On-site hygiene and biosecurity assessment: A new tool to assess live bird stalls in wet markets

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Abstract

Wet markets play an important role in food security and consumers often view the produce as fresher and cheaper. It is highly prevalent in Asia and a source of livelihood for many small and medium businesses. Studies have revealed that highly unsanitary markets, especially those with live bird stalls operating within the wet market could pose a threat to consumer food safety and public health. This study proposed a new, rapid assessment tool for monitoring hygiene and biosecurity measures of live bird stalls. The design of Hygiene and Biosecurity Assessment Tool (HBAT) was supported by the identification of critical hygiene and biosecurity practices based on empirical evidence that suggests such control measures can prevent or reduce the cross contamination or transmission of zoonoses. An observational, cross sectional study of wet markets selling live birds and/or slaughtered birds was conducted to test the tool. Most wet market stalls slaughter and/or sell chicken, followed by quail, duck and amphibians. 50% of the wet market stalls were rated as moderate, 43.2% as poor and require major improvement, 2.3% as good and 4.5% as excellent. Stalls are in general kept in clean condition and no mixing of species or presence of pests or strays were observed. The cleaning and disinfection practices of slaughter area (after each slaughter) and tools require urgent improvement as majority of stalls cleaned the surfaces with water only. Customers have direct access to live bird stalls and should be reminded (with visible signs) to wash their hands before entering other zones. Toilet and handwashing facilities are highly inadequate and improved physical infrastructure and the provision of sufficient hygiene and handwashing facilities are required to facilitate hand hygiene. This study is highly relevant to countries where wet markets and live bird stalls play a crucial food security role to the local communities. The tool could be used to aid policymakers design evidence-based

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assessments to monitor on-site hygiene and biosecurity measures of live bird / animal stalls in wet markets. To our knowledge, this is the first empirical study to propose an on-site hygiene and biosecurity assessment tool to monitor live bird stalls in wet market.

Keywords: biosecurity; cross contamination; handwashing; hygiene; wet markets; zoning

Highlights

- Procedure for assessing on-site hygiene and biosecurity measures of wet market stalls is proposed;
- Cleaning and disinfection practices of slaughter area and tools require urgent improvement to prevent cross contamination;
- Visible notices to prompt workers and public about importance of hand hygiene and procedures for handwashing is required;
- Adequate hygiene and handwashing facilities should be provided to workers and consumers.

Introduction

As of 26 December 2020, a total of 78,383,527 confirmed cases of COVID-19 have been reported globally including 1,740,390 deaths (WHO, 2020). Although the source of 2019-nCoV is yet to be confirmed, early findings suggest a high possibility of a bat origin (Lu et al., 2020; Zhou et al., 2020) and possibly also involving an intermediary animal species (Junejo et al., 2020; Lam et al. 2020). Cohen (2020) and Li et al. (2020) suggested that marketplace played an early role in the spread of COVID-19. Mizumoto et al. (2020) estimated the reproduction number (R) for market-to-human transmission was 0.24 and 2.37 for human-to-human transmission. Moreover, the reporting rate for market-to-human transmission was estimated to be 2 to 34-fold higher than for cases stemming from human-to-human transmission. This strongly suggests that contact history with wet market played a crucial role in identifying COVID-19 cases. Following the spike case of COVID-19 cases, the Huanan Seafood Wholesale Market in Wuhan, China, for example, created a wild animal section where the animals were slaughtered on-site prior to sale (Maron 2020).

Wet market is defined as a place that sell fresh produce, including meat, seafood, fruits and vegetables and sometimes live animals that are slaughtered and sold in open-air environments (Nadimpalli & Pickering, 2020). Domestic and wild animal species including poultry, mammalian, reptiles, amphibians and fish are held in cages / tanks, are stressed and located in close proximity to each other, and that makes for ideal conditions for diseases to multiply. Animals are often slaughtered on-site and hung or placed in the open air without ice or refrigeration (Poland, 2020). In Asia, wet markets are prevalent because consumers view the produce from such environments as fresher, cheaper and highly accessible to low-income communities. It is also an important source of livelihood for many small and medium businesses (Petrikova et al., 2020; Zhong et al., 2020).

Even though viruses causing human foodborne illness cannot grow in foods as reported by Caldwell (2020), various studies have reported high prevalence of foodborne pathogens and viruses found in animal-based and seafood products sold in wet markets. This could be as a result of faecal contamination or handling by infected persons (Seymour & Appleton 2001). *Escherichia coli* and *Salmonella enterica* were isolated from wooden cutting boards used to process raw meat in Hong Kong (Sekoai et al., 2020), avian influenza A virus were found in environmental and animal samples from live poultry markets in China (Yuan et al., 2014), multidrug-resistant *Salmonella* and *Listeria monocytogenes* were isolated from chicken, pork and shrimp sold in Thailand (Minami et al., 2010), influenza virus (H5N1) were detected in live bird markets and food markets in Thailand (Amonsin et al., 2008) and Indonesia (Henning et al., 2019) and *Salmonella* were identified in meat from Philippines (Santos et al., 2020). In Malaysia, multidrug-resistant *Salmonella* were isolated from poultry and processing environments (Chuah et al., 2018; Nidaullah et al., 2017), *Listeria monocytogenes* were detected in chicken offal sold in wet markets (Kuan et al., 2013) and *Vibrio parahaemolyticus* were found in seafood samples in wet markets (Tan et al., 2020). Abatcha et al. (2018) found up to 48% (n=35) of chicken carcasses and 41% (n=202) of environmental samples from wet markers were positive for *Salmonella* spp. *Salmonella* is carried asymptomatically in the gastrointestinal tracts of live birds and contamination can occur whilst slaughtering and handling in the wet markets (Abatcha et al., 2018; Trongjit et al., 2017). A higher prevalence of *Salmonella* spp., including *S.* Enteritidis and *S.* Typhimurium were also reported in wet markets compared to supermarkets (Thung et al., 2016). It is likely that the higher prevalence of foodborne pathogens in wet markets were due to lack of personal hygiene and sanitary conditions in wet markets and high humidity and higher storage temperature in wet markets (Oscar, 2004; Thung et al., 2016). There is an inherent risk of transmitting zoonotic pathogens from live animals to humans and as reported by Poland (2020), it acts as the perfect 'petri dish' environment for a variety of zoonoses to incubate and emerge.

There was a ban on slaughtering of live birds in wet markets by the National Council of Malaysian government to prevent the spread of infectious diseases (The Star, 2013) but slaughtering of live birds in such markets are still prevalent today (Chuah et al., 2018). However, one of the local authorities in Malaysia i.e. the Penang Island City Council had issued a notice to ban the slaughtering and processing of live birds in all stalls (including wet markets) from October 2021 to prevent and control the spread of infectious diseases from slaughtering live birds, prevent cross contamination of water sources as a result of waste from live bird stalls and to control the spread of pests and wastes (Pers. Communication, Penang Island City Council, 2020). A blanket ban on wet markets selling live animals could potentially drive traders to underground markets where monitoring would be impossible (Lynteris & Fearnley, 2020). Instead of banning wet markets, it would be more effective to improve market biosecurity and hygiene of wet markets and use regulated wet markets to enforce the ban of sale of illegal wildlife (Aguirre et al., 2020; Daszak et al., 2020; Petrikova et al., 2020). There exists a number of tools to measure biosecurity measures at farm level. For example, Biocheck.UGent (https://biocheck.ugent.be/en) had been developed to measure biosecurity at broiler farms (Gelaude et al., 2014; Van Limbergen et al., 2018) while Lewerin et al. (2015) developed a risk assessment tool to assess biosecurity measures in cattle and pig farms. BioAsseT (Biosecurity Assessment Tool) was used to measure external, internal biosecurity and diagnostic monitoring of pig farms (Sasaki et al., 2020). To our knowledge, there is a lack of hygiene and biosecurity assessment of live birds delivered post-farm gate including the sale of live birds in wet markets or live poultry markets. This study aims to develop an assessment tool to investigate on-site hygiene and biosecurity measures of live bird stalls in wet markets.

Methodology

Design principles of Hygiene and Biosecurity Assessment Tool (HBAT)

The design of Hygiene and Biosecurity Assessment Tool (HBAT) was guided by previous diagnostic tools utilised in processing environment, slaughterhouses and open-air food markets (Gelaude et al., 2020; Ledo et al., 2016). HBAT was developed in English and supported by the identification of critical hygiene and biosecurity practices based on empirical evidence that suggests such control measures can prevent or reduce the cross contamination or transmission of zoonoses i.e. adequate wet market infrastructure (Chowdhury et al., 2020; Nadimpalli & Pickering, 2020), cleaning and disinfection (Chowdhury et al., 2020; Samaan et al., 2011; Webster, 2004; Yuan et al., 2014; Yuan et al., 2015), zoning (Chowdhury et al., 2020; Indriani et al., 2010; Samaan et al., 2011), waste removal (Indriani et al., 2010), availability of toilets and handwashing facilities (Nadimpalli & Pickering, 2020) and personal hygiene (Ledo et al., 2020). Both authors who are experts in food hygiene, safety and food security reviewed the tool to ensure the content measures the on-site hygiene and biosecurity parameters. The tool is divided into five main sections: Part I: Premises; Part II: Preparation and slaughter area; Part III: Zoning and cross contamination; Part IV: Cleaning and disinfection; Part V: Personal hygiene (Table 1). On-site hygiene and biosecurity scores were quantified by converting the answers in Table 1 into scores where correct application of certain measures = 1 point or 2 points or zero for no application. Food hygiene and biosecurity items that were deemed more likely to result in cross contamination of food and increased public health risks were awarded 2 points. The maximum score of "75" equals full application of hygiene and biosecurity measures on site while "0" represents total absence of any hygienic or biosecurity measures in the live bird / slaughtered bird stall.

Insert Table 1

A score of 2, 1 or 0.5 was applied in certain measures where multiple possible scenarios exist. The points were 'graded' to distinguish different level of good hygiene and biosecurity practices. For example, under Handwashing facilities (in Premises Section of Table 1), if handwashing facilities such as handwashing basin with soap and running water was observed, the stall was awarded 2 points. If only a handwashing basin with running water (but no soap or handwashing liquid) was provided, the stall was awarded 1 point. If only buckets of stagnant water were available, the stall was awarded 0.5 point and if no handwashing facilities were available at all – no marks were awarded. Table 1 also shows that certain criteria were awarded 2 points in Yes, No or Not Applicable sections. A score of 2 is given when the stall demonstrated examples of good hygiene and biosecurity measures. This may include practices where stalls should not be placing animals in overcrowded cages and not slaughtering animals directly near to other live animals.

Design of pilot study

Items in HBAT (Table 1) were adapted into an electronic checklist tool using Online Survey so observation could be carried out using a smartphone (Figure 1). HBAT was pilot tested at two wet markets selling live poultry. Three graduate research assistants well versed in participatory and non-participatory observation skills, food safety and hygiene control measures were trained virtually to use HBAT. Before conducting the actual assessment, all users participated in the observation of wet market stalls selling live or slaughtered animals in the pilot study. Results were reviewed and discordant notifications were discussed.

Insert Figure 1 here

On-site observation

An observational, cross sectional study of hygienic and biosecurity operations at wet markets selling live birds and/or slaughtered birds was conducted. Wet markets were selected using a convenience sampling approach in cities and sub-urban areas of both East and West Coast of Peninsular Malaysia including one town in East Coast of Malaysia. The selection of wet markets was limited by voluntary participation from the stall owners, markets that remained open for business and national restrictions on inter-state travel during the pandemic. The wet markets in the following states were observed: Selangor (n=10), Perak (n=18), Sarawak (n=2) and Kelantan (n=14). Prior to on-site observation, verbal consents were sought from the owners or sellers at the live birds or slaughtered-birds' stalls for the observers to conduct non-participant observation. The study received institutional ethical approval and abide by the Global Code of Conduct for research in low resource settings requirements (TRUST, 2018). The on-site observation was conducted during the period of June – November 2020. This coincides with the Recovery Movement Control Order (RMCO) period where inter-states travel was allowed. The type of market, animals sold, and number of employees per stall were also observed. A total of 2,822 minutes of observations were carried out at 44 live birds and/or slaughtered birds stalls, with each stall averaging 64 minutes of surveillance.

Compliance with Hygiene and Biosecurity Requirements

Hygiene and biosecurity compliance among wet market stall owners selling live and/or slaughtered animals were calculated using the modified formula (Santana et al., 2009; Soon, 2019) below.

$$P = \left(\frac{TS}{\sum 1 - \sum 2}\right) \times K$$

P = Part I to Part V (Part I: Premise; Part II: Preparation and slaughter area; Part III: Zoning and cross contamination; Part IV: Cleaning and disinfection; Part V: Personal hygiene);

TS = Total score;

$\sum 1$ = Total possible points;

$\sum 2$ = Total non-applicable points;

K = constant value (K values for Part I=16; Part II=24; Part III=14.66; Part IV=16; Part V=29.33)

The total score represents the points achieved by a specific section e.g. Premises. $\sum 1$ represents the total possible points that could be achieved. In this case, the total possible points for Premises = 12; Preparation and slaughter area = 18; Zoning and cross contamination = 11; Cleaning and disinfection = 12; and Personal hygiene = 22. Total possible points for all parts = 75. The non-applicable points ($\sum 2$) are points that should be deducted from the equation if the requirement is irrelevant to the wet market stall. This is to avoid potentially distorting the final hygiene and biosecurity score. For instance, if animals are not slaughtered at the stall, then the Preparation and Slaughter section will be noted as non-applicable. The average score for all sections (Part I – V) was calculated as:

$$\left(\frac{\mathrm{PI} + \mathrm{PII} + \mathrm{PIII} + \mathrm{PIV} + \mathrm{PV}}{10}\right)$$

The hygiene and biosecurity scores are classified according to the following scale:

0 – 1.9 = very poor and urgent improvement necessary

2.0 – 4.9 = poor and major improvement necessary

5.0 – 6.9 = moderate and some improvement required

7.0 – 9.0 = Good

9.0 – 10.0 = Excellent

Statistical analysis

Intraclass coefficient correlation (ICC) was calculated to determine inter-rater reliability using SPSS version 27.0. Values scoring < 0.5 = poor reliability, 0.5-0.75 = moderate reliability, 0.75 – 0.90 = good reliability and > 0.90 = excellent reliability (Koo & Li, 2016). Exploratory factor analysis was conducted to determine construct validity. Principal component analysis (PCA) was performed using varimax rotation.

Results and Discussion

More than half of the wet market stalls were situated indoors, and up to 13.6% located partially indoor. However, only 18.2% of the available ventilations systems were working. A number of the wet market stalls were located indoors or partially indoors with limited working ventilation systems in place. All the wet markets were not housed in air-conditioned buildings unlike supermarkets. As the name ‘wet market’ suggests, floors are continually washed down, certain fresh produce are kept moist to ensure freshness and to keep animals alive, hence wet markets posed an extremely humid environment (Ho, 2014). Wet market stalls located indoors require high ventilation rate to remove moisture, heat and contaminant (Lee & Lee, 2013). Failure to ventilate the damp and warm environment pose a risk for foodborne pathogens and zoonoses to emerge and thrive (Chuah et al., 2018; Rahman et al., 2018). Moreover, high ambient temperatures could lead to heat stress among broiler chickens. Heat stress were found to affect chicken immune functions, increasing the risk of infectious disease outbreaks (Hirakawa et al., 2020). Stalls were mostly operated by one or two staff (including the owner). Majority of the wet market stalls slaughter and/or sell chicken (88.6%) followed by quail, duck and other category (i.e. frogs and toads) (Table 2).

Insert Table 2

On-site Hygiene and Biosecurity Assessment Scores

The Intra-Class Correlation Coefficient (ICC) between the users in the pilot tests measured 0.84, $F(1, 3) = 110.08$, $p < 0.05$) indicating high inter-rater reliability. The Kaiser-Meyer-Olkin (KMO) measures of sampling value was 0.75. According to Hair, Black, Babin, Anderson and Tatham (2010), the criterion of validity should be > 0.60 and the KMO fulfils the requirement. All factor loadings were > 0.40 and explained 79.22% of the variance. Based on the observation, market stalls selling live birds and or slaughtered birds were scored using Table 1 and Formulas 1 and 2. 50% of the wet market stalls were rated as moderate, 43.2% as poor and require major improvement, 2.3% as good and 4.5% as excellent (Figure 2).

Insert Figure 2

Twenty-one stalls were found to place live birds in overcrowded cages although none of the birds were mixed with other poultry. Of these 21 stalls with overcrowded cages, 8 stalls were not maintained in a sanitary condition. Overcrowding of animals lead to highly stressed animals and coupled with highly unsanitary conditions, this would serve as breeding grounds for zoonoses (Wiebers & Feigin, 2020). Most stalls were maintained in clean condition (70.5%), cages were kept clean (43.2%) and the stall area free from pests or strays (e.g. rodents, stray cats or dogs and wild birds) (59.1%). One good practice observed amongst all stalls was the absence of mixing different bird species in the same cage. As reported by Chan et al., (2013) other bird species e.g. ducks, geese and quails were segregated from chickens to prevent the spread of avian influenza viruses from asymptomatic birds to chickens. Majority of stalls had some form of handwashing facilities available. However, there is limited number of public handwashing facilities for customers, especially when crossing into zones selling ready-to-eat food. Although 84.1% of the wet market stalls were in a different zone, up to 30% of the live bird stalls were situated less than 3m away from other food stalls (Table 3). In most wet markets, customers were observed to have direct access to the live bird stalls (90.9%). This is a common practice as customers prefer to select their own bird of choice, other than want to see themselves on how the farms/markets handle the birds. However, this increases the opportunity for human transfer of pathogens and zoonoses (Cui et al., 2019) as evidenced by the spill-over of avian influenza virus from infected poultry to humans (Wang et al., 2017; Wang et al., 2020). As the case of avian influenza H5N1 outbreak occurring in 2003 to 2006, it has been reported that most patients who have been infected had recent direct contacts with poultry (Woo et al., 2006). Although customers could access the handwashing facilities at 43.2% of the stalls, this study did not carry out any observation of the customers (i.e. whether they washed their hands after selecting the birds / touching the surfaces at the live bird stall).

Insert Table 3

There is also a lack of public handwashing facilities for customers when entering different zones (e.g. ready-to-eat food stalls). Only nine wet markets provided public handwashing facilities for consumers and staff before entering zones selling ready-to-eat foods. Fourteen wet markets placed visible signs and notices to remind customers and staff to wash their hands. This is concerning given that the observations were carried out during the COVID-19 pandemic. Notices to remind or prompt workers and public about importance of hand hygiene and procedures for handwashing is one of the key strategies proposed by the WHO multimodal hand hygiene improvement strategy (WHO, 2009). The live bird stalls were located in a live animal zone and although most were segregated, up to 30% were within 3 meters of other food stalls. Previous studies have shown that aerosol transmission is an important mode of viral transmission in wet market environment (Wei et al., 2018). In closed environments with minimal ventilation, virus in aerosols may persist in air for longer and at higher concentrations, thus increasing rate of transmission (Wu et al., 2020). Formation of aerosols are further aided with the use of hosepipes (81.8%) and brooms (68.2%) for cleaning which is prevalent in this study. Furthermore, the washing down of stalls resulted in waste run-offs contaminating other non-live bird areas and food stalls.

Table 4 shows the observation of hygiene practices of wet market stalls selling live birds and/or slaughtered birds. Most stalls that sell live birds also slaughtered the birds on-site. Six stalls were observed to slaughter birds on the ground although most staff carried out the process on bench tops that are easy to clean and smooth (e.g. stainless steel, ceramic stone, worktable with aluminium top). Slaughtered birds were often cleaned to remove visible dirt, soil and blood stains before selling them to customers. Only 38.6% of the stalls cleaned the work surface after each slaughter and most stalls only used water to clean the surface area. Similarly, knives and tools were mostly washed with water only. Wastes were collected into dedicated bins with lids (18.2%), without lids (36.4%) but some wastes were also washed into nearby drains (29.5%). Although most stalls used easy-to-clean and smooth surfaces as their work tops (e.g. stainless steel, ceramic stone, worktable with aluminium top). cleaning of work surfaces was not often carried out after each slaughter. Slaughter area and tools were cleaned with water only (> 70%). This posed a risk of transmission of foodborn pathogens and zoonoses if surfaces were not cleaned adequately. *Escherichia coli* and *Salmonella* (Sekoai et al., 2020), *Kelbsiella pneumoniae* (Lo et al., 2020) and H7N9 virus (Wang et al., 2015) were detected in samples collected from surfaces of chopping boards from wet markets. Traditional cleaning method of wooden cutting boards often used by Asian vendors include scraping the surface of the wooden cutting board with a chopping knife until a white layered film has been removed, followed by rinsing with hot water (Lo et al., 2020). In terms of cleaning practices, most stalls used hosepipes and brooms to clean the stall area. It is concerning to note that liquid wastes such as blood were washed into other nearby areas including food stalls (18.2%).

Workers mostly wear apron and boots, but only slightly more than half of the staff wore masks or gloves. In certain wet markets, the use of masks could be due to the enforcement during the Movement Control Order (MCO). A third of the workers were observed to touch their mouth, nose or eyes whilst handling or after handling live animals. A number of staff also tend to use their mobile devices during and after handling live birds (15.9%). Contamination with faecal residues upon handling live birds can occur on such mobile devices. It has been reported in Olsen et al. (2020) that mobile communication devices can serve as possible breeding grounds for microbial organisms.

Handwashing with soap were only observed in small number of stalls (20.5%) in this study. More than half of the staff (56.8%) would wash hands with water only after handling live animals. This finding reflects the study by Alam et al. (2019) where market sellers were found to rarely wash their hands with soap but tend to wipe their hands with a cloth. Although majority wore aprons and boots, slightly less than half of the workers wore face masks and most do not use gloves when handling live animals. One of the main reasons documented by Alam et al. (2019) was that the high temperature and humidity in wet markets discourage workers from wearing protective equipment. Toilet facilities were lacking in half of the wet market stalls and the remaining stalls with access to such facilities were found to be inadequate (dirty, no running water, no soap or hand drying facilities). Although half of the wet market stalls have access to toilet facilities, the provision of clean and adequate potable water supply is seriously lacking and is important to facilitate handwashing practices.

Insert Table 4

The above findings reflect the hygiene and biosecurity measures rating for most stalls. Majority were rated as moderate with some improvements required or poor with major improvements necessary. This study reiterates the recommendations of Nadimpalli and Pickering (2020) and WHO (2006) that called for standardised global monitoring of water, sanitation and hygiene (WASH) and to improve the physical infrastructure of wet markets including the provision of sufficient toilets, handwashing facilities, potable water supply and proper drainage. In April 2020, WHO called for stricter food safety and hygiene standards for wet markets and is developing guidance for the safe operation of wet markets (Briggs, 2020). Our findings can aid the design of evidence-based assessments to monitor on-site safety, hygiene and biosecurity measures of live bird stalls in wet markets. Shi et al. (2020)

conducted a meta-analysis of 19 studies and found that implementation of interventions in live bird market environment significantly reduce zoonotic infections. This is also the first assessment tool to assess level of on-site hygiene and biosecurity measures of live bird stalls in wet markets, providing a rapid indication of the hygiene and biosecurity scores of live bird stalls.

Limitations, practical implications and recommendation for future studies

There are several limitations associated with the study. First, the study was conducted during the Recovery Movement Control Order and Conditional Movement Control Order (stricter measures with no inter-states travel in November 2020). The data collection was restricted to several states, hence limiting the number of sites. The data collected during the on-site observation only provided a snapshot of current hygiene and biosecurity measures. The presence of observers may have introduced the Hawthorne effect among the participants and potentially increases handwashing behaviours. The researchers did not observe other biosecurity measures such as weekly rest days, weekly and monthly disinfection practices, transportation and receipt of live birds at wet markets, whether poultry were kept overnight / days and treatment and transportation of collected wastes. The tool could be easily adapted to suit the local food and requirements of wet markets in different regions. For example, instead of live bird stalls in Malaysia, the tool could potentially be modified and applied to other stalls selling live and/or slaughtered meat and seafood products. If future researchers were to modify the tool, the content and construct validity and inter-reliability must be tested to ensure multiple users could assess the same hygiene and biosecurity measures with no significant differences. One of the key strengths of HBAT is the convenience, ease of use and enables rapid assessments of on-site hygiene and biosecurity measures. Since it could be used in smartphones, it offers covert observation of stalls by veterinary, public health and food safety inspectors. It would be highly valuable to conduct a live poultry supply chain study to assess the hygiene and biosecurity measures from farm to market. The on-site Hygiene and Biosecurity Assessment tool could be further adapted and/or modified to suit other countries and local wet market practices selling live birds.

Conclusion

Wet markets play a crucial food security role for local communities and is a source of livelihood for many small-scale food businesses as well as provide essential social interaction for elderly residents in the area. However, highly unsanitary wet markets with minimal or no biosecurity measures is the perfect 'petri dish' environment for a variety of zoonoses to thrive. Instead of banning or trying to outlaw wet markets with live animals, it would be more effective to ensure that live animal stalls in wet markets are practising good hygiene and biosecurity measures. Physical infrastructures including designated or segregated area for live bird stalls, provision of toilet and adequate handwashing facilities for workers and staff and monitoring of wet markets to ensure hygiene and biosecurity measures are met are crucial interventions needed to ensure the safety and welfare of animals and that public health are not at risk. By practising this, the transmission of the viruses to humans can be controlled and reduced. To our knowledge, this is the first study to propose an on-site hygiene and biosecurity assessment tool to monitor live bird stalls in wet market. The study could be used to aid policymakers in developing guidance and training of staff operating live bird stalls and to design evidence-based assessments to monitor hygiene and biosecurity measures.

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