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Developing a method to estimate aerosol generation during poultry slaughtering and defeathering in Bangladesh: An experimental study

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ABSTRACT

Influenza viruses can be aerosolized when slaughtering infected chickens, which increases the risk of zoonotic transmission. We conducted pilot experiments to measure the concentrations of airborne particles <2.5 μm during slaughtering and defeathering of chickens to help identify methods that can minimize workers' exposure to potentially hazardous aerosol particles. By using two types of airborne particle monitors stationed at different heights and angles in a controlled environment, we measured aerosolized particulate matters during exsanguination of 10 slaughtered chickens and use of a mechanical device for defeathering 10 chickens. For the slaughtering experiments, the median particle concentrations at 148 cm height were 67 μg/m^3 (IQR 44–121) with a baseline count 10 μg/m^3 (IQR 10–10) for the Particle and Temperature Sensor + (PATS+) monitors and 34 μg/m^3 (IQR 34–64) with a baseline count 25 μg/m^3 (IQR 16–44) for the SidePak™ monitor. For the defeathering experiments, the median particle concentrations recorded by the PATS+ monitors were not significantly different between 148 cm (41 μg/m^3, IQR 29–49; baseline 12 μg/m^3, IQR 10–19) and 107 cm height (37 μg/m^3, IQR 29–44; baseline 13 μg/m^3, IQR 10–22). Our protocol can be used to test the generation of airborne particles that are <2.5 μm during different slaughtering and defeathering techniques used in the live bird markets to identify procedures that produce the lowest concentrations of small aerosol particles.

1. Introduction

Influenza viruses can be transmitted through the air, with previous pandemic influenza viruses–likely spreading through airborne routes to the human respiratory system [1,2]. Originating in avian hosts, highly pathogenic avian influenza (HPAI) virus strains cause sporadic infections in humans, often with high mortality, and could mutate over time to spread more efficiently between immune-naïve human hosts [3,4].

Zoonotic strains of avian influenza viruses (AIVs) have been isolated from the air of live bird markets (LBMs), and such locations have been one of the primary sources for human cases [5–8]. In Bangladesh, HPAI

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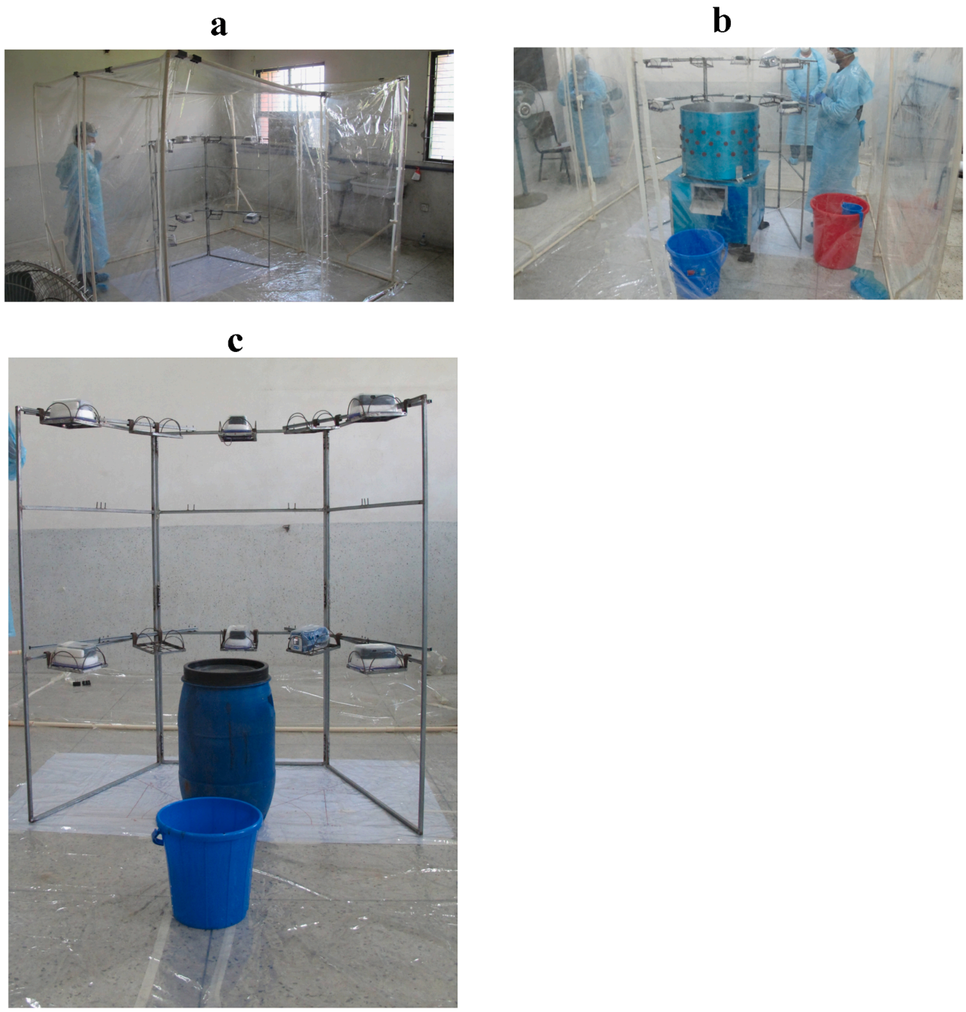


Fig. 1. Placement of equipment inside the portable booth during experiments at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018
a: Portable booth to conduct experiments
b: Placement of particle monitors for slaughtering experiments
c: Placement of particle monitors for defeathering experiments.

H5N1 virus has become endemic in poultry [9]. Ninety five percent of poultry in Bangladesh are sold in LBMs [10]. Live poultry are kept in LBMs until sold, slaughtered, and processed [10]. Poultry in these markets are often infected with a variety of AIVs. A study showed that 47 % of birds tested from LBMs in Bangladesh's two largest cities were actively infected with AIVs, including 5 % with highly pathogenic H5 strains [11]. The handling, slaughtering and defeathering of sick poultry have been implicated as key risk factors for human risk of contracting HPAI [12–16]. In 2012, Bangladesh reported three cases of H5N1 infection among LBM workers [17,18]. In a laboratory setting, the slaughtering of poultry inoculated with AIV has been shown to generate viable viruses in small aerosols (1–4 μm particles) and large aerosols (>4 μm) and to infect and kill immunologically naive birds and ferrets through an airborne route of transmission [19]. In LBMs, the exsanguination of the slaughtered poultry and the use of mechanical defeathering devices are of particular concern because both processes generate aerosol plumes that could potentially contain HPAI and other viruses [19–21]. AIV RNA were frequently detected in nasopharyngeal and arm swabs among LBM workers in Dhaka [22].

AIV and other pathogens have been detected in airborne particulate matter (PM) collected in LBMs, slaughterhouses, and other settings [23–28]. Prior studies have shown that viable airborne HPAI virus particles are generated during the slaughtering process of experimentally infected poultry in a research laboratory [21] but the amount of PM aerosolized during the slaughtering and defeathering processes was not assessed. Measuring aerosolized PM that could carry viable AIV particles may be a more effective proxy method of studying potential exposure than attempting to detect aerosolized AIVs directly, which can be complex and expensive.

In recent years, many models of low-cost aerosol monitors for PM have become available worldwide. These monitors have been in use to assess household, institution-based and occupational exposure to PM [29–32]. The most widely available low-cost monitors measure $PM_{2.5}$, which is a commonly used quantitative measure of air pollution consisting of all airborne particles <2.5 μm in size. Aerosol particles <4 μm, which includes $PM_{2.5}$, are "respirable", which means that they are small enough to reach the alveolar region of the lungs when inhaled [33]. In human lungs, receptors for H5N1 influenza are predominantly found in the alveolar region [34]. Aerosol monitors that only measure $PM_{2.5}$ do not measure the full-size range of particles produced during slaughtering and defeathering. However, within this limitation, the wide availability and low cost of these aerosol monitors potentially provides a method for studying the effectiveness of different interventions to reduce aerosol generation. These low-cost monitors could be used globally in LBMs and similar settings where research-grade equipment is not available. There is insufficient information regarding tools, measurements and procedures necessary or appropriate for comparing particles produced by various poultry slaughtering and defeathering techniques. Therefore, we

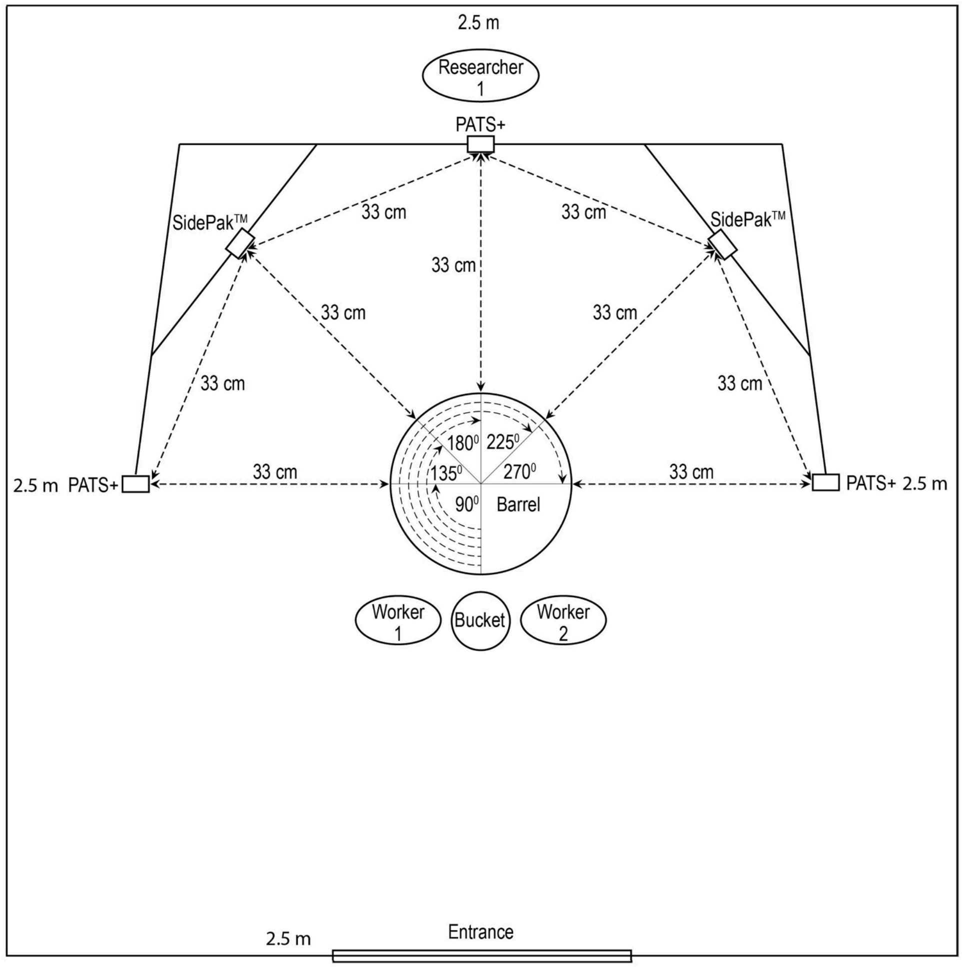


Fig. 2. Diagram of placement of equipment and particle monitors inside the booth for slaughtering experiment at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018. For each angular position shown for the Particle and Temperature Sensor + (PATS+) monitors, one monitor was placed at the height of the mouth of the barrel and a second monitor was placed above it at the workers' breathing height for all events. The SidePak™ monitor was alternated for the two angular positions at the height of the mouth of the barrel for five events and at the workers' breathing height for five events.

conducted pilot experiments by using two types of airborne particle monitors stationed at different heights and angles in a controlled environment to measure aerosolized PM during poultry slaughtering and defeathering, particularly during the exsanguination of the slaughtered poultry and use of mechanical defeathering device, which can help identify methods that can minimize workers' exposure to potentially hazardous aerosol particles [35].

2. Methods

From June to September 2018, a team of researchers from icddr,b conducted the experiments at the National Reference Laboratory for Avian Influenza, Bangladesh Livestock Research Institute (BLRI), Savar, Dhaka, Bangladesh. We conducted experiments in the facility to ensure a controlled environment, which was required to determine the concentration of $PM_{2.5}$ generated during poultry slaughtering and defeathering. To ensure consistency across slaughtering and defeathering activities, we hired three LBM workers, who followed the same slaughtering and defeathering steps as in the LBMs and used the same equipment, including knives, barrels and defeathering machines. The team also conducted a survey from February to March 2018 using a semi-structured observation form in all shops ($N = 716$) of all retail LBMs ($N = 35$) in Dhaka city consisting of at least 10 shops. During the survey, they collected data on various equipment used for containing slaughtered poultry during exsanguination and mechanical defeathering, average weight of slaughtered broiler chickens, and average temperature of hot water used for scalding. Additionally, the team measured breathing heights of randomly selected 20 adult workers, who conducted slaughtering and defeathering, from three conveniently selected LBMs. This study was reviewed and approved by the Institutional Review Boards (IRB) of icddr,b and the US Centers for Disease Control and Prevention (CDC) (see 45 C.F.R. part 46 and 21 C.F.R. part 56). All participants for survey provided oral consent and for experiments provided written consent.

2.1. *Portable booth*

A portable booth (2.5 m width x 2.5 m length x 2 m height) was constructed with 13 mm PVC pipe and transparent plastic films to minimize extraneous air movement and aerosol particle dispersion (Fig. 1). The booth was installed inside an empty air-conditioned (25 °C) room to ensure minimal air movement in and out of the booth.

2.2. *Equipment used in the experiments*

For slaughtering experiments, we used a knife to slaughter the chicken, an empty bucket to capture slaughtering blood, and a plastic barrel (diameter: 27.9 cm, height: 55.9 cm) to contain the chicken during its exsanguination (Figs. 1a, 1b and 2). For defeathering experiments, we used all the equipment used in slaughtering in addition to a

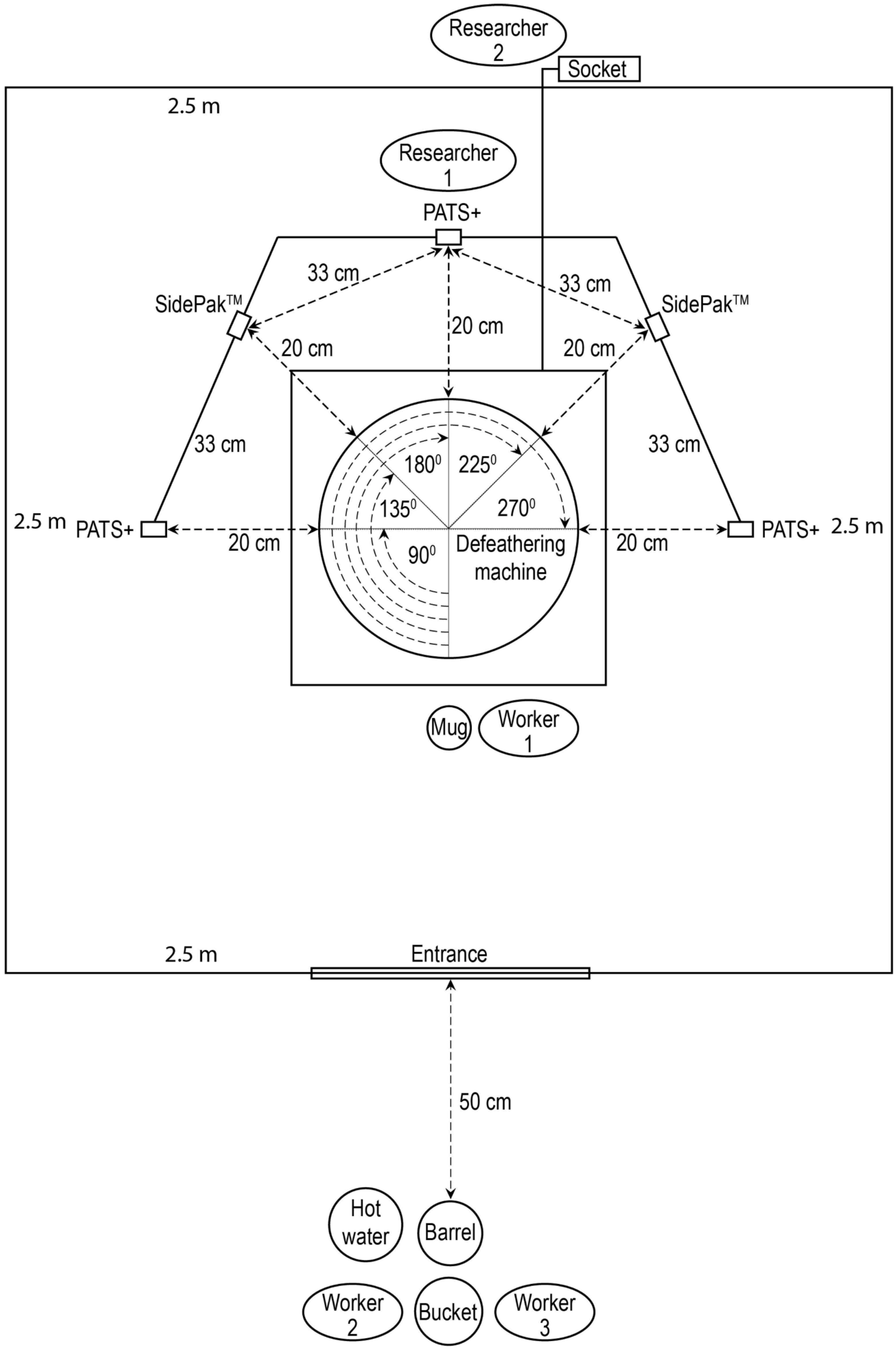


Fig. 3. Diagram of placement of equipment and particle monitors for defeathering experiment at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018. For each angular position shown for the Particle and Temperature Sensor + (PATS+) monitors, one monitor was placed at the height of the mouth of the defeathering machine and a second monitor was placed above it at the workers' breathing height for all events. The SidePak™ monitor was alternated for the two angular positions at the height of the mouth of the machine for five events and at the workers' breathing height for five events.

defeathering machine, which consists of two parts: a square box (length: 68.6 cm, width: 68.6 cm, height: 57.2 cm) at the bottom and a cylinder (diameter: 58.4 cm, height: 49.5 cm) on top (the total height is 106.7 cm), a pot of hot water (about 60 °C measured using a thermometer), a bucket full of room temperature water, a mug (to pour in room temperature water into the defeathering machine from the bucket), a bucket to capture waste and wastewater during defeathering process, an electric socket to turn on the defeathering machine, and the same particle monitors as the slaughtering experiments (Fig. 1c and 3). We used two stand fans to facilitate airflow between experiments. We also used an indoor digital temperature humidity meter to measure temperature and relative humidity of the room. The workers in our experiments used personal protective equipment (PPE) during all experiments, which included hair covers, goggles, respirators, gowns, gloves and boots, although workers in LBMs rarely wear PPE.

2.3. Type and placement of the particle monitors

During the experiments, we used six Particle and Temperature Sensor + (PATS+) (developed by Berkeley Air Monitoring Group Berkeley, USA) and one SidePak™ (model: AM520 Personal Aerosol Monitor; developed by TSI Incorporated, USA) portable aerosol monitors to estimate the total aerosolized $PM_{2.5}$ particles. The PATS+ monitors can measure the concentration of aerosol particles every 10 s from 0.5 μm to 2.5 μm using optical scattering [29,36]. Its lower detection limit for particulate matter is 10 – 20 $\mu g/m^3$, and the upper limit is 30, 000 – 50,000 $\mu g/m^3$ [36]. The SidePak™ monitor, on the other hand, can measure the concentration of aerosol particles every second from 0.1 μm to 10 μm with a particle detection limit of 1 to 100,000 $\mu g/m^3$ [37]. The SidePak™ draws 1.8 liters/minute of air [37]. The PATS+ has a small fan that draws air into the sensing chamber; the flowrate is not specified by the manufacturer. For our experiments, the SidePak™ was used with an internal impactor that restricted the upper limit of the measurement size range to 2.5 μm. We performed a zero calibration on the PATS+ devices using a zeroing box and the SidePak™ device using the zero-filter attached to the inlet to ensure accurate measurements following the user manual instructions [37,38].

Monitors were placed adjacent to the barrel or defeathering machine. An iron frame was used to hold the particle monitors 33 cm away from the barrel or 20 cm from the defeathering machine, horizontally at the opposite side (i.e., at 180º) of the entrance to the booth or workers' position (Fig. 2 and 3). The assessments were conducted at two different heights: one at the height of the mouth of the barrel (Fig. 1b) or defeathering machine (Fig. 1c) and another at the workers' breathing level. The measurements were recorded in three different directions: left, opposite and right side of the entrance (90º, 180º and 270º clockwise from the entrance position respectively) (Fig. 2 and 3). Six PATS+ monitors were placed in six different directions at two different heights. As a comparison, the SidePak™ monitor was placed at the same heights and the same distance (33 cm) from the barrel/defeathering machine and in two different directions (135º or 225º) from the entrance position and between two PATS+ monitors (Fig. 2 and 3). Only one SidePak™ was available during our experiments which was placed at the height of the mouth of the barrel or defeathering machine for five events and at the height of human breathing level for five events. Each monitor was kept in a plastic container separately during data collection to prevent clogging from feathers, blood, or blood-mixed water. Temperature and humidity were recorded at the beginning and end of each experiment and baseline using a digital meter outside the booth, and every 10 s during the event and baseline using PATS+ monitors inside the booth.

2.4. Animals used

We conducted experiments using a total of 22 broiler chickens (*Gallus gallus domesticus*). We used only broiler chickens for this experiment because broiler chickens are the most common bird type sold in Bangladeshi LBMs. All the chickens were of typical market weight and were purchased from local LBMs. For each experiment, we used 10 chickens for slaughtering and defeathering, and two additional chickens were used for demonstration purposes. As this was a pilot study, we did not calculate a specific sample size for the experiments. The Research protocol was reviewed and approved by icddr,b's Animal Experimentation Ethics Committee (AEEC) and the CDC Animal Care and Use Committee (Atlanta, USA; Protocol number 3054KILCHIX).

2.5. Experiments for slaughtering

Before initiating the experiment, the team took a baseline measurement of aerosol particles inside the booth for five minutes. Inside the booth, two workers performed the slaughtering of chickens in the presence of a research team member, who operated the SidePak™ monitor which was required to be turned on and off before and after each experiment. The researcher stood adjacent to the frame inside the booth and started the SidePak™ monitor before the worker entered the booth. The barrel was placed in the middle of the booth and a bucket was placed adjacent to the barrel to hold blood (Fig. 1b). Two workers (one holding the chicken and the other performing slaughtering) slaughtered a chicken with a knife over the bucket and immediately put it inside the barrel for its exsanguination. The particle measurements were taken for five minutes following the worker entering the booth. After five minutes, a worker removed the chicken from the barrel and the booth. The entire experiment was repeated 10 times for 10 chickens. Between slaughtering each chicken, we refreshed the air in the booth by removing the curtains, turning on two stand fans and opening all windows and doors. The team also used two stand fans to facilitate airflow for five minutes. After that, we stopped the fans, reinstalled all of the curtains, shut all of the windows and doors, and waited for five minutes for the particle concentrations to return to baseline level. Then, the team measured the baseline particle concentration by using the monitors in the same position for five minutes.

Table 1
Equipment used for containing slaughtered poultry during exsanguination and mechanical defeathering and other measurements in the live bird markets of Dhaka city, 2018.

Characteristics	No. of shops n (%)
Availability of arrangement in the shop	[*N* = 716]*
Slaughtering	617 (86)
Mechanical defeathering	237 (33)
Equipment to contain slaughtered poultry during exsanguination	[*N* = 617]*
Plastic barrel (small, medium and large size)	568 (92)
Metal cone (made of iron sheet, stainless steel and iron rod)	58 (9)
Defeathering machine	22 (4)
Discarded plastic oil container	5 (0.8)
Discarded plastic water jar	3 (0.5)
Metal barrel	3 (0.5)
Plastic bucket	3 (0.5)
Discarded multi-chambered plastic container of car batteries	1 (0.2)
Lids for slaughtering containers	[*N* = 567]*
Open barrel without lid	283 (50)
Covered barrel with plastic lid: solid	222 (39)
Covered barrel with plastic lid: a hole in the middle	25 (4)
Covered barrel with metal lid (dish, defeathering machine lid, cooking pot lid)	22 (4)
Covered barrel with plastic bowl/bucket	7 (1)
Covered barrel with wooden/plywood lid	6 (1)
Open bucket without lid	2 (0.4)
Covered bucket with plastic lid	1 (0.2)
Covered barrel with lid of oil container	1 (0.2)
Covered barrel with plastic lid: a star-cut in the middle	1 (0.2)
Lids for defeathering machine	[*N* = 237]*
Covered machine with metal lid: solid (dish, tray, cooking pot lid, sheet)	108 (46)
Covered machine with metal hinged lid	80 (34)
Open machine without lid	37 (16)
Covered machine with wooden/plywood lid	6 (3)
Covered machine with cork sheet	5 (2)
Covered machine with metal lid: a hole in the middle	1 (0.4)
Other measurements	Average (range)
Workers' breathing height (cm) [*N* = 20]	148 (143–152)
Scalding water temperature (°C) [*N* = 27]	56.8 (40–65)
Weight of broiler chickens (kg) [*N* = 79]	1.7 (0.9–2.4)
Height of plastic barrel for slaughtering (cm) [*N* = 54]	56 (55.9–61)
Height of defeathering machine (cm) [*N* = 237]	107†

* Multiple answer is accepted.
† All machines' heights were the same.

2.6. Experiments for defeathering

Inside the booth, a worker performed the defeathering in presence of a research team member operating the SidePak™ monitor. In the middle of the booth, a defeathering machine was placed on the ground (Fig. 1c). Next to the machine, a bucket filled with normal room temperature

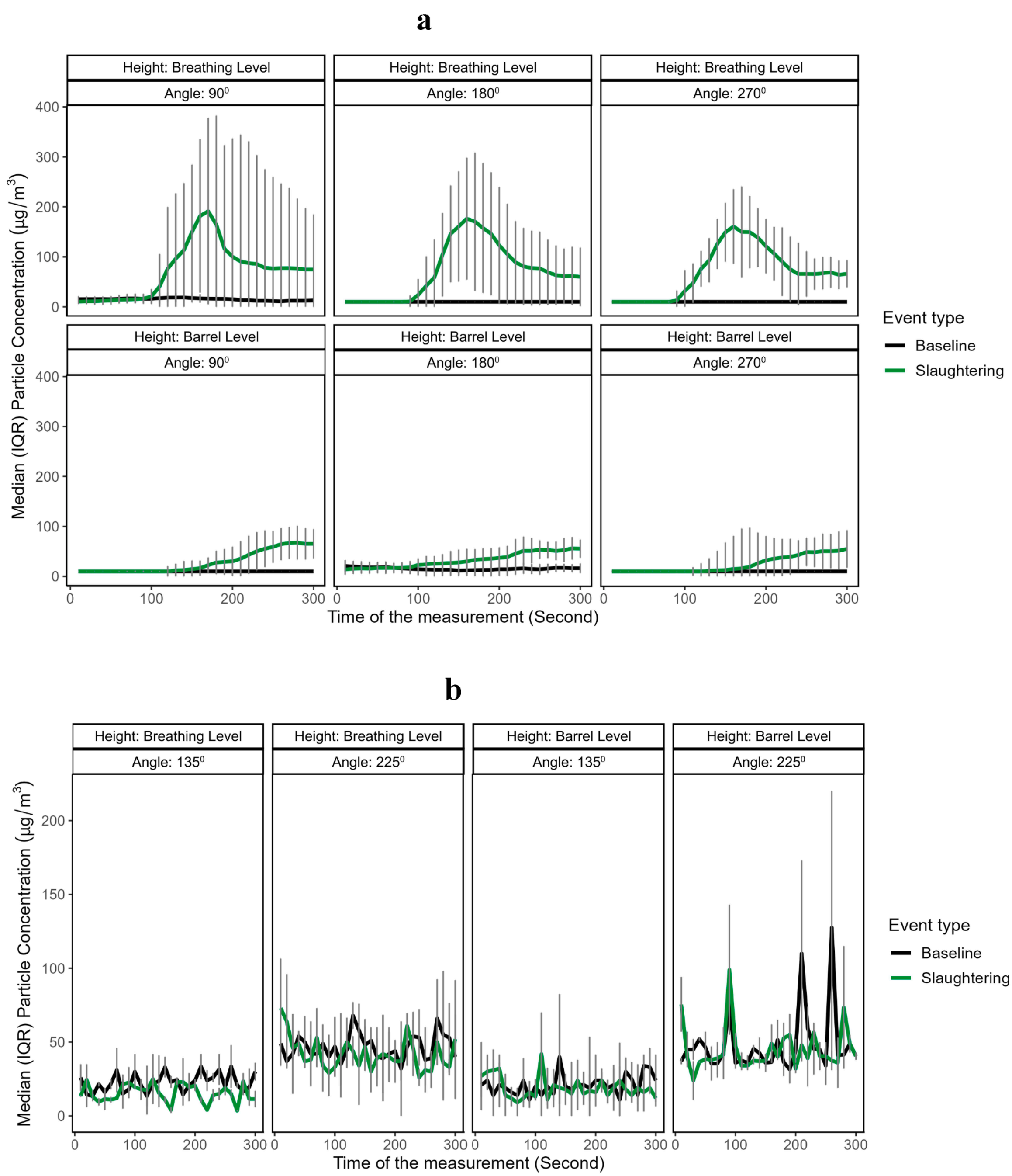


Fig. 4. Average particle concentration at each height and angle during 10 slaughtering events at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018. The graphs show the median and interquartile range of the particle concentration reported by the Particle and Temperature Sensor + (PATS+) and SidePak™ monitors during slaughtering events and baseline with error bars illustrating the variability of data at different time points.
a: PATS+ monitor
b: SidePak™ monitor.

water was placed along with a mug on the ground. A separate bucket was also placed to capture feathers and wastewater from the defeathering machine. Outside the booth, a bucket, a barrel, and a container of hot water (60 °C) were placed 50 cm away from the booth entrance. Two workers slaughtered a chicken outside the booth with a knife over the bucket and placed the chicken inside the barrel for its exsanguination. The carcass was then dipped in hot water for 17 s before being taken inside the booth and placed in the defeathering machine for 20 s, and the worker poured 2.5 liters of normal room temperature water inside the defeathering machine from the water bucket using the mug. The researcher recorded all measurements using the same method as the slaughtering experiments. The same method was used to refresh the air in the booth and room, and to measure the baseline particle concentration.

2.7. Data analysis

The particulate matter generated during exsanguination of slaughtered chickens (referred to as 'slaughtering') and mechanical defeathering was analyzed based on the instrument type and instrument placement and position. We estimated the median and interquartile range (IQR) of PM concentrations for both baseline and slaughter/

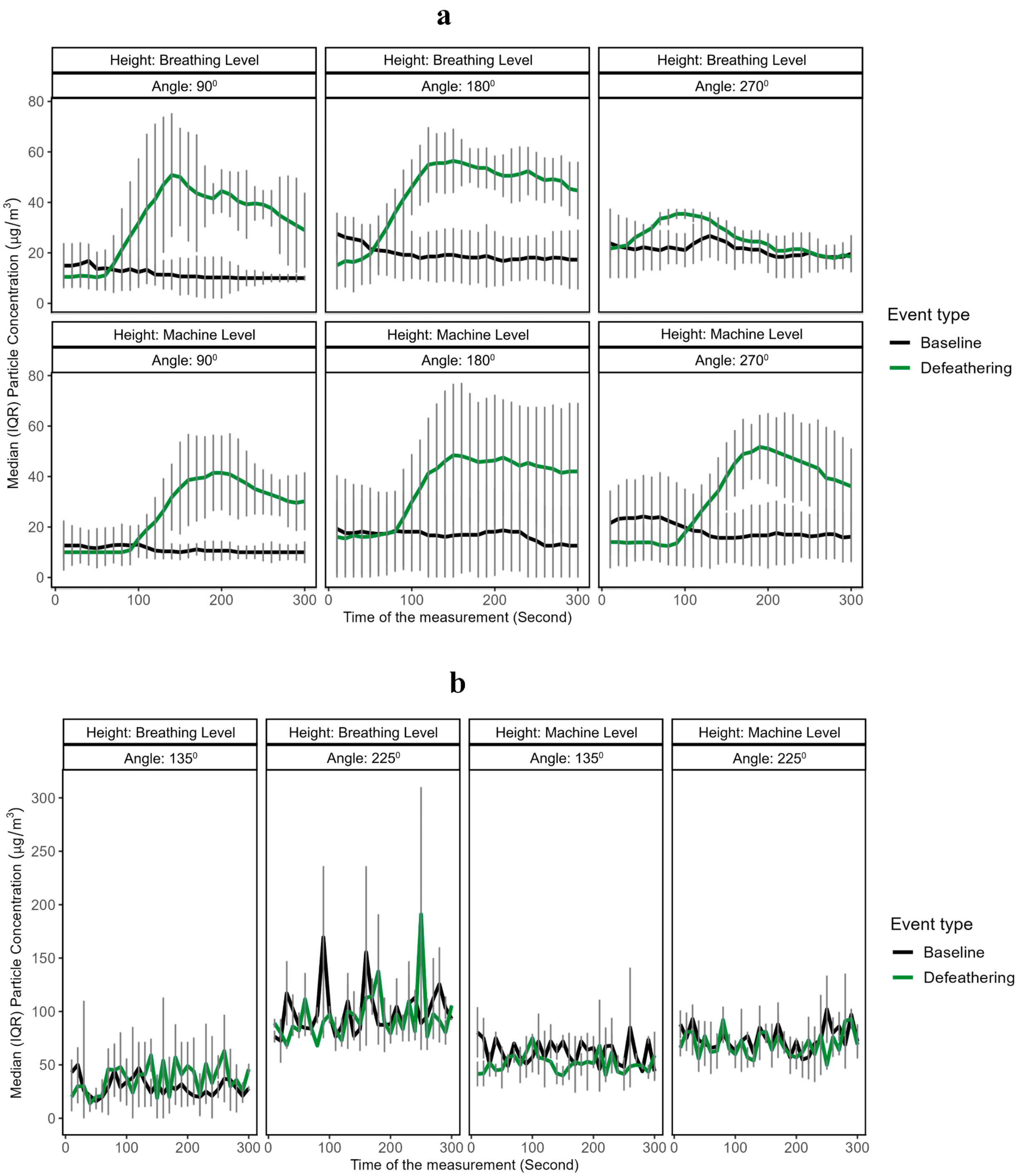


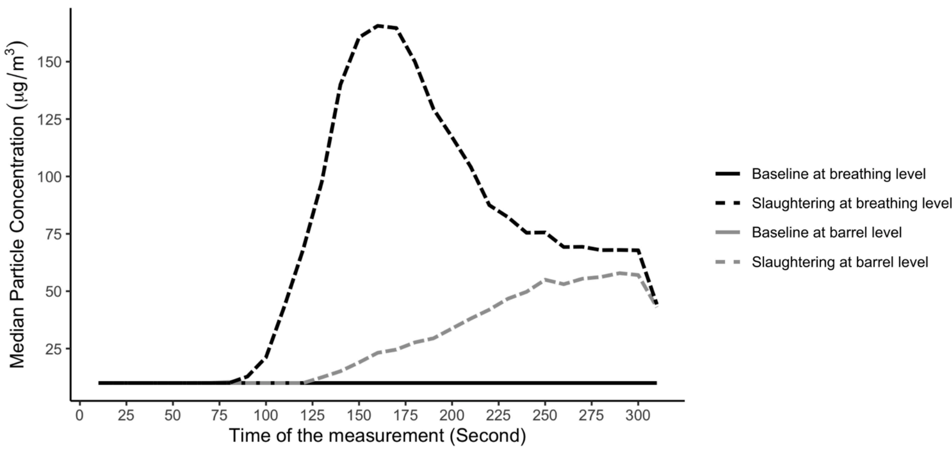
Fig. 5. Average particle concentration at each height and angle during 10 defeathering events at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018. The graphs show the median and interquartile range of the particle concentration reported by the Particle and Temperature Sensor + (PATS+) and SidePak™ monitors during defeathering events and baseline with error bars illustrating the variability of data at different time points.
a: PATS+ monitor
b: SidePak™ monitor.

defeathering events. Data management and analysis were performed using Stata SE (StataCorp LP, College Station, TX, 2017, version 15), and data visualization was conducted with R Statistical Software (R Core Team, 2023, version 4.3.2).

3. Results

The survey identified different types of equipment commonly used in the LBMs, including barrels with and without lids and small and large metal cones to contain slaughtered poultry during the death struggle, and defeathering machines with or without lids for mechanical defeathering (Table 1). The average breathing height of the workers was 148 cm and the average weight of broiler chickens was 1.7 kg. The most commonly used barrel for slaughtering had an average height of 56 cm and all defeathering machines had a height of 107 cm (Table 1).

Variations in particle concentrations were recorded during slaughtering and defeathering of chickens for different heights, positions and particle monitors (Figs. 4 and 5). The average duration of the



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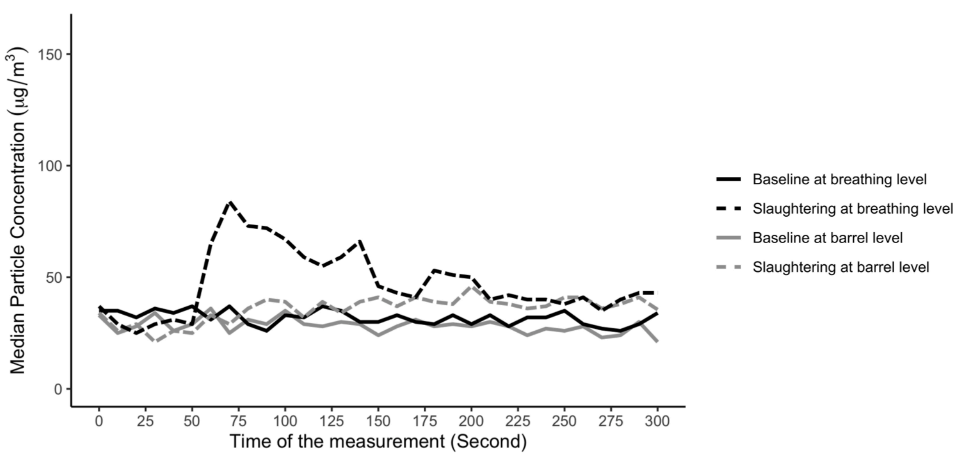

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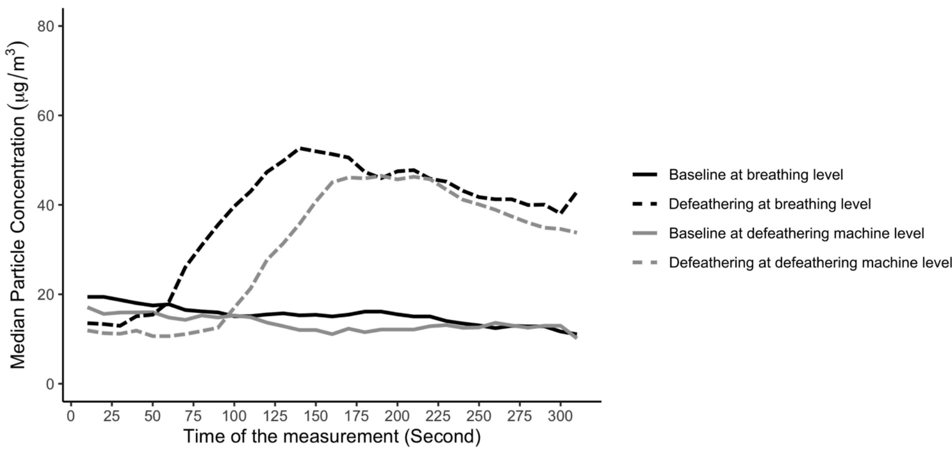
Fig. 6. Average particle concentration at breathing and barrel heights during 10 slaughtering events at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018
a: Particle and Temperature Sensor + (PATS+) monitor
b: SidePak™ monitor.

slaughtering events was 145 (range 105–185) seconds (starting from workers entering the booth until the chicken stopped moving), and defeathering events was 26 (range 22–29) seconds (starting from workers entering the booth until the defeathering machine was turned off). The SidePak™ monitor at both heights and PATS+ monitors at human breathing height recorded higher particle concentrations during the slaughtering process compared to the defeathering process (Fig. 6 and 7).

The baseline median particle concentrations recorded by PATS+ monitors at the human breathing height and barrel height were similar (10 µg/m^3, IQR 10–10) for slaughtering, while slightly increased during defeathering events (Table 2). These readings were close to the background measurements in an empty room and also approached the lower detection limit of the device. The SidePak™ monitor recorded higher particle concentrations (64 µg/m^3, IQR 62–74 and 32 µg/m^{3,} IQR 28–76) at the baseline during defeathering compared to slaughtering events (Table 2, Fig. 7b). The SidePak™ monitor also recorded higher particle concentrations during baseline than the PATS+ monitor throughout the experiments (Table 2, Fig. 6 and 7).

During slaughtering experiments, both types of monitors recorded higher particle concentrations at human breathing height than the barrel height (Fig. 6). At the breathing height, an increase in PM was observed after about 80 s, which reached the maximum after about 160 s in PATS+ monitor (Fig. 6a). At the same level, the SidePak™ monitor detected the changes in concentration of PM faster (after about 50 s), which reached its maximum concentration immediately (after about 70 s) (Fig. 6b). The median particle concentrations at human breathing height were 67 µg/m^3 (IQR 44–121) for the PATS+ monitors and 34 µg/m^3 (IQR 34–64) for the SidePak™ monitor, which were higher than the barrel height (Table 2). The particle concentrations at the 90° position of the breathing height were higher (75 µg/m^3, IQR 46–155) compared to the other two positions for the PATS+ monitor (Table 3). For the SidePak™ monitor, the highest concentration (64 µg/m^3, IQR 34–66) was recorded at the 225° position. For the defeathering experiments, the particle generation was recorded earlier at the breathing height (after about 60 s) compared to the defeathering machine height (at about 90th seconds) by the PATS+ monitors but not noticeably different at these two heights after reaching the peak (Fig. 7a). The SidePak™ monitor recorded a higher particle concentration at the defeathering machine height (71 µg/m^3, IQR 68–86) compared to the breathing height (44 µg/m^3, IQR 40–84) (Table 2, Fig. 7b). The highest particle concentrations were recorded for the PATS+ monitors at the 180° position at the human breathing height (50 µg/m^3, IQR 41–53) (Table 3). For SidePak™, the highest particle concentrations observed were 97 µg/m^3 (IQR 84–109) at the 225° position at human breathing height (Table 3).

The relative humidity and temperature inside the booth were



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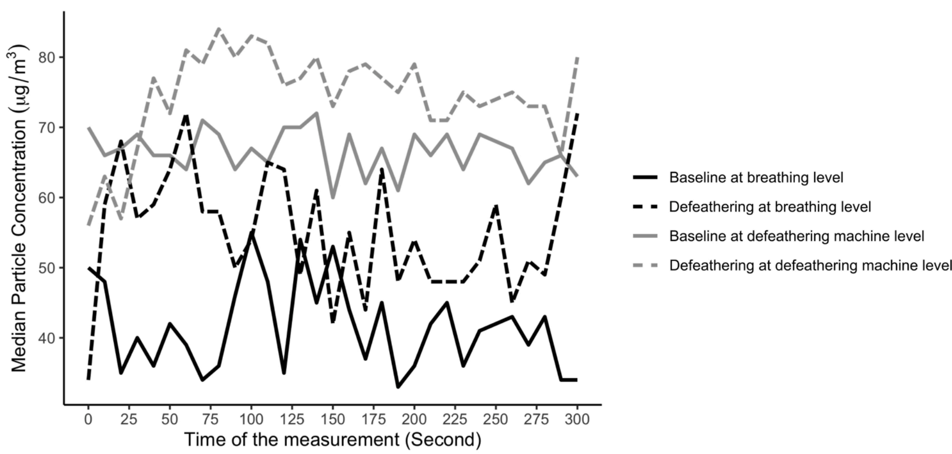

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Fig. 7. Average particle concentration at breathing and defeathering machine heights during 10 defeathering events at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018
a: Particle and Temperature Sensor + (PATS+) monitor
b: SidePak™ monitor.

consistently higher than outside the booth throughout all slaughtering and defeathering events (Fig. 8). In both types of experiments, an increasing trend in relative humidity and room temperature were observed from noon to evening (Fig. 8). A noticeable difference in the relative humidity observed between inside and outside the booth during the defeathering experiments.

4. Discussion

In the controlled environments, both PATS+ and SidePak™ monitors were able to record variations in $PM_{2.5}$ particle concentration during poultry slaughtering and defeathering experiments. Both monitors recorded greater particle concentrations at human breathing height (148 cm) compared to barrel height (107 cm) during slaughtering experiments. However, the concentrations observed at breathing height were similar in PATS+ and lower in SidePak™ monitors compared to machine height during defeathering experiments.

We expected to see high concentration of $PM_{2.5}$ particles generated during slaughtering and defeathering processes due to continuous particle aerosolization from poultry movement, feathers, and dander. Therefore, our assumption was that particle measurement devices with comparatively lower maximum limits, which are intended for use in relatively clean environments, might not be suitable to measure particle concentrations during poultry slaughtering and defeathering processes. The PATS+ and SidePak™ monitors are photometer-based instruments that are able to measure much higher aerosol particle concentrations than are optical particle counters and similar devices [39]. The PATS+ monitor is part of a generation of low-cost aerosol particle measurement devices that have been developed to allow inexpensive monitoring of indoor aerosols [29].

The particle concentrations recorded by the SidePak™ monitor were more variable and generally lower than the PATS+ data. The SidePak™ monitor responded to changes in concentration faster than the PATS+ monitor, partly because the SidePak™ monitor reports data every second, while the PATS+ monitor reports every 10 s. The SidePak™ monitor also recorded higher particle concentrations at baseline than the PATS+ monitor. It might be that the SidePak™ responds differently to particles of different sizes than does the PATS+. These results suggest that the SidePak™ monitors are more sensitive than PATS+; the PATS+ data might be reliable in a relative sense, i.e., if the results from experiment A are 50 % lower than the results from experiment B, then the actual concentration during experiment A was probably 50 % lower than the concentration during experiment B. However, the data might be less reliable in an absolute sense, i.e., if the results say that the

Table 2
Particle concentration observed during slaughtering and defeathering experiments at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018.

Event type	Particle concentration (μg/m^3) at different heights of the particle monitor	
	at slaughtering barrel/defeathering machine height Median (IQR)	at human breathing height Median (IQR)
Slaughtering experiments		
PATS+	(N = 30)	(N = 30)
Baseline	10 (10 – 10)	10 (10 – 10)
Slaughtering	22 (11 – 43)	67 (44 – 121)
SidePak™	(N = 5)	(N = 5)
Baseline	22 (16 – 34)	25 (16 – 44)
Slaughtering	33 (28 – 41)	34 (34 – 64)
Defeathering experiments		
PATS+	(N = 30)	(N = 22*)
Baseline	13 (10 – 22)	12 (10 – 19)
Defeathering	37 (29 – 44)	41 (29 – 49)
SidePak™	(N = 5)	(N = 5)
Baseline	64 (62 – 74)	32 (28 – 76)
Defeathering	71 (68 – 86)	44 (40 – 84)

* One PATS+ monitor collected data only for two events and was not functional for the rest of the experiment.

concentration during experiment A was 100 μg/m^3, the actual concentration may be higher or lower depending upon how the instruments were calibrated. We deployed six PATS+ monitors during each experiment, which allowed us to compare aerosol concentrations in multiple locations. Overall, we concluded that the PATS+ monitors can be used in our experiments so long as their limitations are understood. When possible, it is helpful to also collect data with a research-grade instrument for comparison.

The SidePak™ monitor recorded higher particle concentrations during slaughtering events compared to defeathering events. This may have occurred because the defeathering time is much shorter than the slaughter and exsanguination time, and also because of the use of water during defeathering, which could suppress the aerosolization of small particles. Using water/hot water during defeathering might produce particles larger than 2.5 μm, which the aerosol monitors would not have detected. Moreover, during the defeathering experiments, the particle concentrations did not reach a peak until well after the defeathering was completed, which might reflect the time it took for the particle cloud to reach the particle monitors. This contradicts with prior expectations which often depict defeathering as the riskiest activity for aerosolization of the virus [20]. Nevertheless, no study could be found comparing the particle generation between different steps of the poultry slaughtering and processing procedure. This pilot experiment provides preliminary evidence of the importance of slaughtering in $PM_{2.5}$ aerosol particle generation, but further research is needed to confirm this finding.

The SidePak™ monitor recorded lower differences between the aerosol concentration from the events and the baseline levels. The SidePak™ has a built-in impactor that removed particles greater than 2.5 μm from the air before the measurements are made, while the PATS+ does not, which may have influenced the results. The instruments also may respond differently to aerosols like those produced during slaughtering and defeathering, which do not have the same composition as the particles used to calibrate the instruments by the manufacturers.

The findings of these experiments provide baseline information on the generation of $PM_{2.5}$ aerosols during poultry slaughtering and defeathering processes, which can be helpful to design methods for future experiments. The findings also provide insight about the appropriateness of booth refreshing time and method, and allowable distance between the monitors to avoid airflow interference with each other. Although the booth was refreshed after each experiment to reduce particle levels and maintain conditions similar to background

Table 3
Particle concentrations observed at different positions of the particle monitors during slaughtering and defeathering experiments at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018.

Particle monitor type	Angles of the particle monitors	Event type	Heights of the particle monitors*	Particle concentration (μg/m^3) Median (IQR)
Slaughtering experiments				
PATS+	90 °	Baseline	SBH (N = 10)	10 (10 – 10)
			HBH (N = 10)	12 (10 – 16)
		Slaughtering	SBH (N = 10)	15 (11 – 32)
			HBH (N = 10)	75 (46 – 155)
	180 °	Baseline	SBH (N = 10)	16 (10 – 19)
			HBH (N = 10)	10 (10 – 10)
		Slaughtering	SBH (N = 10)	30 (21 – 46)
			HBH (N = 10)	63 (39 – 121)
	270 °	Baseline	SBH (N = 10)	10 (10 – 10)
			HBH (N = 10)	10 (10 – 10)
		Slaughtering	SBH (N = 10)	14 (10 – 43)
			HBH (N = 10)	65 (40 – 82)
SidePak™	135 °	Baseline	SBH (N = 3)	16 (14 – 22)
			HBH (N = 2)	15 (15 – 16)
		Slaughtering	SBH (N = 3)	28 (27 – 33)
			HBH (N = 2)	30 (25 – 34)
	225 °	Baseline	SBH (N = 2)	41 (34 – 47)
			HBH (N = 3)	44 (34 – 47)
		Slaughtering	SBH (N = 2)	43 (41 – 44)
			HBH (N = 3)	64 (34 – 66)
Defeathering experiments				
PATS+	90 °	Baseline	HBH (N = 10)	10 (10 – 12)
			DMH (N = 10)	10 (10 – 14)
		Defeathering	HBH (N = 10)	35 (22 – 46)
			DMH (N = 10)	29 (27 – 32)
	180 °	Baseline	HBH (N = 10)	18 (11 – 24)
			DMH (N = 10)	13 (10 – 27)
		Defeathering	HBH (N = 10)	50 (41 – 53)
			DMH (N = 10)	41 (38 – 65)
	270 °	Baseline	HBH (N = 2†)	19 (12 – 26)
			DMH (N = 10)	17 (10 – 22)
		Defeathering	HBH (N = 2†)	24 (21 – 28)
			DMH (N = 10)	37 (31 – 44)
SidePak™	135 °	Baseline	HBH (N = 3)	28 (13 – 32)

(continued on next page)

Table 3 (*continued*)

Particle monitor type	Angles of the particle monitors	Event type	Heights of the particle monitors*	Particle concentration (μg/m^3) Median (IQR)
			DMH (*N* = 2)	52 (40 – 64)
		Defeathering	HBH (*N* = 3)	40 (22 – 44)
			DMH (*N* = 2)	60 (51 – 68)
	225 °	Baseline	HBH (*N* = 2)	89 (76 – 101)
			DMH (*N* = 3)	74 (62 – 76)
		Defeathering	HBH (*N* = 2)	97 (84 – 109)
			DMH (*N* = 3)	86 (71 – 86)

* HBH: Human breathing height (at 148 cm from the floor)
SBH: Slaughtering barrel height (at 56 cm from the floor)
DMH: Defeathering machine height (at 107 cm from the floor).

† One PATS+ monitor collected data only for two events and was not functional for the rest of the experiment.

measurements, the baseline concentration in the booth increased as the experiments progressed. This probably occurred because the particle concentration in the room containing the booth went up each time the booth was flushed out into the room. The fact that the baseline reached highest in the afternoon on the day of defeathering experiments may have been caused by an increase in humidity due to rain on that day. Several studies found positive relationships between PM and humidity because hygroscopic particles can hydrate and increase in size and mass under humid conditions, and because humid conditions can induce the formation of secondary fine particles [40,41]. Using portable air filters may help reducing the baseline particles, especially when the particle concentrations being detected during the experiments are not much greater compared to the baseline levels. If the portable filters are run in the room outside the booth, then the particle concentrations in the booth will be reduced when the air is refreshed between experiments.

This experiment recorded greater particle concentration at human breathing height than measured at the slaughtering barrel height. The most likely explanation for this result is that the flapping and other movements of the chicken in the barrel produce a vertical plume that carries the particles upward before they disperse sideways towards the monitors. Similar results were also reported from computational fluid dynamics modeling of defeathering processes at LBMs which showed an upward plume of aerosol generation from the defeathering process [42]. This result illustrates the importance of placing monitors within the breathing zone of a worker when estimating worker exposures to airborne hazards. Using breathing height monitor placement is also considered best practice for estimating worker exposures according to the CDC, as it more accurately assesses worker's exposure than a monitor placed out of the breathing zone [43].

The study had some limitations. Our study only examined the concentrations of $PM_{2.5}$. Slaughtering and defeathering produce aerosol particles across a very broad size range, and differences seen in $PM_{2.5}$ concentrations may not apply to larger aerosols. Further research is needed to understand how $PM_{2.5}$ concentrations relate to aerosol concentrations in other size ranges. In addition, the use of $PM_{2.5}$ as a proxy for AIV or other aerosolized bioaerosols is not well understood, and it is impossible to estimate the amount of infectious AIV in particles generated in this environment. The amount of infectious AIV generated may also depend upon the viral strain and other zoonotic factors. Calibration of the light scattering monitors are set by companies and not in the field, which could lead to inaccuracies of $PM_{2.5}$ estimations when dealing with

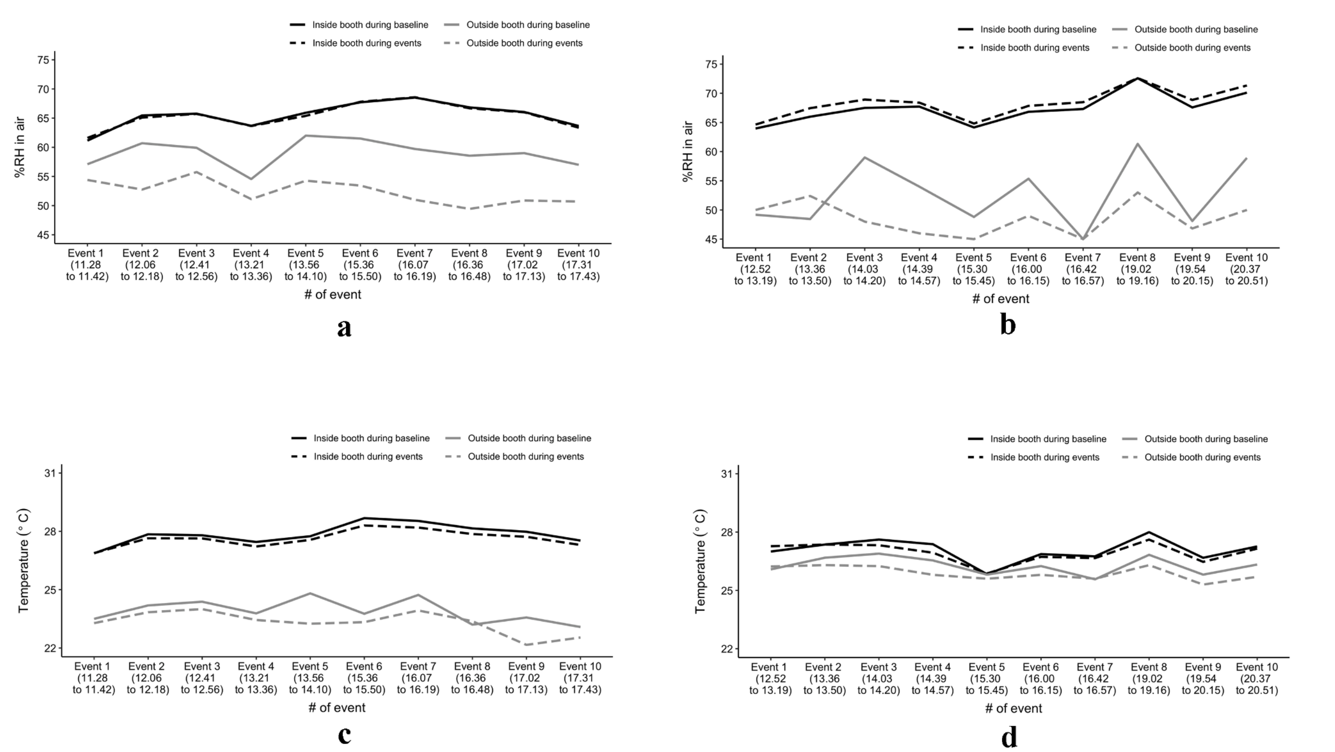

Fig. 8. Average humidity and temperature recorded inside and outside the booth during slaughtering and defeathering experiments at Bangladesh Livestock Research Institute, Savar, Dhaka, 2018.
a: Humidity during slaughtering experiments
b: Humidity during defeathering experiments
c: Temperature during slaughtering experiments
d: Temperature during defeathering experiments.

different particle size distribution than company calibration samples. It is also likely that the calibrations were not done with an aerosol with the same material composition as the dust in the LBM. The type of aerosol material affects the optical properties of the aerosol. The PATS+ monitors also probably measured some particles that were larger than 2.5 μm, while the SidePak™ has an inlet impactor that removes particles larger than 2.5 μm. Bangladeshi slaughtering and defeathering processes may be different from those used in other countries, therefore, these findings may not be applicable to other contexts. Nevertheless, it provides useful insight for the settings with similar practices and equipment in the continent. During the experiments, only one SidePak™ monitor was available for data collection in icddr,b. Consequently, there was limited opportunity to cover experiment data in each position using the SidePak™ monitor compared to PATS+. After the 8th defeathering experiment, one of the PATS+ monitors (positioned at 270°) had stopped functioning due to a technical issue. Data was only obtained from this monitor for two experiments, which was not adequate for conducting a comprehensive comparison with the data from the other positions. Despite following the booth-refreshing procedure, the baseline particle concentrations could not be brought down to minimum level for both monitors.

5. Conclusion

Our experiments showed that poultry slaughtering and defeathering aerosolized significant amounts of $PM_{2.5}$ particles, and that the concentrations depend upon the type of activity and the locations of the monitors. In our experiments, slaughtering generally produced higher particle concentrations than did defeathering. During the slaughtering experiments, we found that the aerosol concentration at the height of the worker's breathing zone was higher than the concentration at the height of the barrel mouth, which emphasizes the need to measure worker exposures within the breathing zone. This series of experiments provides evidence of potential exposure pathways of airborne viruses from poultry to humans during the slaughtering and defeathering activities in LBMs and the optimization of methods to test these mechanisms of exposure further in the future. This information can be used to help prioritize mechanisms for aerosolized virus control, model risk of AIV transmission within LBMs, and test the effectiveness of different available slaughtering and defeathering methods for reducing airborne particulate generation during slaughtering and defeathering.

CRediT authorship contribution statement

Nadia Ali Rimi: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Md. Habibullah Fahad:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Investigation, Formal analysis, Data curation. **Andrew Clark:** Writing – review & editing, Methodology, Conceptualization. **Rebeca Sultana:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Kamal Hossain:** Writing – review & editing, Visualization, Validation, Formal analysis, Data curation. **Md. Khaled Saifullah:** Writing – review & editing, Writing – original draft, Validation, Resources, Investigation, Formal analysis, Data curation. **Ireen Sultana Shanta:** Writing – review & editing, Supervision, Investigation. **David E. Swayne:** Writing – review & editing, Methodology, Conceptualization. **Md. Zakiul Hassan:** Writing – review & editing, Methodology, Conceptualization. **Syed Mohammad Golam Mortaza:** Writing – review & editing, Validation, Resources, Investigation, Formal analysis, Data curation. **Sayeda Tasnuva Swarna:** Writing – review & editing, Validation, Resources, Investigation, Formal analysis, Data curation. **Md. Giasuddin:** Writing – review & editing, Resources. **Christopher LeBoa:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **M. Sajjadur Rahman:** Writing – review & editing, Resources. **Debashish Biswas:** Writing – review & editing, Resources. **Mahbubur Rahman:** Writing – review & editing, Methodology, Conceptualization. **James C. Kile:** Writing – review & editing, Methodology, Conceptualization. **Erin D. Kennedy:** Writing – review & editing, Methodology, Conceptualization. **William G. Lindsley:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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