International Journal of Hygiene and Environmental Health xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Mitigation strategies to reduce the generation and transmission of airborne highly pathogenic avian influenza virus particles during processing of infected poultry

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ARTICLE INFO

Keywords:
Avian influenza
Airborne transmission
Poultry
Public health
Safe processing

ABSTRACT

Airborne transmission of H5N1 highly pathogenic avian influenza (HPAI) viruses has occurred among poultry and from poultry to humans during home or live-poultry market slaughter of infected poultry, and such transmission has been experimentally reproduced. In this study, we investigated simple, practical changes in the processing of H5N1 virus-infected chickens to reduce infectious airborne particles and their transmission. Our findings suggest that containing the birds during the killing and bleeding first step by using a disposable plastic bag, a commonly available cooking pot widely used in Egypt (halla), or a bucket significantly reduces generation of infectious airborne particles and transmission to ferrets. Similarly, lack of infectious airborne particles was observed when processing vaccinated chickens that had been challenged with HPAI virus. Moreover, the use of a mechanical defeatherer significantly increased total number of particles in the air compared to manual defeathering. This study confirms that simple changes in poultry processing can efficiently mitigate generation of infectious airborne particles and their transmission to humans.

1. Introduction

Since 2003, over 850 human cases of H5N1 Eurasian A/goose/Guangdong/1/1996 (Gs/GD) lineage virus have been reported, with a 53% case fatality rate (Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A Virus et al., 2008; World Organisation for Animal Health (OIE), 2018; Lai et al., 2016). The majority of human infections with H5N1 highly pathogenic avian influenza (HPAI) virus have occurred following direct or indirect exposure to infected poultry in live-poultry markets (LPM) of developing countries, especially in rural settings (Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A Virus et al., 2008; World Organisation for Animal Health (OIE), 2018; Lai et al., 2016). The main risk factors associated with human infections include visiting a LPM or participating in activities with intensive contact with infected poultry like slaughtering, defeathering, or preparing sick poultry for cooking (Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A Virus et al., 2008; Samaan et al., 2011; Kirunda et al., 2015; Richard and Fouchier, 2016; Biswas et al., 2017).

The LPM setting plays a critical role in maintaining, amplifying, and disseminating avian influenza (AI) viruses among poultry and from poultry to humans (World Organisation for Animal Health (OIE), 2018; Lai et al., 2016; Suarez, 2016), with indirect evidence of potential transmission via fomites, as supported by the detection of AI viruses from the environment (Indriani et al., 2010; Kang et al., 2015; Zhou et al., 2016); and airborne exposure, as supported by the recent detection of viral RNA (Wei et al., 2018) and isolation of infectious AI viruses (Zhou et al., 2016; Wu et al., 2018) from air sampled at Chinese LPMs. Furthermore, viable AI viruses can be detected in the air where live poultry are kept and processing activities, such as slaughtering and defeathering, are performed (Zhou et al., 2016). Recently, we have

Abbreviations: AI, avian influenza; BHI, brain heart infusion; CL, cloacal; dpe, days post-exposure; ECE, embryonating chicken eggs; EID_{50}, mean egg infectious dose; FAO, Food and Agriculture Organization; GMT, geometrical mean titers; Gs/GD, H5N1 Eurasian A/goose/Guangdong/1/1996; HI, hemagglutinin inhibition; HPAI, highly pathogenic avian influenza; LPM, live-poultry markets; NIOSH, National Institute for Occupational Safety and Health; OP, oropharyngeal; PVC, polyvinyl chloride; SPF, specific pathogen free; USNPRC, US National Poultry Research Center; VN, virus neutralization; VN/04, A/Vietnam/1203/04(H5N1) HPAI

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https://doi.org/10.1016/j.ijheh.2018.05.013
Received 20 March 2018; Received in revised form 29 May 2018; Accepted 29 May 2018

confirmed that viable airborne HPAI virus particles are generated during simulated processing of asymptomatic HPAI virus-infected poultry, and such airborne virus can be transmitted to naïve poultry and ferrets (Bertran et al., 2017), emphasizing the high risk involved in processing infected poultry.

In countries that are enzootic to influenza in poultry (Padmawati and Nichter, 2008), slaughtered poultry can be incubating or carrying the HPAI virus without clinical signs (Spickler et al., 2008), or they can be sick or dead and still be processed for human consumption as a result of financial concern (Rimi et al., 2014). Several research studies have focused on understanding poultry processing practices within the village setting and identifying specific behaviors around processing sick poultry that may represent opportunities for transmission (Kirunda et al., 2015; Rimi et al., 2014; Van Kerkhove et al., 2011; Sultana et al., 2012a, b; Fasanmi et al., 2016). These studies indicate that all the steps of the processing of sick poultry (killing, scalding, defeathering, eviscerating, and cleaning up) are exposing villagers and other animals to potential risk of HPAI virus transmission, and they recognize that practical and culturally acceptable interventions are needed to reduce such risk (Kirunda et al., 2015; Rimi et al., 2014; Van Kerkhove et al., 2011; Sultana et al., 2012a, b; Fasanmi et al., 2016). Several authors have suggested generic interventions that could be evaluated for effectiveness, such as using a bucket to contain blood, carcass, offal, skin, feathers, and waste water, and burying the bucket contents to reduce potential environmental contamination (Samaan et al., 2011; Rimi et al., 2014). For the processing of sick poultry, a more restrictive set of recommendations could be explored, such as using hot water for defeathering and disinfection of the bucket and processing tools at the end of the process, since AI virus is susceptible to high temperatures (World Organisation for Animal Health (OIE), 2017). Defeathering typically exposes several persons to the processed poultry for a prolonged period of time (Rimi et al., 2014); therefore, skinning sick poultry along with feathers instead of defeathering may also reduce the exposure from defeathering process (Samaan et al., 2011; Rimi et al., 2014). Moreover, technical training in appropriate processing techniques should be provided; evisceration is a critical step in the slaughter process because it exposes villagers directly to intestinal contents (Rimi et al., 2014) that may rupture, thus resulting in spillage of fecal material, viruses, and bacteria (Samaan et al., 2011).

In the developing world, poultry at the village level are a critical source of protein. Many households have individual flocks, managed by women from hatching to consumption. In village situations of limited income and limited literacy, proposed interventions for any development activities must take into account a variety of factors, e.g., proven efficacy, low complexity, affordability (no or low cost), use of existing equipment, sustainability, cultural standards, and religious acceptability. This study is taking into account these considerations. The broad goal of this study was to investigate the use of normal household equipment for simple, practical, affordable, and straightforward changes in poultry processing methods to prevent or reduce infectious airborne particles during the processing of H5N1 HPAI virus-infected chickens. We also determined the impact of some of these strategies on reducing airborne virus and their transmission to ferrets, the animal model for human influenza infection.

2. Materials and methods

2.1. Viruses

Eurasian Gs/GD lineage clade 1 virus A/Vietnam/1203/04 (H5N1) HPAI (VN/04) was used (GenBank accession numbers HM006756-63). This virus was selected because: i) it is representative of zoonotic avian-origin viruses (Writing Committee of the Second World Health Organization Consultation on Clinical Aspects of Human Infection with Avian Influenza A Virus et al., 2008; World Organisation for Animal Health (OIE), 2018); and ii) it is capable of generating infectious airborne particles during the processing of infected chickens and transmit to naïve hosts exposed to the same air space (Bertran et al., 2017). The virus was propagated and titrated in embryonating chicken eggs (ECE) using standard methods (Spackman and Killian, 2014). The virus was diluted to the target dose with brain heart infusion (BHI) broth (Becton, Dickinson and Company, Sparks, MD).

2.2. Animals

Adult (60 weeks old) specific pathogen free (SPF) White Leghorn chickens (*Gallus domesticus*; US National Poultry Research Center, USDA-ARS (USNPRC)) were utilized. All birds were serologically negative for antibodies against influenza A virus infection by hemagglutinin inhibition (HI) test (OIE, 2016) before HPAI virus challenge. In Experiment 2, naïve, 3-month-old female domestic ferrets (*Mustela putorius furo*; Marshall, North Rose, NY) were utilized as the mammalian model for HPAI virus transmission to humans (Richard and Fouchier, 2016). Ferrets were anesthetized with an intramuscular injection of a ketamine (25 mg/kg)-xylazine (2 mg/kg)-atropine (0.05 mg/kg) cocktail before nasal wash collection or euthanasia by intracardiac injection of sodium pentobarbital. Ferrets were H5-seronegative by HI test and virus neutralization (VN) test (OIE, 2016), and nasal washes were negative for influenza A virus based on ECE isolation testing (Swayne et al., 2008) before the study. All procedures were performed according to the requirements of protocols approved by the Institutional Animal Care and Use Committee, and Institutional Biosecurity Committee.

2.3. Environmental conditions in the processing enclosure

All the experiments were conducted in animal biosafety level 3-enhanced facilities at the USNPRC. The processing area was a HEPA enclosure (Class Biologically Clean Ltd., Madison, WI) 1.5 m wide x 6.7 m long x 2.1 m high with unidirectional and single pass airflow of 8.3 air changes/h (340 m^3/h) at 0.046 m/s from the processing area towards the air samplers or the naïve animals (Fig. 1). The mean temperature in the enclosure during the slaughter runs was 24.2C ± 0.4C, with a mean relative humidity of 81.0% ± 1.7%. All procedures were performed using adequate personal protective

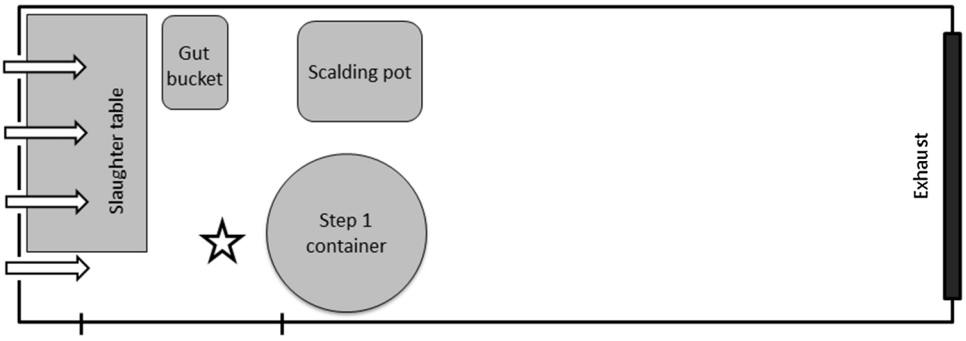

Fig. 1. Diagram of the processing area. The enclosure was 1.5 m wide x 6.7 m long x 2.1 m high, with 8.3 air changes/h (340 m^3/h) and a velocity of 0.046 m/s. The star represents the location of the air samplers and particle counters (Experiments 1, 3, and 4) or the naïve hosts (Experiment 2). The container method for the kill step varied depending on the experiment. The arrows indicate the airflow within the HEPA enclosure.