

Biosecurity Assessment and Seroprevalence of Respiratory Diseases in Backyard Poultry Flocks Located Close to and Far from Commercial Premises

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SUMMARY. Raising backyard chickens is an ever-growing hobby in the United States. These flocks can be a substrate for respiratory disease amplification and transmission to commercial facilities. Five hundred fifty-four chickens from 41 backyard flocks were sampled in this study. ELISA kits were used to detect antibodies against avian influenza (AI), infectious laryngotracheitis (ILT), Newcastle disease (ND), infectious bronchitis (IB), *Ornithobacterium rhinotracheale* (ORT), *Mycoplasma gallisepticum* (MG), and *Mycoplasma synoviae* (MS). All visited flock owners answered a biosecurity questionnaire that assessed biosecurity measures. The questionnaire revealed that backyard poultry owners lack simple biosecurity measures such as use of dedicated shoes, their chicken sources are unreliable, and few of them benefit from veterinary oversight. Only one flock had a clear vaccination history against ND and IB. ORT, ND, IB, MS, MG, and ILT were the most seroprevalent in backyard poultry flocks with 97% (41/42), 77.5% (31/40), 75% (30/40), 73% (31/42), 69% (29/42), and 45% (19/42), respectively. The vaccinated flock was not considered in these calculations. When examining the distance between backyard flocks and the nearest commercial poultry facility, ND and MG were significantly more likely to be found in backyard flocks close to (<4 miles) whereas ORT was significantly more likely in backyard chickens located far from (>4 miles) commercial poultry. Birds purchased directly from National Poultry Improvement Plan hatcheries showed a reduced ND, MG, and MS antibody prevalence. Wearing dedicated shoes decreased MS antibody-positive birds. Finally, history of wild bird contact had a clear effect on an increased seroprevalence of NDV and MG. Serological results suggest that backyard poultry flocks have the potential to serve as a reservoir or amplifier for poultry respiratory diseases. The information generated in this project should direct extension efforts toward emphasizing the importance of small flock biosecurity and chick acquisition sources.

RESUMEN. Evaluación de la bioseguridad y seroprevalencia de enfermedades respiratorias en parvadas de traspatio localizadas en proximidad o a distancia de instalaciones avícolas comerciales.

La crianza de pollos de traspatio es una afición que está aumentando cada vez en los Estados Unidos. Estas parvadas pueden ser un sustrato para la amplificación de enfermedades respiratorias y la transmisión a instalaciones comerciales. Se recolectaron muestras de 554 pollos de 41 parvadas de traspatio en este estudio. Se usaron estuches ELISA para detectar anticuerpos contra la influenza aviar (IA), laringotraqueítis infecciosa (ILT), enfermedad de Newcastle (ND), bronquitis infecciosa (IB), *Ornithobacterium rhinotracheale* (ORT), *Mycoplasma gallisepticum* (MG) y *Mycoplasma synoviae* (MS). Todos los propietarios de las parvadas visitadas respondieron un cuestionario que evaluó las medidas de bioseguridad. El cuestionario reveló que los dueños de aves de traspatio carecen de medidas simples de bioseguridad, como el uso de zapatos especiales, los proveedores de sus aves no son confiables y pocos de ellos se benefician de la supervisión de un médico veterinario. Solo una parvada tenía un claro historial de vacunación contra la enfermedad de Newcastle, bronquitis infecciosa, *O. rhinotracheale*, *M. gallisepticum*, and *M. synoviae* y laringotraqueítis aviar, con los datos más altos de seroprevalencia con 97% (41/42), 77.5% (31/40), 75% (30/40), 73% (31 / 42), 69% (29/42) y 45% (19/42), respectivamente. La parvada vacunada no fue considerada en estos cálculos. Al examinar la distancia entre las parvadas de traspatio y las instalaciones avícolas comerciales más cercanas, la enfermedad de Newcastle y *M. gallisepticum* fueron significativamente más propensos a encontrarse en parvadas de traspatio cercanas (<4 millas) mientras que *O. rhinotracheale* fue significativamente más probable en pollos de traspatio ubicados lejos de (> 4 millas) las instalaciones avícolas comerciales. Las aves compradas directamente de incubadoras que estaban sujetas al Plan Nacional de Mejoramiento Avícola mostraron una prevalencia reducida de anticuerpos contra la enfermedad de Newcastle, *M. gallisepticum*, *M. synoviae*. El uso de zapatos especiales disminuyó el número de aves positivas a la presencia de anticuerpos contra *M. synoviae*. Finalmente, la historia del contacto con aves silvestres tuvo un efecto claro sobre una mayor seroprevalencia para la enfermedad de Newcastle y *M. gallisepticum*. Los resultados serológicos sugieren que las parvadas de aves domésticas tienen el potencial de servir como un reservorio o amplificador para las enfermedades respiratorias de las aves. La información generada en este proyecto debe dirigir los esfuerzos de extensión para enfatizar la importancia de la bioseguridad en parvadas pequeñas y de las fuentes para la adquisición de pollos.

Key words: respiratory diseases, seroprevalence, biosecurity, backyard flock, commercial flock

Abbreviations: AI = avian influenza; CAHFS = California Animal Health and Food Safety Laboratory; CDFA = California Department of Food and Agriculture; END = exotic Newcastle disease; HPAI = highly pathogenic AI; IBV = infectious bronchitis virus; ILT = infectious laryngotracheitis; MG = *Mycoplasma gallisepticum*; MS = *Mycoplasma synoviae*; ND = Newcastle disease; NPIP = National Poultry Improvement Plan; ORT = *Ornithobacterium rhinotracheale*; RT-qPCR = reverse transcriptase quantitative polymerase chain reaction; UCCE = University of California cooperative extension; USDA = U.S. Department of Agriculture

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In the United States owning poultry as a hobby is an ever-growing trend. A survey conducted by the U.S. Department of Agriculture (USDA) showed that four of the largest cities in the United States (Los Angeles, New York, Miami, and Denver) had 0.8% of homes owning poultry in 2010 and 4% planned on owning chickens by 2015 (15). While this study shows the fast growth rate of backyard poultry ownership in large cities, this growth might be even faster in suburban areas where land is readily available. An increase in food prices, specifically eggs, and a trend of knowing food sources has been used as arguments to own backyard poultry (4). This fast-growing trend has not been accompanied by the generation of reliable resources to owners, specifically related to management, health, and biosecurity. In addition, among the poultry community, a lack of substantial studies on these matters is acknowledged (7). A popular source of information for owners continues to be nonacademic websites, which are not characterized as a reliable source for poultry information (2). This situation translates into poor biosecurity, health, and management practices when backyard flock owners raise their birds (4).

Respiratory pathogens are a common cause of disease in poultry. Poor performance, increased condemnation in meat birds, drop in egg production, and decreased quality in egg layers are common outcomes. In addition, high mortality and severe losses including trade restrictions can be associated with respiratory diseases such as exotic Newcastle disease (END) and highly pathogenic avian influenza (HPAI). The role of backyard flocks in END and HPAI has been extensively documented (1,11,12,14); e.g., the 2002 outbreak of END in California originated in a backyard flock and disseminated throughout the state, affecting commercial poultry and costing more than \$160 million in eradication and control efforts (12).

Through a program allowing backyard flock owners to submit deceased chickens for necropsy free of charge, the California Animal Health and Food Safety laboratory (CAHFS) has reported a prevalence of 13.8% of respiratory pathologies from all submitted noncommercial birds (10). Among the most common respiratory causes, the same laboratory, using ELISA for antibody detection, reported *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) antibodies as the most prevalent with 75% and 42%, respectively, followed by infectious bronchitis virus (IBV) (36%) and Newcastle disease virus (NDV) (7%) (Dr. Bruce Charlton, personal communication, 2012). Other seroprevalence studies in backyard poultry have encountered antibodies against further respiratory diseases. Using a backyard chicken database in Maryland, 39 flocks totaling 262 birds were tested using commercial ELISA antibody detection kits. Results showed 12%, 49%, and 7% of birds showing antibodies against ND, infectious laryngotracheitis (ILT), and MG, respectively (8). In another study that examined backyard poultry flocks within one mile of commercial turkey facilities, 39%, 25%, 39%, and 27% of backyard flocks tested positive for IB, MG, MS, and ND, respectively (9). In Belgium a study of 56 fancy poultry breeders found that 95.4%, 75.6%, 76.3%, 63.5%, 36.7%, and 30% of chickens tested positive for antibodies against *Ornithobacterium rhinotracheale* (ORT), IB, MS, avian metapneumovirus, MG, and ILT, respectively (7).

The purpose of this cross-sectional study was to gain a better understanding of the management and biosecurity measures that backyard poultry owners use in their backyard flocks. In addition, we examined the seroprevalence of several respiratory diseases—avian influenza (AI), ND, IB, MG, MS, ORT, and ILT—in these flocks. The distance to nearest commercial poultry operations was incorporated in

the analysis of the flock's antibody profile as a way of understanding the risk that backyard flocks pose to commercial facilities.

MATERIALS AND METHODS

Research authorization. All animal experimental procedures were approved by the University of California, Davis Institutional Animal Care and Use Committee (IACUC, approval no. 18918). The biosecurity survey was considered exempt by the University of California, Institutional Review Board.

Poultry flocks. Backyard chicken flocks were located and contacted using the California Backyard Poultry Census (http://ucanr.edu/sites/poultry/California_Poultry_Census/) created in collaboration with the University of California Cooperative Extension (UCCE). This census was distributed through the UCCE website (<http://www.ucanr.edu/sites/poultry>) and in every outreach activity performed by UCCE throughout the state of California.

Backyard poultry flocks were also found through owners referring to other owners. All surveyed and sampled flocks were in Yolo, Sonoma, Napa, Alameda, and Yuba counties in California. Visits occurred between January and September of 2016.

Commercial poultry facilities. The distance of commercial poultry facilities in relation to backyard poultry flocks was investigated using latitude and longitude coordinates obtained from databases provided by the UCCE and authorized by the CDFA. Facilities were considered commercial if they contained more than 3000 chickens. Backyard flocks were considered close to or far from commercial poultry facilities if they were less or more than four miles' distance, respectively. Distances were calculated mapping flock locations using ArcGIS software (ArcGIS, Redlands, California). The cutoff distance to determine if premises were close to or far from commercial flocks was decided based on the USDA/APHIS highly pathogenic avian influenza response zones. We considered their buffer zone (approx. 4.37 miles) as a distance where flocks were at risk of getting infected with respiratory diseases (16).

Survey. A survey was created to assess management and biosecurity of backyard chicken flocks. The survey was crafted based on a biosecurity self-assessment for commercial poultry facilities constructed during the 2015–16 avian influenza outbreak in the United States (3). It consisted of 34 questions covering management, health, and biosecurity of small flocks and was conducted to the participants by the same interviewer during our visit to their properties. The entire survey can be found at https://docs.google.com/forms/d/e/1FAIpQLSci0Jy_WZLMGwBIOZsGcNXrWq4gbH7DI1bUzP8GSHkfBDxvQ/viewform?usp=send_form.

Serological tests. Sera were obtained from blood drawn from the ulnar vein of the chickens at the visited flocks. Birds up to a maximum of 30 per flock were sampled to obtain a representative sample. Antibody titer determination was performed using commercial ELISA kits for MG, MS, IB, ND, ILT, and AI (IDEXX, Westbrook, ME) and ORT (BioCheck Scarborough, ME) following manufacturers' instructions. Optical density was measured using a spectrophotometer (BioTek Instruments, Winooski, VT). Birds with a positive AI ELISA result were oropharyngeal swabbed, and reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) was performed looking for the presence of the agent (13).

Statistical analysis. Statistical analysis was performed in R 3.2.3 using chi-squared test (R Core Team, Vienna, Austria). Significant differences were detected when $P < 0.05$.

RESULTS

A total of 41 backyard flocks were sampled totaling 554 birds. Of the 41 flocks, considering the four miles distance, 14 (255 birds)

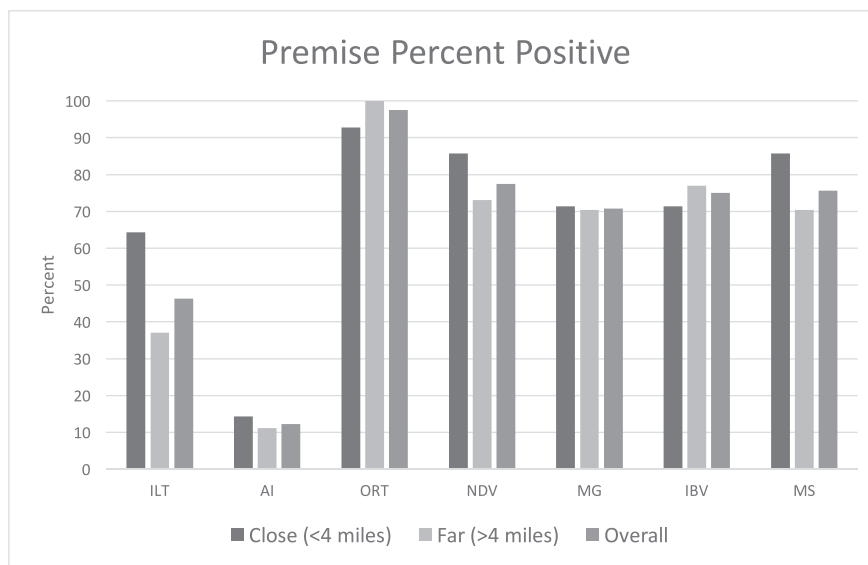


Fig. 1. Percentage of antibody positive backyard poultry flocks ($n = 41$) against different respiratory diseases. From a total of 41 flocks, 14 were close to commercial poultry and 27 far from commercial premises. Vaccinated flock was removed from this graph.

were close to a commercial poultry facility, and 27 (299 birds) were far from a commercial poultry facility. The flock sizes ranged from 2 to 400 chickens, and multiple ages were represented from 4 months to 10 years of age. The flocks consisted of many different breeds obtained from a variety of sources. One flock (30 birds), which was located far from commercial poultry, was vaccinated four times (23 and 44 days and 10 and 16 weeks) with a live attenuated IB + ND vaccine. In addition, at 10 weeks of age a live attenuated Pox-AE vaccine was given via the wing web. This flock was not considered in the seroprevalence study of IB and ND.

The owners of all sampled premises (41/41) answered the biosecurity and management survey. Results showed that 36 (88%) backyard flock owners observed contact with wild birds while five flocks did not observe contact. Twelve (29%) of the backyard flock owners used dedicated shoes for working around their birds, while 29 (71%) wore normal shoes and clothing when around their flock. Eight (20%) of the backyard flock owners obtained their birds directly from a National Poultry Improvement Plan (NPIP) - certified hatchery, 14 (34%) received their chickens from a friend, 16 (39%) of the participants bought their chickens from a feed store, two (5%) participants obtained them through humane society adoptions, and one (2%) hatched their own chickens. Fifteen (37%) of the participants have used a diagnostic lab or veterinarian to monitor the flock health and or to necropsy a deceased bird.

Antibodies against all tested respiratory pathogens were found in backyard flocks. ORT, ND, IB, MS, MG, and ILT were the most seroprevalent in backyard poultry flocks with 97.5% (40/41), 77.5% (31/40), 75% (30/40), 75.6% (29/41), 70.7% (29/41), and 46.3% (19/41), respectively (Fig. 1). Six samples from five flocks tested positive for antibodies against AI using the commercial ELISA kit. Subsequent RT-qPCR tests from oropharyngeal swabs of the same birds tested negative for presence of viral RNA.

No differences in respiratory disease antibody prevalence were found when comparing flocks close to and far from commercial poultry premises (Fig. 1). Statistical analysis based on the number of positive birds demonstrated that birds located near commercial poultry facilities were more likely to have antibodies against ND and

MG ($P < 0.05$), while chickens far from commercial poultry facilities were more likely to have antibodies against ORT ($P < 0.05$) (Fig. 2).

Serological profile of backyard chickens was associated with the survey results to demonstrate the real effect of biosecurity in respiratory disease seroprevalence. Birds purchased directly from NPIP hatcheries showed a lower ND, MG, and MS antibody prevalence ($P < 0.05$) compared with birds obtained through other sources. Wearing dedicated shoes decreased the number of MS antibody-positive birds ($P < 0.05$) compared with owners not using them. Finally, history of wild bird contact had a clear effect on a higher seroprevalence of NDV and MG ($P < 0.05$).

DISCUSSION

During the detection, surveying and sampling efforts of the 41 different small flocks we observed much variability in these systems, particularly in management. Dissimilar flock sizes, ages, breeds, management conditions, and biosecurity status reflected the complexity of the urban poultry flocks. This reassures the importance of the concept “population medicine” when doing poultry medicine and extension work with backyard poultry for anamnesis, diagnosis, prevention, and treatment, since most of the problems in these settings arise from flock management dysfunctions.

The results from the biosecurity survey portion of this study indicated that many backyard poultry owners follow poor biosecurity practices placing their flocks at risk of disease exposure. One of the most overlooked biosecurity measures is using dedicated shoes when servicing the flock. Using dedicated shoes is an easy practice that can reduce the introduction and dissemination of pathogens into or from a flock. We have previously shown that AI can stay in boot crevices even after boot disinfection using footbaths (6). This is not the first time lack of biosecurity practices have been recognized in backyard poultry flocks (11).

The bird source of these flocks is worth analyzing. While NPIP hatcheries, for the most part, ensure good breeding and hatching

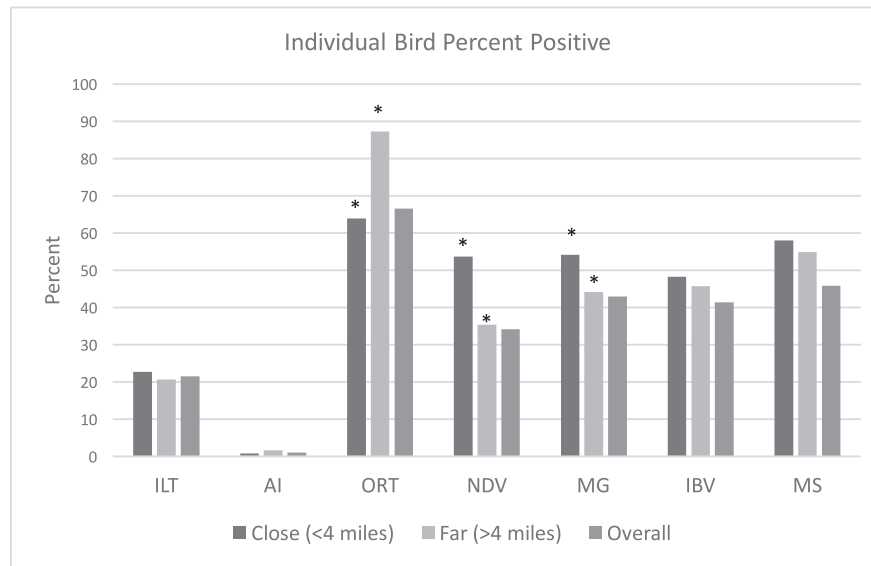


Fig. 2. Percentage of antibody positive backyard poultry birds testing positive for antibodies against respiratory diseases. From a total of 554 birds, 255 were close to commercial poultry and 299 were far from commercial premises. Vaccinated birds were removed from this graph. Asterisk indicates significant differences ($P < 0.05$).

practices, less than 20% of the birds from the surveyed flocks came from that source. A high percentage of chickens are obtained from unreliable sources such as friends (36%) or self-hatched (2.4%). These practices pose risks to the perpetuation of vertically transmitted diseases such as MG, MS, and the reappearance of diseases eradicated long ago from the poultry industry such as *Salmonella Pullorum* and *Gallinarum*. Currently, NPIP audit chick sources for H5 and H7 AI in addition to *Salmonella Pullorum* and *Gallinarum* monitoring (17). The most common chick source was feed stores (39%). If feed stores buy their chicks from NPIP-certified hatcheries, the risk of introducing pathogens to a flock can be reduced. This makes outreach and education extremely necessary especially for feed store managers, instructing them on the reasons and importance of obtaining chicks from NPIP-certified sources.

Flock owners who did not use a veterinarian or laboratory to monitor their flocks or mortality (63.4%) stated that they did not know whom to contact. The survey responses reflect the outreach need for these urban agriculture systems and confirm conclusions of work by others showing that backyard poultry owners are avid seekers of poultry resources and information (4). New poultry extension strategies should consider working in parallel with diagnostic laboratories toward promoting their service and importance among small poultry holders.

Our seroprevalence assessment showed that antibodies against all tested pathogens (ORT, IB, MS, MG, ILT, and AI) were found in the sampled backyard poultry flocks. These results are comparable with other studies in the United States and Europe that looked at the serological profile of backyard/hobby poultry. Reports from Belgian backyard poultry showed higher seroprevalence for IB, ILT, MS, and ORT on an individual bird basis (5). Madsen, after performing seroprevalence studies in Maryland, reported fewer antibody presences in backyard birds for ND and MG but many more seropositive birds for ILT and AI (7,8). Previous studies in California reported similar seroprevalence for AI, IB, ND, and MS but lower seroprevalence for MG (9). While the reviewed seroprevalence studies do not align perfectly with one another, in terms of what diseases are most prevalent, the overall conclusion

appears to be that there is a high prevalence of antibodies against respiratory pathogens in backyard poultry. In regard to the AI-positive birds it might be that those birds were exposed to avian influenza viruses through their exposure to the environment and lack of biosecurity. An ELISA test is highly sensitive to detect antibodies generated after infection; positive ELISA results will indicate prior exposure to the pathogen and will not tell exactly when the infection occurred. The RT-qPCR will detect a conserved segment of the viral genome during the acute phase of the infection, limiting the detection of the pathogen. However, it is likely that those results might be part of the error of the test (false positives), since mostly single birds in different flocks were positive and viral particles were not detected by RT-qPCR. In cases where you do not know the source of AI antibodies, the use of tests like hemagglutination inhibition are helpful to determine H5 or H7 AI exposure. Unfortunately, this testing requires the use of H5 and H7 AI viruses and needs to be performed in select agent laboratories.

Commercial poultry producers have always been concerned about the proximity of backyard flocks to their premises. We evaluated the effect of the distance to commercial poultry premises in association with seroprevalence to the above-mentioned respiratory pathogens. No significant differences were found in the number of flocks testing positive for antibodies against the respiratory pathogens if flocks were located less than four miles from a commercial poultry facility. However, when looking at individual chickens rather than flocks, chickens located near commercial poultry facilities were significantly more likely to have antibodies against ND and MG, while chickens far from commercial poultry premises were significantly more likely to have antibodies against ORT ($P < 0.05$). While seroconversion for ND and MG in chickens close to commercial premises might be related with vaccination protocols on commercial farms in the vicinity, ORT antibodies in birds far from commercial premises could be reflecting cross-reactivity with ORT like bacteria commonly present in wild birds.

An association between the chicken's serological profile and surveyed biosecurity practices was able to demonstrate that simple biosecurity measures, such as the use of dedicated shoes, can

significantly reduce disease exposure and subsequently antibody presence particularly against MS ($P < 0.05$). The same effect was found if birds were not exposed to wild birds, reducing their ND and MG prevalence ($P < 0.05$). Even though it is difficult to eliminate the interaction of wild birds and backyard birds, deterrent measures might be an option in areas where there is high risk of exotic diseases. Flocks from birds purchased from NPIP hatcheries had lower antibodies against ND, MS, and MG ($P < 0.05$) compared with chickens acquired by other means. Since MG and MS are vertically transmitted pathogens, these results highlight the role of NPIP certification in preventing the vertical transmission of those pathogens in new flocks (17). This information should direct extension efforts toward emphasizing the importance of acquiring chicks from NPIP-certified hatcheries.

Only one of the surveyed and sampled flocks had a clear vaccination history. These birds were obtained from a commercial hatchery and had veterinary oversight. These birds received a combined live attenuated IB + ND vaccine in the field four times before laying onset. This flock was resampled five months after the first sampling to estimate the effect of the environment. The second sampling data were not included in the seroprevalence analysis. In the first sampling the birds tested positive for antibodies against ORT, ND, and IB. In the second sampling the flock tested positive for the same respiratory pathogens, and, in addition, we found antibodies against ILT, MG, and MS. These results demonstrate the role of the environment in these flocks and the need of adequate biosecurity measures to prevent them from being a problem to the poultry industry.

This study detected the lack of simple biosecurity practices in small flocks in urban and periurban areas. Poultry outreach efforts need to be focused on proving to small holders how biosecurity, the use of diagnostic laboratories, and reputable chick sources will benefit the health, well-being, and productivity of small poultry flocks. In addition, the results from the serological assessment showed that small poultry flocks are clearly a good sentinel of their environment and have the potential to serve as a reservoir or amplifiers of respiratory diseases that might affect commercial poultry.

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