A novel partial lid for mechanical defeatherers reduced aerosol dispersion during processing of avian influenza virus infected poultry

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Abstract  

Infectious virus-laden aerosols generated during poultry processing may mediate airborne transmissions of avian influenza at live poultry markets. To develop effective control measures to reduce aerosol dispersion, we characterised the aerosol flow pattern of the mechanical defeatherers, a major source of aerosol dispersion during poultry processing at live poultry markets in China. Mechanical defeatherers create a strong air circulation during operation with inflow and outflow velocities over 1 m/s. A partial lid was designed to suppress the outflow and reduce aerosol dispersion. Computational fluid dynamics simulations confirmed that the partial lid prototype reduced the aerosol escape rate by over 65%. To validate the effectiveness of the partial lid in reducing aerosol dispersion, a field study was conducted at a retail poultry shop in Guangzhou and the concentrations of influenza viral RNA and avian 18S rRNA dispersed in air were monitored during poultry processing, with and without the use of the partial lid. At the breathing zone of the poultry worker, the use of the partial lid effectively suppressed the upward airflow and reduced the concentration of avian 18S rRNA in the air by 57%. The economic and practical partial lid can be easily implemented to reduce generation of influenza virus-laden aerosols at live poultry markets.

Introduction  

Among the multiple subtypes of avian influenza viruses (AIV) that have reportedly infected humans, H5N1, H5N6, H10N8, H7N7 and H7N9 have caused lethal infections in humans since 1997 [1–4]. H5N1 and H7N9 subtypes are of the most concern as these viruses have repeatedly caused zoonotic human infections with high case fatality rates [5,6]. The majority of human infections occurred following direct or indirect exposure to infected poultry at the human–poultry interface, including poultry farms and live-poultry markets [7].

Live poultry markets (LPMs) play a critical role in maintaining, amplifying and disseminating AIV among poultry and from poultry to humans. Previous epidemiological studies
have demonstrated the transmission potential of avian influenza virus via contact, droplets and airborne routes at LPMs [8–15]. AIV may replicate in the intestinal and respiratory tracts of the infected poultry, and infectious viruses can be shed in faeces or released in exhaled breath. Highly pathogenic influenza viruses may replicate in multiple tissues and further cause systemic infections in the infected poultry, posing additional risks for processing poultry carcasses. In countries where AIV is enzootic, surveillance studies have demonstrated that genetically diverse AIV are highly prevalent among poultry at LPMs. As such, AIV are frequently detected on contaminated surfaces at LPMs [8–11]. Viral RNA or infectious AIV were also detected in the air inside LPMs [13–15] and downwind from a wholesale LPM [12]. LPMs are widely distributed in both urban and suburban areas in China, and impose a significant threat on neighbouring residents, poultry market/shop workers and visitors.

Human behaviour at LPMs may also facilitate the generation and dispersion of AIV-laden particles during the processing of AIV-infected poultry, which increases the infection risks for both poultry workers and customers. Infectious AIV have been detected in aerosols dispersed by mechanical defeatherers during poultry processing at LPMs in China [15]. In a recent study, Bertran et al. [16] experimentally demonstrated that aerosols generated during the manual processing of infected poultry mediated AIV transmission to chickens and ferrets placed within the same airspace. Most retail shops at LPMs are commonly equipped with mechanical defeatherers to process poultry for customers. The use of a mechanical defeatherer significantly increased the total number of aerosols compared to manual defeathering [17]. The motor-driven disk of the defeatherer produces strong air circulation, which is favourable for aerosol generation and dispersion. Despite its common availability and the potential risk of dispersing AIV-laden particles, there has been no study that systematically investigated the flow characteristics of the mechanical defeatherers. Such knowledge is essential to develop practical and effective control measures to reduce the risk of AIV risk at LPMs. In this study, we characterised the flow pattern and dispersion of AIV from a mechanical defeatherer using a smoke test and computational fluid dynamics (CFD). A novel partial lid was developed, and its effectiveness in reducing aerosol dispersion was validated at a LPM in Guangzhou, China.

**Materials and methods**

Our studies involved laboratory flow pattern analysis, CFD simulations, and a field study. The flow pattern of a mechanical defeatherer was characterised in the building environment lab of The University of Hong Kong, and the CFD simulation was performed to further reveal the flow characteristics and quantify the reduction of aerosol dispersion using a partial lid. Finally, a field study was carried out in a poultry retail shop of a wholesale market in Guangzhou, China.

**Flow pattern characterisation by lab experiment**

The 2200 W mechanical defeatherer (MK-2200, Zhaoji, Foshan, China) had overall dimensions of 84 cm (L) × 68 cm (W) × 93 cm (H). The interior diameter of the cylindrical part was 62 cm with an opening of 54 cm. The rotating disk located at the bottom of the cylinder was driven by a motor, which ran at a speed of 185 r/min during operation. There were a number of rubber rods (10 cm in length, 2 cm in diameter) both on the cylinder wall and the disk for defeathering purposes (Fig 1). Feathers were washed away through the gap between the disk and the cylinder wall and collected at the outlet of the drainage slot.
The experiment was conducted in a room with the dimensions of 7.0 m (length) × 4.0 m (width) × 2.5 m (height). Air-conditioners were turned off to reduce disturbance. The measured background velocity was below 0.01 m/s.

A smoke generating machine (Concept Air Trace, Berks, UK) that generated fine particles below 0.5 μm was used for the visualisation experiment, as illustrated in Fig 1. The mechanical defeatherer was turned on, and smoke was released after it reached a steady state condition. As smoke from the generator has initial momentum, a mixing chamber was used as a buffer to reduce its disturbance to the air flow. The smoke out of the defeatherer was illuminated by lamps, and a black screen was used to enhance the contrast. In addition, a laser sheet was produced by a computer-controlled 3 W DPSS 532 nm laser projector (Ourslux Lighting Technology Co, Ltd) to reveal the flow dynamics. It illuminated the sagittal plane above the defeatherer as shown in Fig 1. Data were recorded using a Canon 6D camera with 24–105 lens at 25 fps.

To quantitatively explore the air flow, velocities above the cylinder were measured with a hot-sphere anemometer (AirDistSys 5000, SENSOR Electronic, Gliwice, Poland). At each point, the velocity was continuously monitored for three minutes. Three tests were carried out, and the results were averaged.

**Computer simulations using CFD**

Three-dimensional models of the mechanical defeatherer with and without a partial lid were reconstructed and meshed with the aid of SolidWorks 2014 and ANSYS ICEM CFD 14.5, respectively. Flow velocity field and aerosol trajectories while the defeatherer was under
operation were solved with the commercial CFD software, ANSYS Fluent 14.5. This study adopted the computing-efficiency and robust RNG k-ε model for simulating the flow [18,19]. The velocity field was obtained, and the aerosol motion equation was solved. The volume of the chicken and its rubbing with the rubber rods were not included in the simulation. For simplicity, the rubber rods of the rotating disk were treated as a uniform aerosol source. The number of released aerosols in each simulation was over 36,000 (Table 1). A humid environment in both the defeatherer cavity and surrounding room was expected. Hence, droplet evaporation was not considered. The background airflow in the room was assumed to be still.

The study used a second-order upwind scheme for all the variables except pressure for the Reynolds averaged Navier-Stokes (RANS) equations, and discretisation of pressure was based on a staggered scheme PRESTO. The so-called SIMPLE algorithm was employed to couple the continuity and momentum equations. The final computational mesh contained 9.13 million tetrahedral cells for the original configuration of the mechanical defeatherer and 10.21 million tetrahedral cells for that with a partial lid after careful grid independent tests.

**Field study in a poultry retail shop**

Four runs of field experiments were carried out in a poultry retail shop on 27 and 28 July 2016 in Guangzhou (Fig 2). Animal ethics approval has been obtained from The Committee on the Use of Live Animals in Teaching and Research (CULATR) at the University of Hong Kong (CULATR# 4115–16) to collect poultry swabs at live poultry markets. Poultry slaughtering and defeathering procedures were performed by the poultry shop owner while the air was sampled by our research team. In each experiment, the poultry retail shop was first ventilated for approximately 30 minutes to remove background virus-laden aerosols from the air. Afterwards, ten chickens were subjected to oropharyngeal swab sampling to monitor the level of avian influenza infection. The chickens were slaughtered by the shop owner by the severing of the jugular vein and kept inside a flapping bucket. The mechanical defeatherer was turned on, and the chickens were defeathered one by one within 30 minutes. The standard procedure for defeathering a chicken was as follows: scalding (about 40 s), defeathering in the mechanical defeatherer (about 20 s), cleaning, and dressing. Water temperature in the scalder was kept in the range 64 to 68˚C during each experiment. The defeatherer was operated by a skilled poultry worker to simulate the routine poultry slaughtering process. Water was poured into the defeatherer frequently to wash away feathers during the defeathering process.

Room temperature was 32.5˚C and 35.8˚C on July 27 and July 28, respectively. Relative humidity was 71.0% and 55.5% on these two days, respectively. The background velocity was monitored at site Velocity1 (1.0 m and 1.6 m in height, respectively) by hot-sphere anemometers. Aerosol concentrations were measured using the Aerodynamic Particle Sizer (APS, TSI

| Configuration | Aerosol diameter (μm) | No. of released aerosols | No. of escaped aerosols | Reduction by the lid |
|---------------|----------------------|-------------------------|-------------------------|---------------------|
| No lid        | 5                    | 36,572                  | 2,163                   | /                   |
| No lid        | 10                   | 36,572                  | 3,096                   | /                   |
| No lid        | 20                   | 36,572                  | 2,864                   | /                   |
| No lid        | 50                   | 36,572                  | 360                     | /                   |
| With a lid    | 5                    | 36,410                  | 725                     | 66.3%               |
| With a lid    | 10                   | 36,410                  | 1,068                   | 65.4%               |
| With a lid    | 20                   | 36,410                  | 714                     | 75.0%               |
| With a lid    | 50                   | 36,410                  | 2                       | 99.4%               |

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Fig 2. Layout of the defeathering room. The mechanical defeatherer, scaler, cleaner and flapping bucket used for the experiment are highlighted in red.

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3320) at site APS1 (6 cm away and 8 cm above the top of the mechanical defeatherer). Cyclone-based NOISH samplers (model BC251) were operated during the 30-minute defeathering process at sites NIOSH1 to 4 (NIOSH1 was 10 cm from the defeatherer and 12 cm above the cylinder top, and NIOSH2 to 4 were 1.6 m above the ground). The NIOSH samplers could collect aerosols from three size categories (i.e., > 4 \( \mu \)m, 1 to 4 \( \mu \)m, and < 1 \( \mu \)m) with a designated flow rate of 3.5 L/min [20]. After the experiment, 1 mL of MEM with 4% BSA was added to each of the collection tubes and PTFE filters of the NIOSH samplers to resuspend airborne particles collected from the air. Samples were analysed by real-time PCR to obtain the concentration of avian influenza virus and avian 18S rRNA, as described previously [12]. Briefly, total RNA was extracted by RNeasy Mini kit (Qiagen); AgPath-ID One-Step RT-PCR Reagents (Life Technologies) and QuantiTech SYBR Green RT-PCT reagents were used to target influenza A virus M genes and 18S rRNA, respectively.

Results

Visualisation and measurement of the airflow patterns of operating mechanical defeatherers

Rotation of the disk generated strong air circulation above the mechanical defeatherer. Figs 3 and 4 reveal its flow pattern (see more details in S1 Video), and the velocity magnitude is shown in Fig 5. The centrifugal effect of the rotational disk and bounding effect of the cylinder wall created an outflow that spiralled out of the defeatherer. The thickness of the annular upward air outflow from the defeatherer cylinder (see Fig 4A) was approximately 7 cm for the system that we tested. Meanwhile, there was also a downward flow into the defeatherer because the pressure above the disk centre was relatively low. The inflow spiralled towards the centre of the top opening and accelerated rapidly after it entered the cylinder. At the interface of the outflow and inflow, the large shear stress induced a strong vortex, which shed from the outflow and enhanced the horizontal spread of the air (Fig 4B). The amount of outflow from the defeatherer was considerable, and the space above it was almost filled by smoke within 10 s after the smoke was released. The smoke readily spread after it reached the ceiling.

A disk of 60 cm in diameter (see Fig 1) was rotated at a speed of 185 rpm. Therefore, the maximum air velocity near the disk was as large as 5.8 m/s. At the defeatherer-room interface, namely the very top of the defeatherer cylinder, the spiral outflow had a thickness of about 7 cm, and crossed point (23.5, 1.0) cm at a speed of about 1.40 cm/s. The maximum speed of inflow was 1.04 m/s (Point \( O_1 \) in Fig 5). The air velocity decayed as the distance from the defeatherer increased and was about 0.20 m/s at \( z_1 = 60.0 \) cm.

Development of a partial lid to reduce the dispersion of aerosols from an operating mechanical defeatherer

The spiral outflow carried virus-laden aerosols. Therefore, the most direct method to reduce the spread of viruses from the mechanical defeatherer was to block the upwards air current. The thickness of the annular outflow of the defeatherer was approximately 7 cm. Thus, a partial lid made from polystyrene (0.5 cm in thickness) was added to the defeatherer cylinder (Fig 6). There was an opening in the lid to make sure that the defeathering process was not obstructed.

Although air in the mechanical defeatherer could still escape, the effect of the lid (\( d = 32 \) cm; \( d \) is the opening diameter) was significant as shown in Fig 7A. Compared to the case without a partial lid, only a small amount of smoke was visible and did not spread upwards efficiently. Thus, the worker was much less exposed. The velocity at \( O_1 \) was reduced to 0.48 ± 0.01
Fig 3. Illustration of the airflow pattern of operating mechanical defeatherers.

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m/s, which was less than half of that without the lid. A further decrease in the cover opening (e.g., \( d = 24 \text{ cm} \)), did not extinguish the flow circulation out of the cylinder; the velocity at \( O_1 \) was measured to be \( 0.40 \pm 0.03 \text{ m/s} \). The opening of 24 cm in diameter was relatively small for

Fig 4. Visualisation of the air pattern above the mechanical defeatherer. (A) side view, illuminated by the laser sheet (left) or by the lamps (right) and (B) top view, illuminated by the laser sheet.

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a poultry worker to operate the defeatherer. Therefore, a lid with a 32-cm opening was chosen for the CFD simulations.

The CFD simulations further confirmed the effect of the partial lid on the suppression of airflow (Fig 7B). In particular, the outflow that carried the virus-laden aerosols tended to escape from the mechanical defeatherer and travel horizontally (Fig 8). As a result, the partial lid efficiently prevented particle-laden air travelling to the breathing zone of the poultry workers. The partial lid also reduced the quantity of escaped aerosols. Table 2 shows the statistical results of the aerosols released from the rubber rods of the rotating disk. Taking the 5-μm aerosols as an example, 2.163 out of 36,572 aerosols escaped from the top opening of the defeatherer. In contrast, 725 out of 36,410 escaped when the partial lid was attached, resulting in a reduction rate of 66.3%. The partial lid reduced the 10-μm aerosol escape rate by 65.4%, 20-μm aerosol escape rate by 75.0%, and almost contained 50-μm aerosols.

A field study to validate the effect of the partial lid at a retail poultry shop

The effect of the partial lid on the release of airborne particles containing AIV viral RNA or avian 18S rRNA was evaluated at a retail poultry shop. Forty chickens (10 chickens per experiment run) were purchased from the same poultry supplier in the early mornings of 27 and 28 July 2016. AIV was detected from the oropharyngeal swab of the chickens by RT-PCR, at the positive rate of 10%-50% (Table 2). The result is in agreement to our longitudinal surveillance data collected at the LPM where this field study was performed, as we expected that approximately 39.4% of the chickens (N = 1,801) would be infected with AIV [21]. The AIV positive rate and the viral load in the poultry swab samples was the lowest in the 1st experiment. The highest AIV viral load was detected in the 4th experiment but only from four out of ten chickens. APS was applied to monitor the quantity of aerosol release during the experimental runs. The strong airflow of the operating mechanical defeatherer facilitated the dispersion of aerosols into the room (Fig 9). The aerosol concentration measured at site APS1 during the 30-
minute run of experiment No. 1 was $3.8 \times 10^3$ to $2.44 \times 10^4$ particles/L. The aerosol concentrations increased sharply when one chicken was thrown into the defeatherer and dropped after the 20 s defeathering process. Ten sequential peaks of the aerosol concentration were recorded, which corresponded to the defeathering process of 10 chickens during the experimental run. The result corroborates the role of mechanical defeatherers as important sources for aerosol generation at the LPMs.

AIV viral RNA was readily detected in 15 out of 16 air samples collected by the NIOSH bioaerosol sampler, which separated the aerosols into three size fractions (Table 2). The AIV M gene was predominantly detected in aerosols of $\geq 4 \mu$m (14/16), less frequently in aerosols of 1 to 4 \(\mu\)m (4/16), and rarely in submicron sized aerosols (1/16). The highest virus concentration was detected at $5.0 \times 10^4$ copies/m\(^3\) (Site NIOSH4, Experiment 3). Concentrations of AIV viral RNA detected in air varied between experiments, possibly due to the variable AIV positive rates detected among the chickens used for the four experimental runs (Table 2).

Because not every chicken used for the field study was infected with AIV, it might not be ideal to evaluate the performance of the partial lid by comparing the AIV M gene quantity dispersed in the air. Therefore, we further quantified the avian 18S rRNA copy number in air as a surrogate [12] to test the control effect of the partial lid. Avian 18S rRNA was a suitable housekeeping gene for quantifying genetic materials of avian species. The highest concentration of 18S rRNA in the air was detected in the 4\(^{th}\) experiment as $2.5 \times 10^6$ copies/m\(^3\) at site NIOSH1 where the air sampler was immersed in the outflow of the mechanical defeatherer. It is followed by $1.1 \times 10^8$ copies/m\(^3\) at site NIOSH2. The poultry worker stood close to the defeatherer...
Fig 7. Partial lid ($d = 32$ cm) to suppress the mechanical defeatherer outflow. (A) visualisation with the laser sheet, (B) velocity vectors by CFD simulation.

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during the operation and was readily exposed to AIV if there is any. With the use of a partial lid \((d = 32 \text{ cm})\), the 18S rRNA concentration at the poultry worker’s breathing zone (site NIOSH2) was reduced by 57% (Fig 10; see more details in S1 Table), which was different from the simulated aerosol dispersion pattern in Fig 8B that indicated that the 18S rRNA-laden aerosols in the field study were able to reach the breathing zone due to the background ventilation. Before the use of the partial lid, 18S rRNA concentrations at site NIOSH 3 and NIOSH 4

![Fig 8. Aerosol (5 μm in diameter) dispersion from the mechanical defeatherer. (A) without or (B) with a partial lid \((d = 32 \text{ cm})\).](https://doi.org/10.1371/journal.pone.0216478.g008)

Table 2. Air sampling results from the defeathering experiments.

| Experiment No. | Use of the lid | Number of AIV positive chickens (mean ± SD, viral RNA copy number) | Concentrations of AIV M gene sampled in air (copies/m³) |
|----------------|----------------|-----------------------------------------------------------------|-----------------------------------------------------|
| 1              | No             | 1/10 \((1,224 ± 4,569)\)                                        | ≤ 1 μm Neg* Neg Neg Neg |
|                |                | 1–4 μm 3.79×10² Neg Neg Neg Neg |
|                |                | ≥ 4 μm Neg 5.41×10³ 1.28×10⁴ 4.41×10² |
| 2              | Yes            | 5/10 \((4,004 ± 6,445)\)                                        | ≤ 1 μm 8.33×10² Neg Neg Neg Neg |
|                |                | 1–4 μm Neg Neg Neg Neg 7.02×10² |
|                |                | ≥ 4 μm 1.34×10⁴ 1.65×10³ 1.06×10⁴ 8.65×10³ |
| 3              | Yes            | 4/10 \((20,531 ± 41,753)\)                                      | ≤ 1 μm Neg Neg Neg Neg Neg |
|                |                | 1–4 μm Neg Neg Neg Neg 3.85×10⁴ |
|                |                | ≥ 4 μm 5.61×10³ 2.23×10³ Neg 1.15×10⁴ |
| 4              | No             | 4/10 \((56,402 ± 103,813)\)                                     | ≤ 1 μm Neg Neg Neg Neg Neg |
|                |                | 1–4 μm Neg Neg Neg Neg 1.49×10⁴ |
|                |                | ≥ 4 μm 1.51×10⁴ 9.18×10³ 4.77×10³ 8.91×10³ |

* Neg = Negative

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were much lower compared to those at site NIOSH 1 and NIOSH2 because they were located relatively far from the defeatherer. However, the level of 18S rRNA reduction in the air with a partial lid was not significant at sites NIOSH3 and NIOSH4.

**Discussion**

The mechanical defeatherer operates with a rotating disk and removes poultry feathers by fixed rubber rods. This process is efficient in producing relatively large aerosols. Viral RNA and 18S rRNA were predominantly detected in aerosols larger than 4 µm. Consistent with our results, the simulated slaughter of infected poultry generated viable virus predominantly in droplets (> 4 µm) and aerosols (1 to 4 µm), but not in particles (< 1 µm) [1]. The highest concentration of viral RNA detected in the air at the poultry stall was 5.0×10³ copies/m³, which was two orders of magnitude lower than that detected inside the LPM (4.4×10⁵ copies/m³), where 10,000 to 20,000 live poultry were held daily [12]. Although we did not quantify the infectious dose of AIV in the air, viral RNA detected at 5.0×10⁷ copies/m³ in the air may still pose a significant AIV infection risk for poultry workers and nearby customers. It is also expected that during outbreak conditions when most of poultry are infected with AIV, the quantity of AIV-laden aerosols in air may be further increased.

Fig 9. Concentration of aerosols (> 0.523 µm) during the defeathering process.

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With the use of a partial lid, the exposure risk to the poultry worker was effectively reduced. It was not practical to completely seal the top of the mechanical defeatherer to contain the generated aerosols because poultry workers need to pour water in regularly during the defeathering process to wash away the feathers. To further reduce aerosol dispersion, one possible improvement is to add a pivot door to the opening of the current partial lid (Fig 11). The use of the pivot door would allow the poultry workers to easily open and cover the mechanical defeatherer while needed. The partial lid can be made at low cost with polystyrene, plastic or steel, can be easily installed for existing mechanical defeatherers, and provides a practical method to reduce the dispersion of virus-laden aerosols. It is worth mentioning that the concentration of influenza viral RNA or avian 18S rRNA at sites NIOSH3 and NIOSH4 were not greatly affected by the partial lid (see Fig 10 and Table 2). The partial lid effectively suppressed upward outflow, but the aerosol generation by rubbing of poultry and rubber rods was barely affected. As a result, the aerosol concentration inside the mechanical defeatherer and within the outflow may increase correspondingly. In addition, the partial lid potentiated the release of

![Graph showing concentration of 18S rRNA](https://doi.org/10.1371/journal.pone.0216478.g010)
horizontal air flow (see Figs 7 and 8), which might have contributed to the increased detection of influenza virus-laden or avian 18S rRNA-laden aerosols at sites NIOSH3 and NIOSH4 while the lid was in use. However, with the addition of the pivot door, it is expected that the horizontal air flow will be effectively reduced.

During our experiment, we demonstrated that the drainage slots could draw air from the room into the mechanical defeatherer. Meanwhile, aerosols may also escape from the drainage slot (Fig 8). With the use of a partial lid, our CFD simulations indicated that the escaped aerosol numbers from the drainage slot were less than 29 and 56, respectively, for the original configuration and that with a partial lid. Methods to prevent aerosol escape from the drainage slot will be tested in a future study. Furthermore, it may be possible to refine the shape or geometry of the partial lid to further reduce both upward and horizontal aerosol dispersion. Before the live poultry markets are replaced by centralized poultry slaughterhouses, the application of simple interventions to reduce aerosol dispersion of mechanical defeatherers will help to reduce the transmission risk of avian influenza viruses.

**Conclusions**

The flow pattern above the mechanical defeatherer is characterised by strong downward and upward spiral flows with velocities recorded over 1 m/s. The machine efficiently produces and disperses aerosols that carry avian influenza virus into the ambient air. Viral RNA was readily
detected in 15 out of 16 air sampling events with the highest concentration recorded at $5.0 \times 10^3$ copies/m$^3$. Viral RNA and avian 18S rRNA were predominantly detected in aerosols larger than 4 µm. The partial lid prototype that we designed suppressed the release of the upward airflow from the mechanical defeatherer while potentiated the release of horizontal airflow. The partial lid reduced the aerosol escape rate by over 65% according to the CFD simulations and may offer partial protection for poultry workers by reducing their potential exposure to aerosolised AIV by 57%. Our detailed analyses of the aerosol dispersion pattern support the rationale of the lid design, which can be easily implemented to reduce dispersion of influenza-laden aerosols during poultry processing at live poultry markets.

**Supporting information**

**S1 Video. Visualization of the defeathering machine’s flow.** The machine was first started, and smoke was released after it reached the steady condition. (MP4)

**S1 Table. Air sampling results of 18S rRNA from the defeathering experiments.** (XLSX)

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