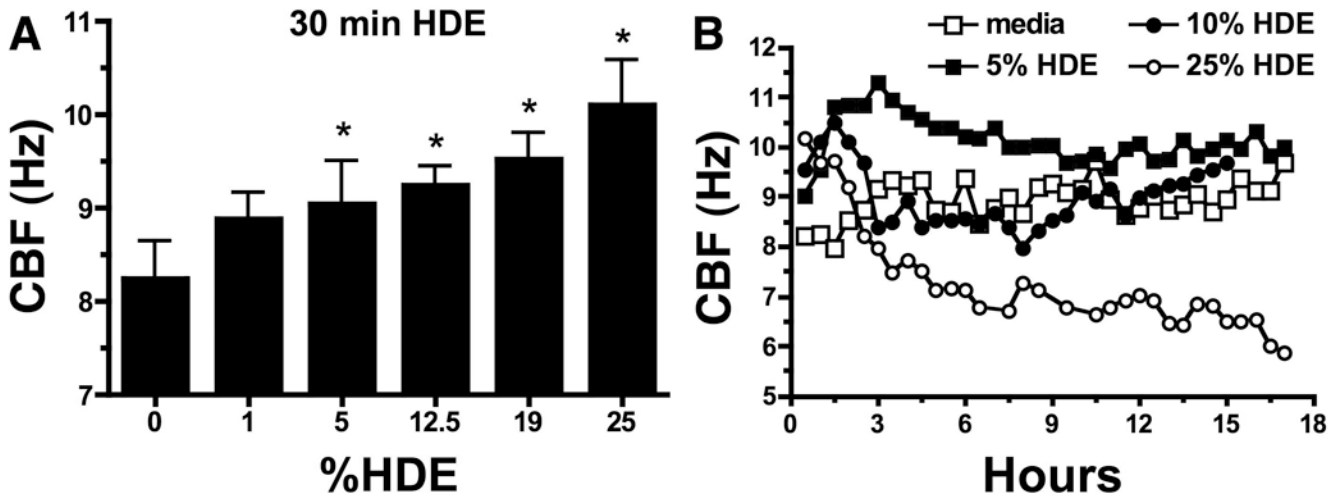

Figure 2. The elevation of baseline ciliary beat frequency (CBF) by hog barn dust extract (HDE) is time- and concentration-dependent

Ciliated bovine bronchial epithelial cells were treated with 0–25% dilutions of HDE and CBF was measured at 30 minutes and 24 hrs. As shown in Panel A, HDE increases baseline CBF vs. media control at 30 min exposure to 5–25% HDE (**P*<0.05). However, overnight exposures with 5–10% HDE result in no significant changes in baseline CBF (Panel B). Higher concentrations of HDE (25%) resulted in lowered CBF from 4–24 hrs.

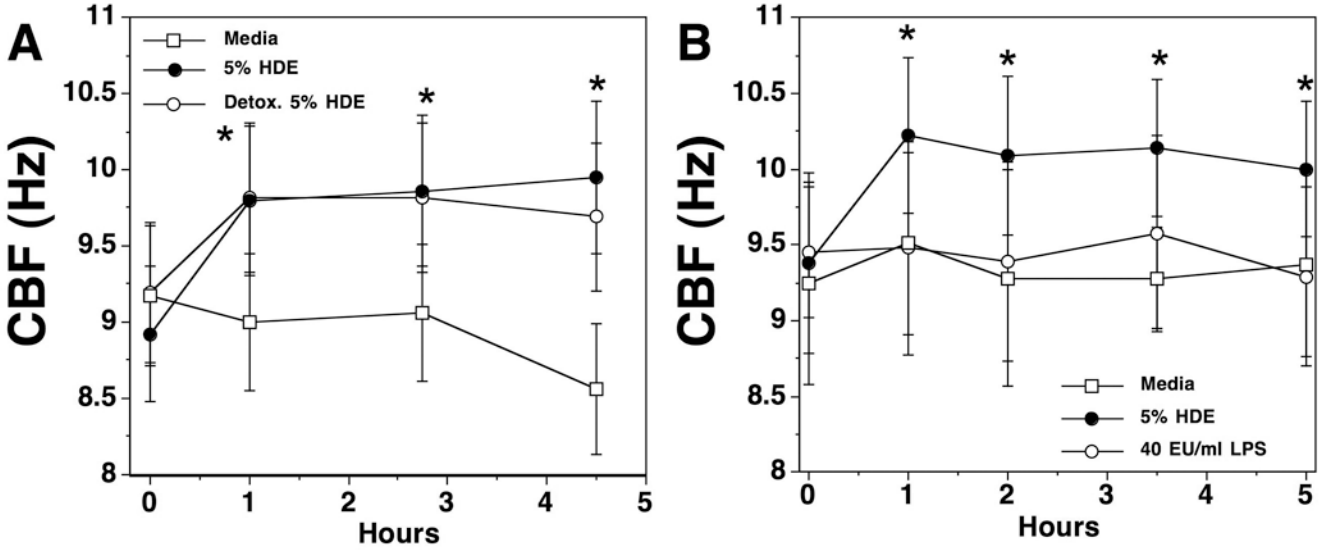

Figure 3. Elevation of ciliary beat frequency (CBF) by hog barn dust extract (HDE) is not due to endotoxin

In Panel A, ciliated bovine bronchial epithelial cells were treated for up to 5 hrs with control media (Media), a 5% dilution of hog dust (5% HDE), or 5% diluted hog dust solution eluted from endotoxin-binding polymyxin B column (Detox. 5% HDE) and CBF measured. Endotoxin-depleted HDE significantly elevated baseline CBF vs. media control at each time point from 1–4.5 hr (*P<0.05). No significant differences were observed between 5% HDE vs. Detox. 5% HDE at similar time points. In Panel B, the addition of the average amount of endotoxin measured in the HDE (40 EU/ml LPS) to the cell media resulted in no significant change in CBF vs. control media (Media) at any time point assayed.

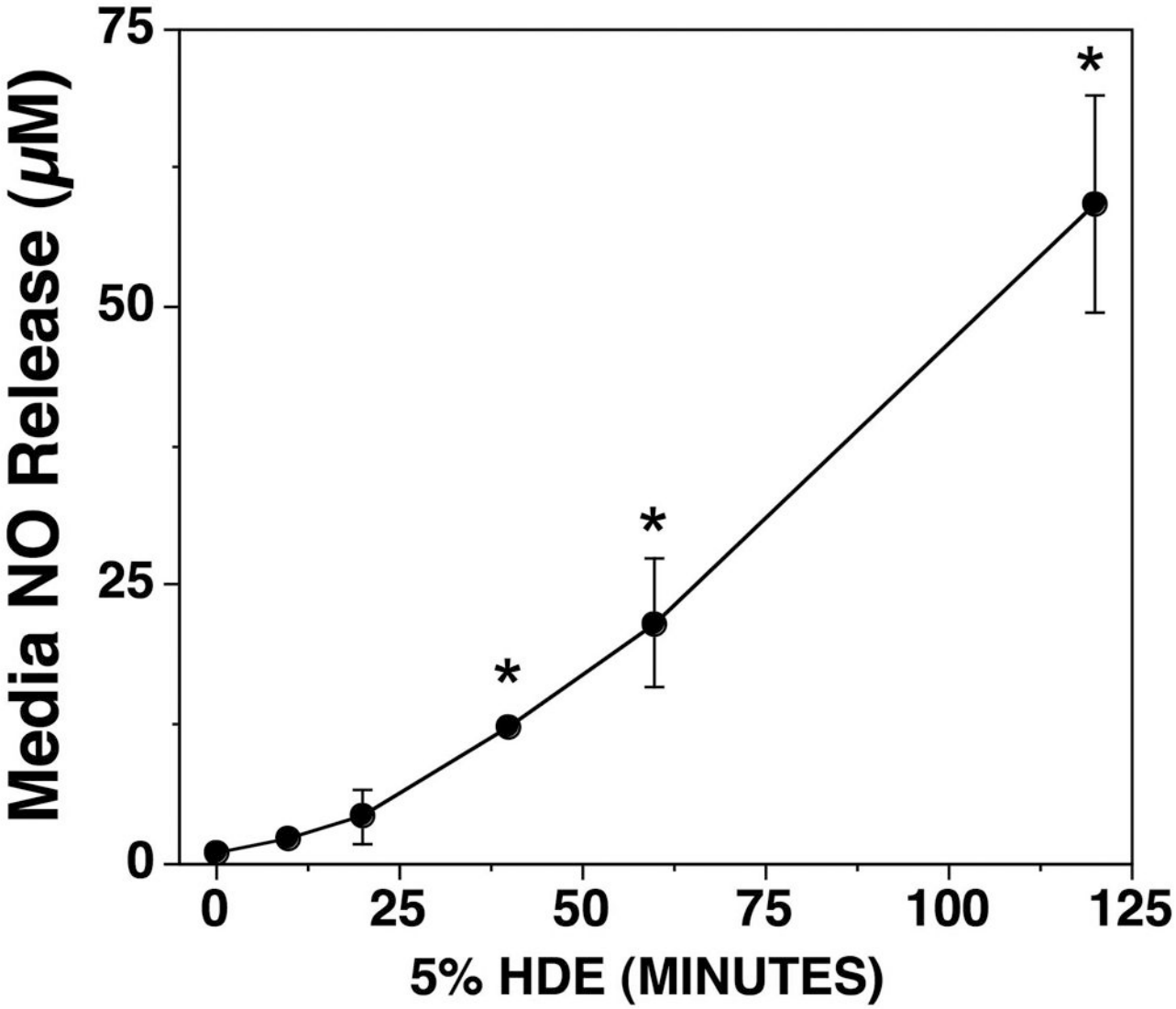

Figure 4. Hog barn dust extract (HDE) stimulates nitric oxide (NO) production in airway epithelial cells

Ciliated bovine bronchial epithelial cells were treated with a 5% dilution of HDE in a nitrogen-free cell media (5% HDE) for up to 2 hrs. Total increase in media nitrites and nitrates were assayed. Exposure to 5% HDE increased the level of accumulated nitrites and nitrates rapidly vs. control media-treated cells with a sustained increase up to 2 hrs (*P<0.05).

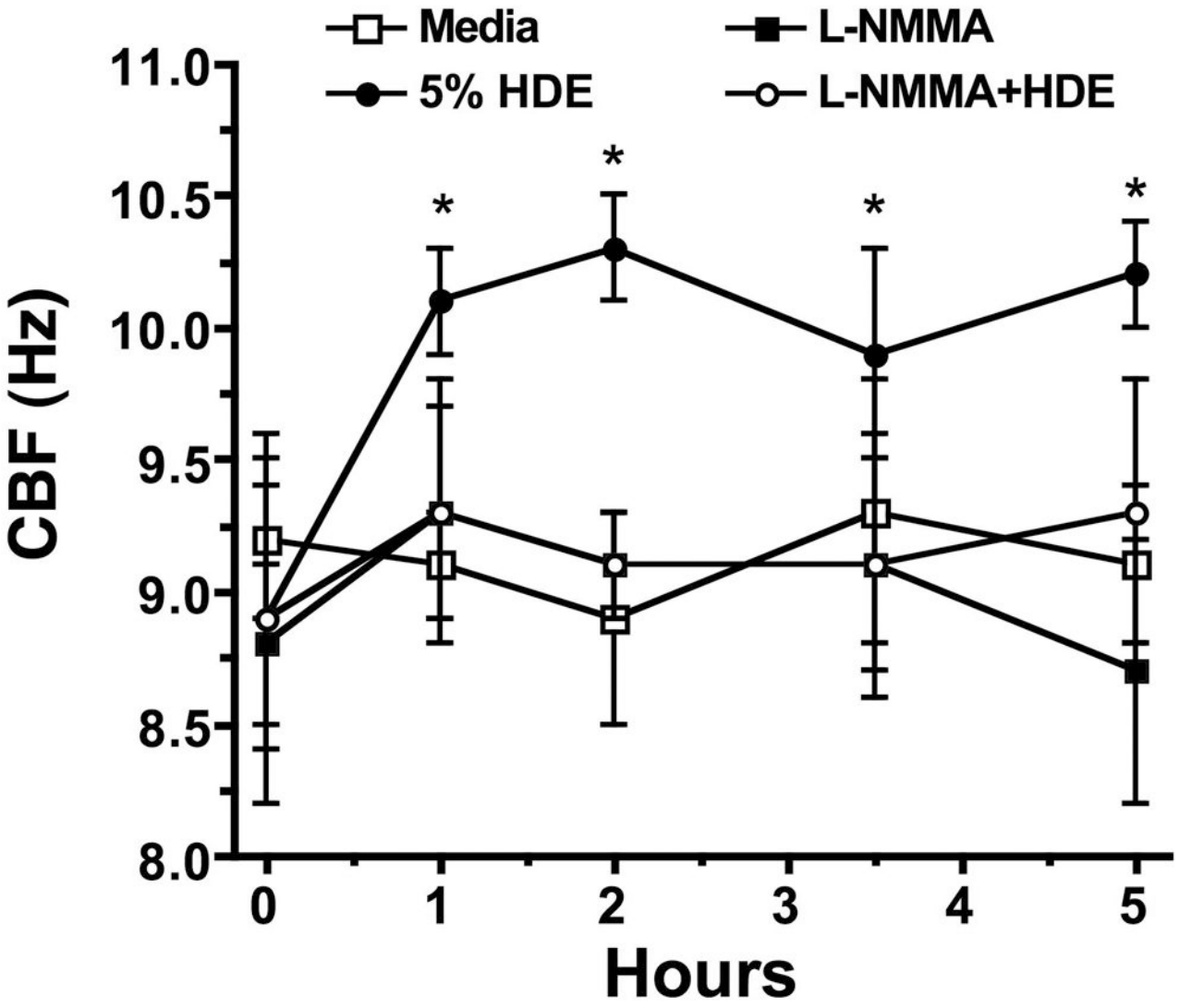

Figure 5. Hog barn dust extract (HDE) stimulation of ciliary beat frequency (CBF) is nitric oxide dependent

Ciliated bovine bronchial epithelial cells were treated for up to 5 hrs with control media (Media) or a 5% dilution of hog dust (5% HDE) in the presence or absence of 10 μM of a nitric oxide synthase inhibitor (L-NMMA) and CBF was measured. 5% HDE increased baseline CBF vs media control cells at each time point (**P*<0.05), but this HDE-induced elevation was blocked when the cells were pretreated for 1 hr with L-NMMA (L-NMMA+HDE).

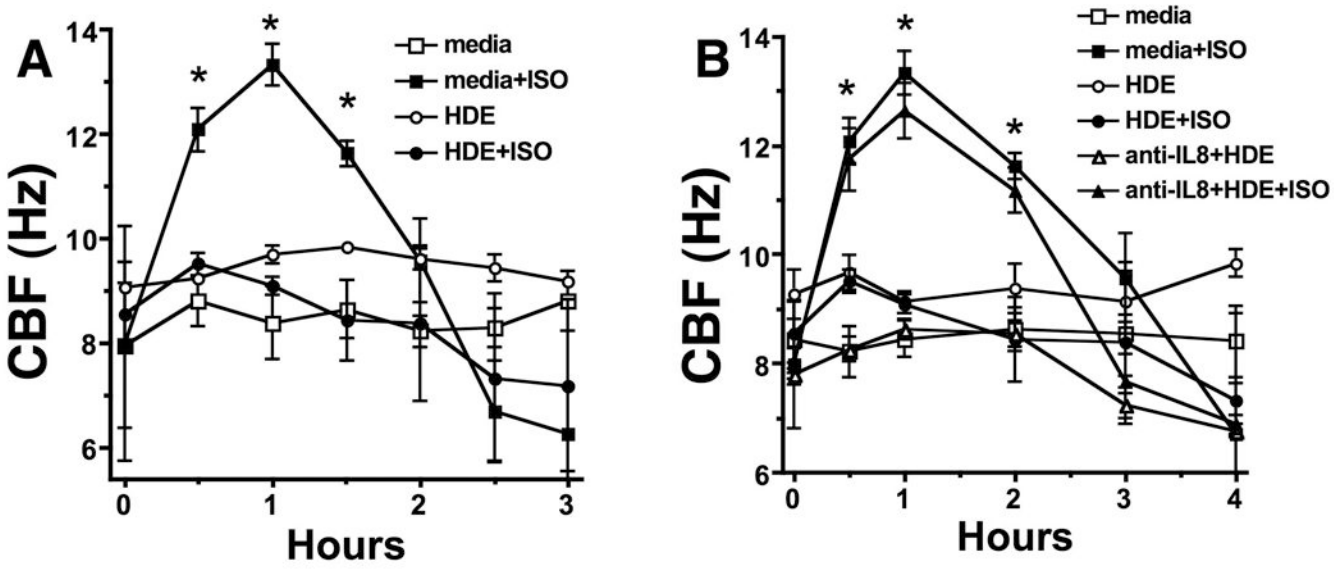

Figure 6. Hog barn dust extract (HDE) inhibition of beta agonist-stimulated increases in ciliary beat frequency (CBF) is blocked by antibodies to IL-8

Ciliated bovine bronchial epithelial cells were pre-treated with or without a 5% dilution of HDE for 1 hr prior to exposure to 100 μM isoproterenol (ISO) for up to 4 hr. In Panel A, isoproterenol significantly stimulates increases of 3–4 Hz in CBF vs. control media-treated cells over a period of 30–90 minutes exposure (**P*<0.005). Preincubation with 5% HDE blocks the isoproterenol stimulation of CBF and results in no changes vs. control media. In Panel B, overnight pre-incubation of HDE with antibodies to interleukin-8 (anti-IL8) restored the ability of isoproterenol to stimulate CBF vs. isoproterenol stimulated cells exposed to HDE and not pretreated with anti-IL-8 (**P*<0.005).

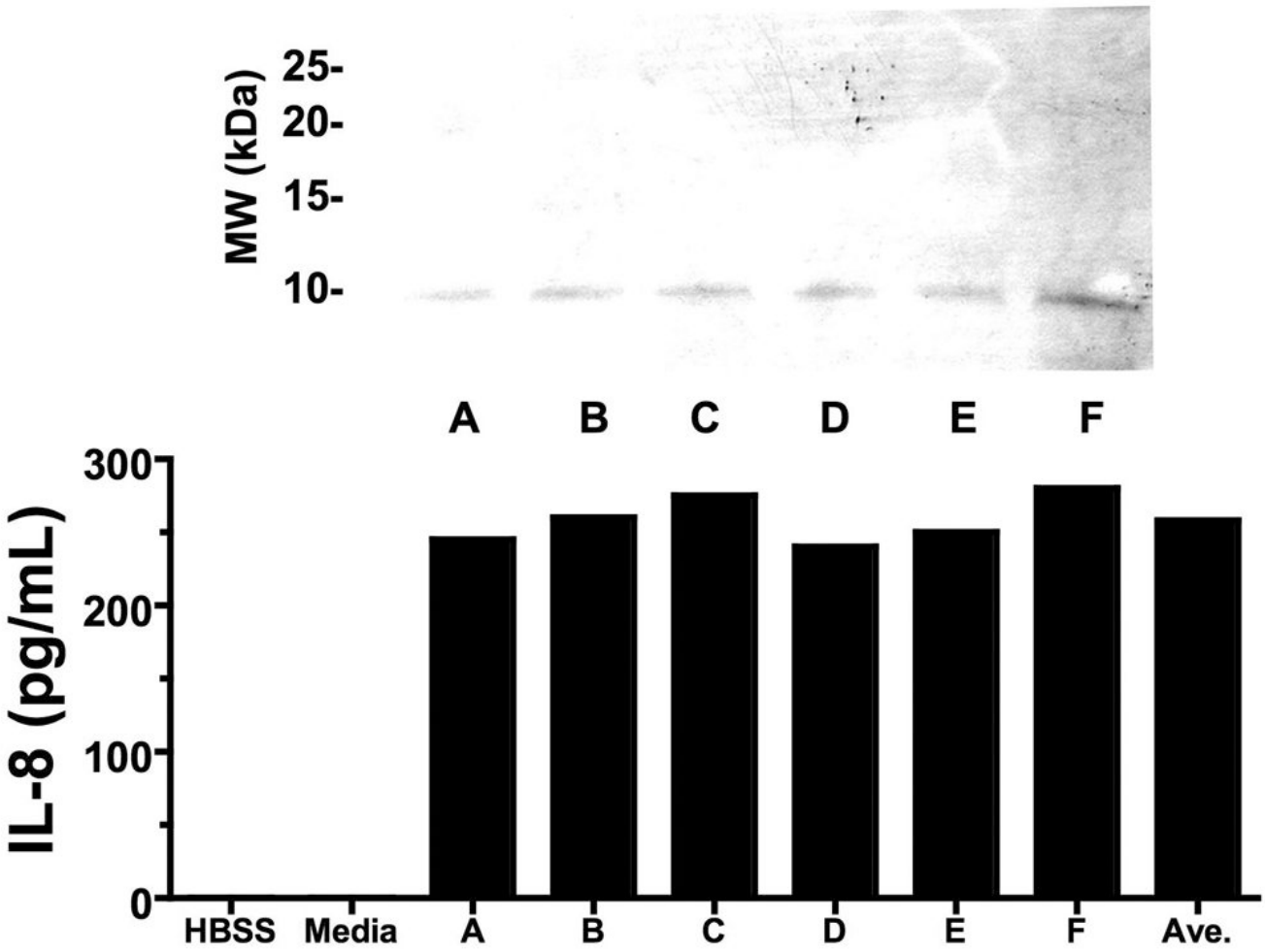

Figure 7. Identification of interleukin-8 in hog barn dust extract (HDE)

Six different samples of HDE (A–F) were resolved by polyacrylamide gel electrophoresis and subjected to Western blotting using antibodies against porcine interleukin-8 (Top panel). HDE demonstrated a reactive protein band at approximately 8–9 kDa recognized by anti- IL-8 antibodies in all dust samples assayed. HDE samples A–F were assayed for IL-8 by ELISA (Bottom panel). An average of 258.3 pg/mL IL-8 was detected in the six HDE samples.