





The abstracts in this handbook are numbered as followed:

- 1. One Health for tackling real-world complex challenges Dirk Pfeiffer
- 2. Programme 1: People, poultry & production Guillaume Fournié
- 3. Gender in preventing and controlling infectious diseases in chicken production and distribution networks in North Vietnam Nguyen Thi Dien, Pham Thi Thu Ha, Nguyen Thi Minh Khue, Ayako Ebata
- 4. The impact of COVID-19 and the subsequent economic crisis on the poultry development and distribution networks in Western and North Western provinces in Sri Lanka <u>De Zoysa M.I.L.</u>, Silva-Fletcher A., Kalupahana R.S., Jayasekara N.K., Herath H.M.A.S., Munasinghe D.M.S.
- 5. Gender dimensions in the backyard poultry sector in Sri Lanka S.A.M.K.P. Abeykoon, H.D. Wickramapathirana, W.M.C. Kamal, H.M.D.N. Navarathne, <u>Poornika</u> <u>Seelagama</u>, Ruwani Kalupahana
- 6. Food safety threats of poultry products: linking production to consumption in India Lavinia Scudiero, Mehroosh Tak
- 7. Global capital and big poultry in South Asia <u>Mehroosh Tak</u>, Mat Hennessey, Ambarish Karamchedu and Ivo Syndicus
- 8. Value extraction dynamics and zoonotic disease risks: Roles, practices, and relations along poultry production and distribution networks in Bangladesh <u>lvo</u> <u>Syndicus, Eve Houghton</u>, Nusrat Irin, Abdullah Al Sattar, Afsana Sultana, Shariar Saif,, Meherjan Islam, Easrat Jahan Esha, Syeda Munira Dilshad, Fatema Jannat, Mahmudul Hasan, Md Helal Uddin, Jinnat Ferdous, Mahabub Alam, Md Abdus Samad, Joerg Henning, Pablo Alarcon, Guillaume Fournié,, Rashed Mahmud, Md Ahasanul Hoque, Tony Barnett
- Animal health expenditures and losses in poultry farms in Chattogram, Bangladesh <u>Fatema Jannat</u>, Ivo Syndicus, Mahabub Alam, Rashed Mahmud, Md Ahasanul Hoque[,] Pablo Alarcon
- 10. Movements of commercial poultry traders and their impact on avian influenza virus transmission in Bangladesh Jinnat Ferdous, Helal Uddin, Justine S. Gibson, Suman Das Gupta, Md Ahasanul Hoque, Fournié Guillaume, Joerg Henning
- 11. A mobility study among Chennai-based traders to understand the spatial data of procurement and delivery of broiler chickens from farm to market <u>Vigneshvaran</u> <u>Paramasivam</u>, Ganesh Janarthanan, Ivo Syndicus, Kumaravel Pappaiyan, Joerg Henning, Guillaume Fournié, B Samuel Massilomony Ronald
- 12. **Predictive gravity models of live poultry trade networks in Bangladesh** <u>Dupas</u> <u>M.C.</u>, Moyen N., Gilbert M., Fournié G.
- 13. Programme 2: Host-pathogen dynamics Damer Blake
- 14. Overview of biological field studies in farms and markets in Bangladesh Paritosh Kumar Biswas, Mahmudul Hasan, Pangkaj Kumar Dhar, Chandan Nath, Saira Butt, Lorcan Carnegie, Anne Conan, Md. Helal Uddin, Rashed Mahmud, Mohammed Abdus Samad, Md. Giasuddin, Md. Ahasanul Hoque, Damer Blake, Guillaume Fournié, Fiona Tomley
- 15. Prevalence of avian influenza A(H5) and A(H9) viruses in broiler endpoints and farms in northern Việt Nam <u>Mathew Hennessey</u>, Thuy Hoang Thi, Anne Conan, Younjung Kim, Hoa Thi Thanh Pham, Pham Thi Ngoc, Bui Nghia Vuong, Ashley Banyard, Ian H. Brown, Tom Lewis, Joe James, Josh Lynton-Jenkins, Nicola Lewis, Damer Blake, Fiona Tomley, Lorraine Chapot, Guillaume Fournié

- 16. The genetic and antigenic diversities of H9N2 avian influenza viruses infecting poultry in Bangladesh <u>Rebecca Daines</u>, Toby Carter, Jean-Remy Sadeyen, Fiona Tomley, Munir Iqbal, Bangladesh team of One Health Poultry Hub Consortium.
- 17. Transmission dynamics of H9N2 avian influenza virus in a live-bBird market in Chattogram, Bangladesh Francesco Pinotti, Lisa Kohnle, José Lourenço, Sunetra Gupta, Md. Ahasanul Hoque, Rashed Mahmud, Paritosh Biswas, Dirk Pfeiffer, Guillaume Fournié
- 18. Drivers of avian influenza virus movement within a city: preliminary insights from Dhaka, Bangladesh Jayna Raghwani, Marie-Cécile Dupas, Francesco Pinotti, Guillaume Fournié, APHA, One Health Poultry Hub
- Prevalence of antimicrobial residues in broiler meat in Vietnam, Bangladesh and India Pelligand L., Seow K., Butt S., Thuy H.T., Huong L.Q., Son D.T., Ngoc P.T., Hoa P.T.T., Conan A., Golaviya A., Paleja H., Koringa P., Hasan M., Samad M.A., Roy R.K., Uddin M.H., Dhar P., Mahmud R., Islam S.K.M.A., Biswas P.K., Hoque M.A., Cheah Y.C., Zhang P., Wang Y., Blake D., Hurtaud-Pessel D., Conway P., Fournié G., Tomley F.
- 20. Study on trend of antibiotic resistance of indicator bacteria *E. coli* during production cycle In Vietnam's chicken farms Truong Thi Quy Duong, Tran Thi Nhat, Truong Thi Huong Giang, Vu Thi Kim Hue, <u>Dang Thi Thanh Son</u>*, and the GCRF One Health Poultry Hub team
- 21. Campylobacter isolated from LMIC poultry production networks show reginal links to antimicrobial resistance and novel *C. coli* clusters <u>Burhan Lehri</u>, Richard Stabler
- 22. Diarrheal disease in children in a rural Indian setting incidence, causes and attributable risks from poultry using a One Health approach <u>Reshma Raju</u>, Rohan Michael Ramesh, Selvakumar Prasad, Zayina Zondervenni Manoharan, Gowthaman Vasudevan, Alagesan Alagersamy, Balakrishnan A, Rajeshkumar Rajendiran, Dhanalakshmi Solaimalai, Guillaume Fournié, Fiona Tomley, Balaji Veeraraghavan, Sitara Swarna Rao Ajjampur
- 23. Insights into comprehensive antimicrobial resistance pattern of poultry caeca in Gujarat, India Khushbu Rana, Ankit Hinsu, Akash Golaviya, Ketankumar Panchal, Bhoomika Joshi, Subhas Jakhesara, Haidaruliman Paleja, Damer Blake, Fiona Tomley, Prakash Koringa
- 24. Microbiome diversity and antibiotic resistance gene distribution in poultry and vegetable shop workers: Tales from Gujarat and Tamil Nadu <u>Chavan Monica</u>, Sadik Dantroliya, Rameshchandra Pandit, Chaitanya Joshi, Sitara Ajjampur, Madhvi Joshi, Damer Blake, Fiona Tomley
- 25. Genetic characterisation of indigenous chicken ecotypes from India and Bangladesh Ankit Hinsu, Prakash Koringa, Md Ahasanul Hoque, Hoa Thi Tham Pham, Anne Conan, One Health Poultry Hub consortium^{11,10}, Guillaume Fournié¹, Damer Blake, Fiona Tomley, Androniki Psifidi
- 26. Policy engagement: supporting improved policy design and implementation Robyn Alders
- 27. Animal slaughter, wet markets and food safety in the context of rapid economic transformation: how can Vietnam make animal slaughter safer? Ayako Ebata, <u>Dinh Ton Vu</u>
- 28. Mapping existing animal industry-related policies and developing a poultry policy for Sri Lanka; towards a sustainable and competitive poultry industry

<u>Chamari Kannangara</u>, Palika Fernando, Doreen Anene, Robyn Alders and <u>Ruwani</u> <u>Kalupahana</u>

- 29. One Health, multiple impacts: a review of 10 years of One Health work in Bangladesh and the region with a focus on the poultry industry in Bangladesh Esha E.J., Mahmud R., Alders R., Hoque M.A.
- 30. Poultry development policies in Bangladesh: Stakeholders practices, challenges and way forward Syeda Munira Dilshad, Rashed Mahmud[,] Robyn Alders, Md. Ahasanul Hoque
- 31. Contribution analysis of complex research programmes: the case of the One Health Poultry Hub Giel Ton, <u>Syed Abbas</u>, Gerry Bloom, Keir Mcdonald, Daan Vink, Fiona Tomley
- 32. Programme 4b: Training and evaluation Ayona Silva-Fletcher
- 33. Comparison between biosecurity practices on commercial broiler and layer farms in Gujarat <u>Khushbu Rana</u>, Akash Golaviya, Bhoomika Joshi, Priyal Dakhore, Prakash Koringa, Ayona Silva-Fletcher, Fiona Tomley, Haidaruliman Paleja
- 34. Pre-post training evaluation analysis on biosecurity awareness of small-scale backyard poultry farmers in Sri Lanka <u>Ihalage D.</u>, Silva- Fletcher A. Satharasinghe S. and Kalupahana R.S.
- 35. Assessment of farm profiles, knowledge gain and adaptation behaviors of biosecurity practices in small and medium scale commercial poultry farms in Western agro-climatic zones of Tamil Nadu, India <u>Alagarsamy Alagesan</u>, Arumugam Balakrishnan, Vigneshwaran Paramasivam, Ganesh Janarthanam, Vasudevan Gowthaman, Kumaravel Papaiyan, Thippichettipalayam Ramasamy Gopala Krishna Murthy, Ayona Silva Fletcher, Fiona Tomley, Samuel Masilamoni Ronald
- 36. **Biosecurity in poultry farms: How to change production behaviors** <u>Pham Thi</u> <u>Thanh Hoa</u>, Truong Thi Duong, Nguyen Thi Lien Huong
- 37. Sensitizing molecular researchers towards the threat of antimicrobial resistance (AMR) by increasing knowledge and skills through training programs <u>Jakhesara S</u>., Koringa P., Joshi M., Paleja H., Silva-Fletcher A.
- 38. Evaluation of poultry farmers' knowledge, attitude, and practices regarding production and biosecurity in poultry farming in Bangladesh Meherjan Islam, Easrat Jahan Esha, Syeda Munira Dilshad, Md. Ershadul Haque, Rashed Mahmud, Ayona Silva-Fletcher, Md. Ahasanul Hoque
- 39. Qualitative social sciences at the Hub: results and legacy for future research <u>lvo</u> <u>Syndicus</u> and the One Health Poultry Hub social science teams
- 40. Concurrent study of poultry enterotypes and resistomes may enable the detection of acquired antimicrobial resistance <u>Melanie C. Hay</u>, Ankit Hinsu, Khushbu Rana, Ramesh Pandit, Chaitanya Joshi, Madhvi Joshi, Sophie Hedges, Saira Butt, Haidaruliman Paleja, Burhan Lehri, Dong Xia, Prakash Koringa, Androniki Psifidi, Ludovic Pelligand, Guillaume Fournié, Fiona M. Tomley, Richard Stabler, Patricia Conway, Damer P. Blake and the One Health Poultry Hub Consortium
- 41. Risks of human spillover infections Punam Mangtani
- 42. Poultry farming livelihood strategies and their relevance for zoonotic disease management in Northern Viet Nam Eve Houghton, Nguyen Minh Thi Khue, Tony Barnett, Ivo Syndicus and Nguyen Thi Dien
- 43. Business models and dynamics of change among Chennai's broiler wholesalers: an ethnographic exploration of live bird distribution networks <u>lvo</u>

<u>Syndicus</u>, Vigneshvaran Paramasivam, Ganesh Janarthanan, Eve Houghton, Pallavi Mishra, Kavita Yadav, Vimal Rajkumar, Rajib Dasgupta, Tony Barnett, Kumaravel Papaiyan, Guillaume Fournié[,]

- 44. The research mobility Tracking App Joerg Henning
- 45. SWOT analysis on the current status of family poultry vaccination program implemented in the western province of Sri Lanka Ananda G.V.T., Sathsarani D.M.Y., Kalupahana R.S., Satharasinghe S., Kalupahana A.W., Alders R.
- 46. Spatial distribution of poultry farms using point pattern modelling: a methodology to address livestock environmental impacts and disease transmission risks <u>Dupas M.C.</u>, Pinotti F., Joshi C., Joshi M., Vink D., Tomley F., Gilbert M., Fournié G.
- 47. Exploring motivations of poultry farmers for antimicrobial use in Bangladesh: A qualitative investigation Abdullah Al Sattar, <u>Ivo Syndicus</u>, Nusrat Irin, Md. Helal Uddin, Shariful Islam, Fatema Jannat, Chandan Nath, <u>Eve Houghton</u>, Guillaume Fournié, Md. Ahasanul Hoque
- 48. Selling and management process of sick birds: a qualitative investigation Nusrat Irin, Abdullah Al Sattar, <u>Ivo Syndicus</u>, Anne Conan, Guillaume Fournié, Md. Ahasanul Hoque
- 49. **Spatial traceable maps and management of live bird transportation along chicken value chains** <u>Nguyen Van Dai</u>, Le Thi Thanh Huyen, Pham Thi Thanh Hoa, Han Anh Tuan, Do Van Duc, Dao Thi Binh An, Le Tien Dung, Duong Thi Oanh, Nguyen Thi Phuong Anh, Nguyen The Vinh, Vu Dinh Ngoan, Ta Van Can, Syndycus Ivo, Houghton Eve, Henming Joerg, Fiona Tomley and Guillaume Fournié
- 50. H9N2 avian influenza virus: emergence of immune escape mutant with no haemagglutination activity Thusitha K. Karunarathna, Jean-Remy Sadeyen, Sushant Bhat, Pengxiang Chang, Jiayun Yang, Mehnaz Qureshi, Joshua E. Sealy, Rebecca Daines and Munir Iqbal
- 51. Non-typhoidal Salmonella in humans in India, Vietnam, Bangladesh and Sri Lanka: A systematic review Reshma Raju, Luke O'Neil, Burhan Lehri, Charlotte Kerr, Anne Conan, Jane Falconer, Guillaume Fournié, Nguyen Dong Tu, Sudipta Sarkar, Twinkle Soni, Tran Thi Mai Hung, Fiona Tomley, Damer Blake, Sitara Swarna Rao Ajjampur, Patrick Nguipdop-Djomo, Punam Mangtani, <u>Richard Stabler</u>
- 52. A description of the disease-associated morbidity and mortality in semiintensive chicken farms in Bangladesh, India, and Vietnam Chun Ting Lam, Ying Pei Zhang, Yu Jie Ai, Xin Chen, Guillaume Fournié, Anne Conan
- 53. Longitudinal study of antimicrobial residue in broiler meat in Vietnamese and Bangladeshi farms <u>Pelligand L</u>, Seow K., Butt S., Thuy H.T., Huong L.Q., Son D.T., Ngoc P.T., Hoa P.T.T., Conan A., Hasan M., Samad M.A., Roy R.K., Uddin M.H., Dhar P., Mahmud R., Islam S.K.M.A., Biswas P.K., Hoque M.A., Cheah Y.C., Zhang P., Wang Y., Blake D., Hurtaud-Pessel D., Conway P., Fournié G., Tomley F.
- 54. Genotypic characterization of antimicrobial resistance in non-typhoidal *Salmonella* poultry isolates using multilocus sequence typing Harith Rosh, Elamurugan Appavoo, Kumar K.S., <u>Ronald B.S.M.</u>, Jobin John Jacob, Kirubaharan J.J., Tirumurugaan K.G., Balaji Veeraghavan, Vidhya Mahalingam
- 55. Occurrence and antimicrobial resistance in zoonotic food-borne bacterial pathogens isolated from broiler farms and live bird shops in Tamil Nadu, India Arumugam Balakrishnan, M. Anandhachitra, B.S.M. Ronald, Alagarsamy Alagesan,,

Vasudevan Gowthaman, T.R. Gopala Krishna Murthy, Saravanan Gunaseelan, Damer Blake, Fiona Tomley

- 56. Genomic profiling of antimicrobial resistance in poultry litter bacterial community Animesh Tripathi, Anjali Jaiswal, Dinesh Kumar, Madhvi Joshi, Chaitanya G Joshi, Damer Blake, Fiona Tomley, and <u>Suresh Kumar Dubey</u>
- 57. Endophytic bacterial community reveals antimicrobial resistance in response to poultry-manure application Animesh Tripathi, Anjali Jaiswal Dinesh Kumar, Priyank Chavda, Ramesh Pandit, Madhvi Joshi, Damer P. Blake, Fiona M. Tomley, Chaitanya

G. Joshi, <u>Suresh Kumar Dubey</u>

- 58. The prevalence of poultry-related foodborne pathogens along the farm-to-fork continuum in the poultry industry in Sri Lanka Gayani weerasooriya, M.A.R. Priyantha Damer Blake, Guillaume Fournié, Nilukshi Liyanagunawardena, P.S. Fernando, P.S. de Alwis, Sadun Bandara, K.R.P.S.Premarathne, H.M. Madushi Thilakshika, P.A. Udeshika Sewwandi, Hemal Rasanjana Peiris, Ruwani Kalupahana
- 59. Prevalence of zoonotic food-borne pathogens across commercial broiler farms and live bird shops in South India Vasudevan Gowthaman, Murugesan Ananda Chitra, B.S.M. Ronald, , Alagarsamy Alagesan, Arumugam Balakrishnan Muthusamy Raman, Vijayasanthi, Ramalingam, T.R. Gopala Krishna Murthy, Damer Blake, Guillaume Fournié, Fiona Tomley
- 60. Colonization of zoonotic bacterial pathogens and associated resistance in village chickens in Sri Lanka Kavindya Gaveshani, Udeshika Sewwandi, Madushi Thilakshika, Roshan Abayawansa, Sanda Kottawatta, Ruwani Kalupahana
- 61. Seroprevalence of SARS-CoV-2 in live poultry market workers in Dhaka, Bangladesh in 2022 <u>Mahbubur Rahman</u>, Ahmed Nawsher Alam, <u>Sudipta Sarkar</u>, Manjur Hossain Khan Punam Mangtani, Saira Butt, Anne Conan, Damer Blake, Fiona Tomley, Guillaume Fournié, Tahmina Shirin, Patrick Nguipdop-Djomo on behalf of the One Health Poultry Hub team
- 62. The use of nanopore sequencing to identify plasmid-borne and chromosomally integrated antimicrobial genes from chicken microbiomes in Gujarat <u>Melanie</u> <u>C. Hay</u>, Ankit Hinsu, Haidaruliman Paleja, Chaitanya Joshi, Madhvi Joshi, Burhan Lehri, Dong Xia, Fiona M. Tomley, Richard Stabler, Patricia Conway, Prakash Koringa, Damer P. Blake and the One Health Poultry Hub Consortium
- 63. Exploring an establishment chronicles of microbial community and antimicrobial resistance genes in broilers through high-throughput sequencing <u>Prakash G. Koringa</u>, Ketankumar J. Panchal, Ankit T. Hinsu, Akash V. Golaviya, Haidaruliman Paleja, Rais M. Rajpura, Rafyuddin. A. Mathakiya, Subhash J. Jakhesara, Damer Blake, Fiona Tomley
- 64. Investigating the presence of stalk directed avian influenza antibodies in highly exposed occupational workers in live bird markets in Bangladesh Rebecca <u>Daines</u>, Patrick Nguipdop-Djomo, Mahbubur Rahman, Sudipta Sarkar[,] Ahmed Nawsher Alam, Tahmina Shirin, Saira Butt, Anne Conan, Guillaume Fournié, Munir Iqbal, Punam Mangtani, on behalf of the One Health Poultry Hub consortium.
- 65. Mapping antigenic diversity to investigate putative antigenic residues of H5 avian influenza viruses <u>Rebecca Daines</u>, Jean-Remy Sadeyen, Pengxiang Chang, Nicola Lewis, Munir Iqbal
- 66. *Campylobacter* and non-typhoidal *Salmonella* infection among chicken shop workers in Tamil Nadu, India <u>Rohan Michael Ramesh</u>, Reshma Raju, Venkateshprabhu Janakaraj, Selvakumar Prasad, Zayina Zondervenni Manoharan,

Rajeshkumar, Gowthaman Vasudevan, Alagesan Alagersamy, Dhanalakshmi Solaimalai, Balaji Veeraraghavan, Sitara Swarna Rao Ajjampur

- 67. Estimating the prevalence of avian influenza in chickens in Bangladesh Pangkaj Kumar Dhar, Mahmudul Hasan, <u>Saira Butt</u>, Anne Conan, Md. Helal Uddin, Rashed Mahmud, Mohammed Abdus Samad, Md. Ahasanul Hoque, Paritosh Kumar Biswas, Damer Blake, Guillaume Fournié, Fiona Tomley
- 68. Conjugation mediated transfer of Extended Spectrum of β-lactamases resistance among poultry enterobacteriaceae isolated from health native chicken Indrajith Saravanan, Harith Rosh, <u>B.S.M.Ronald</u>, Vidhya Mahalingam, Muthukrishnan Madhanmohan, Manimaran K., John Kirubaharan J., Elamurugan Appavoo
- 69. Virulence profiling to differentiate avian pathogenic and avian fecal *Escherichia coli* in poultry Sumathra Mani, Elamurugan Appavoo⁻, <u>B.S.M. Ronald</u>, Vijayarani K., Srithar A.
- 70. Quantifying the use of antimicrobials across the poultry production system in South and Southeast Asia Sophie Hedges, Guillaume Fournié, Damer Blake, José Lourenço, Ludovic Pelligand
- 71. Air sample contamination with avian influenza (A/H5N1 and A/H9N2) in live poultry markets in Dhaka, Bangladesh, and its association with market and stalllevel characteristics: an ecological analysis S. Sarkar, P. Nguipdop-Djomo, I. Muntasir, L. Carnegie, S. Butt, A. Conan, A. Nawsher, T. Shirin, M. Hasan, A. Samad, G. Fournié, M. Rahman, P. Mangtani
- 72. Prevalence of *Campylobacter* and non-typhoidal *Salmonella* in chicken in Bangladesh Mahmudul Hasan, Keya Ghosh, Tahia Ahmed Logno, Pangkaj Kumar Dhar, Saira Butt, Anne Conan, Md. Helal Uddin, Rashed Mahmud, Mohammed Abdus Samad, Md. Ahasanul Hoque, Damer Blake, Guillaume Fournié, Paritosh Kumar Biswas, Fiona Tomley
- 73. Spatial and temporal distribution patterns of avian influenza in live bird markets near migratory and non-migratory birds population in Bangladesh <u>Hasnine I.,</u> <u>Uddin M.H., Dhar PK, Logno T.A., Ghosh K., Mahmud R., Chakraborty P., Biswas</u> <u>P.K., Chakraborty P., Carnegie L., Fournié G., Hoque M.A</u>.
- 74. A longitudinal epidemiological investigation on poultry pathogens and farm biosecurity standard in and Sonali chicken production in Chattogram, Bangladesh Saima Akter, Shariful Islam, Md. Helal Uddin, Chandan Nath,Pangkaj KumarDhar, Tahia Ahmed, Keya Ghosh, Rashed Mahmud, Guillaume Fournié, Paritosh Kumar, Anne Conanand Md. Ahasanul Hoque
- 75. Investigation of risk factor for avian influenza virus contamination of live bird markets in Chattogram, Bangladesh Md. Helal Uddin, Anne Conan, Saira Butt, Pangkaj Kumar Dhar, Mahmudul Hasan, Angus Lam, Jinnat Ferdous, Rashed Mahmud, Paritosh Kumar Biswas, Guillaume Fournié[,], Md. Ahasanul Hoque
- 76. Understanding prevalence of key food borne pathogens in selected poultry distribution networks from Gujarat-India Sadik Dantroliya, Monica Chavan, Satyamitra Shekh, Chaitanya Joshi, Madhvi Joshi, Damer Blake[,] Fiona Tomley
- 77. Antimicrobial use patterns in broiler & sonali poultry production in Chattogram, Bangladesh Shariful Islam, Saima Akter, Fatema Jannat, Md. Helal Uddin, Pangkaj Kumar Dhar, Tahia Ahmed, Keya Ghosh, Rashed Mahmud, Guillaume Fournié, Paritosh Kumar, Pankaj Chakraborty and Md. Ahasanul Hoque

- 78. Opening the black-box of multisector partnerships: A theory of action for implementing One Health at country level Syed Shahid Abbas, Gerry Bloom, Lewis Husain
- 79. Exploring export potential: Can Sri Lanka export chicken and related products <u>Dilan Satharasinghe</u>, Ayona Silva-Fletcher, Kaundika Wanigasundara, Dananjaya Karunarathne, Eranda Abeywickrama, Nalin de Silva and Ruwani Kalupahana
- 80. Mind the gap: An assessment of scope for collaboration between public and private veterinary services for effective disease management in Bangladesh's poultry sector <u>Meherjan Islam</u>, Eve Houghton, Ivo Syndicus, Robyn Alders, Rashed Mahmud, Md. Ahasanul Hoque
- 81. Capacity enhancement on biosecurity in poultry farming for line supervisors in contractual commercial broiler farming across Gujarat <u>Binal Prajapati</u>, Akash Golaviya, Khushbu Rana, Prakash Koringa, Fiona Tomley, Ayona Silva-Fletcher, Haidaruliman Paleja
- 82. Community involved biosecurity model: A new approach to reduce infectious diseases including avian influenza in small-scale poultry farming Md. Giasuddin, Mahmudul Hasan
- 83. Flock fortress: Bio-security practices by backyard poultry keepers <u>Shivangi</u> <u>Patel</u>, Akash Golaviya, Khushbu Rana, Bhoomika Joshi, Prakash Koringa, Fiona Tomley, Ayona Silva-Fletcher, Haidaruliman Paleja
- 84. Investigating biosecurity protocols in commercial broiler poultry farms in Gujarat Bhoomika Joshi, Khushbu Rana, Akash Golaviya, Prakash Koringa, Ayona Silva-Fletcher, Fiona Tomley, Haidaruliman Paleja
- 85. Effectiveness of on-site training on good management practices for poultry transporters in hygienic handling and transport of poultry <u>Kumaravel P.</u>, Ganesh J., Vigneshvaran P. Senthilkumar G., Vimal Rajkumar N., B. Samuel Masilamoni Ronald
- 86. Impact assessment of poultry farmer's training regarding poultry rearing and farm biosecurity measures in Bangladesh Meherjan Islam, Rashed Mahmud, Ayona Silva-Fletcher, Md. Ahasanul Hoque
- 87. Examining the influence of training on biosecurity: A comparative analysis of biosecurity scoring between commercial layer and broiler farms in Gujarat, India <u>Akash Golaviya</u>, Khushbu Rana, Bhoomika Joshi, Prakash Koringa, Fiona Tomley, Ayona Silva-Fletcher, Haidaruliman Paleja
- 88. Training on food safety and hygienic meat practices as a tool for empowering poultry meat handling butchers in Chennai Ganesh Janarthanan, Vigneshvaran Paramasivam, Kumaravel Papaiyan, Azhahianambi Palavesam and Aravind Babu Ramasamy Parthiban
- 89. **Biosecurity approaches in commercial layer poultry farming: A Gujarat perspective** <u>Priyal Dakhore,</u> Bhoomika Joshi, Khushbu Rana, Akash Golaviya, Prakash Koringa, Ayona Silva-Fletcher, Fiona Tomley, Haidaruliman Paleja

Cover images

Top – Tony Barnett **Middle** – Ramdas Aswale / Pexels (pexels.com/photo/chickens-in-a-coop-12995535/) **Bottom** – TANUVAS

One Health for Tackling Real-World Complex Challenges

Dirk Pfeiffer

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The global community is experiencing a range of highly complex challenges, including geopolitical risks, climate change, reduction in biodiversity and pandemic risk. Many of these risks are interrelated but tend to be managed separately and, therefore, inefficiently.

The One Health approach aims to encourage integrated research that is linked to an effective science-policy interface. The transition from science to policy being primarily conducted within disciplines or sectors proves to be very challenging for all stakeholders involved. The COVID-19 pandemic demonstrated the essential importance of outbreak response policies that are based on true integration of knowledge from a wide range of areas, not only science. The importance of effective stakeholder engagement has been demonstrated, as well as how easy it is to lose public trust.

The communication between scientists and policymakers has often been dominated by pathogen specialists, thereby biasing the weight of different types of scientific evidence such that policymakers were unable to make decisions based on a holistic consideration of all relevant evidence. An important lesson for countries has been that the ethical aspect of decision-making needs to be emphasized more by consulting appropriate specialist expertise. But it is also important to realise that for One Health knowledge to be translated into good policies it will require development of appropriate competencies not only among scientists but also among policymakers.

The One Health Poultry Hub has addressed several the above challenges. It has benefited immensely from the collaboration of scientists and policymakers from the four project countries, with each of them representing particular epidemiological and socio-cultural contexts, as well as having their own political economy.

Programme 1: People, Poultry and Production

Guillaume Fournié

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This presentation will provide a brief overview of the activities that have been conducted as part of Programme 1 (P1).

P1's core objective was to assess how the production and distribution of chickens was operated in each study area. The mapping of production and distribution networks (PDNs) and link tracing studies conducted at the project onset informed the selection of the network segments, and actual sites, in which the first round of biological and ethnographic investigations took place. Detailed ethnography aimed to characterise the roles and interactions, such as power relations, amongst actors operating within, or even shaping, the PDNs of interest, including the trajectory of poultry and other commodities through those networks.

A second round of social science studies was then launched. Their scopes and methods were diverse and were developed in synergy with or independently from biological studies. While most built on insights generated by the first round, others aimed, for instance, to explore the impact of external shocks on PDN configurations, or the role of global capital in shaping the industry. In parallel, field studies aiming to characterise finely the spatial mobility of traders were implemented, and statistical models simulating the spatial distributions of farms and flows of chickens from production to consumption sites were developed.



Programme 1: People, poultry & production

Guillaume Fournie - National Research Institute for Agriculture, Food and the Environment (INRAE), France; RVC, UK.

- Core hypothesis: Configurations of production and distribution networks (PDNs)
 - influence the transmission and evolutionary dynamics of zoonotic hazards
 - result from social and economic contexts within which stakeholders operate
- Aim: To describe PDN configurations across a spectrum of intensification and production types
 - Roles and interactions along PDNs
 - Rounds 1 & 2
 - Consumers' demand and preferences
 - External shocks
 - Global capital and industrialisation
 - Chicken population dynamics & actors' mobility
 - Spatial structures

Gender in preventing and controlling infectious diseases in chicken production and distribution networks in North Vietnam

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Women working in chicken production and distribution networks in Vietnam practice their activities differently from men. Their activities and behaviours are shaped by gender – the social constructed roles, behaviors, and identities that are shaped by cultural norms and imposed by their biological sex. Exposure to diseases including SARS, avian influenza and other zoonoses threatening human health that Vietnam has experienced are linked to gender-based practices and place men and women at different levels of health risks, potentially driving them towards helpful or harmful behaviors. However, gender is often neglected in public health responses and in formulating policies and interventions for disease prevention and control.

Based on a gender in One Health approach, this research analyses gendered practices in the production and distribution of chicken that affect health risks and provides gender sensitive policy suggestions and interventions to mitigate the health risks. It is based on 6 focus group discussions, 30 key informant interviews and a survey of 132 stakeholders in chicken production and distribution in Northern Vietnam carried out between June 2022 and June 2023.

Results show that labour division in chicken farming and trading show the gender stereotypes in which most men run large-scale businesses with high investment and women participate much more in small and medium businesses. In access to resources, 96.8% of men and 79.2% of women have ownership over land, and 39.3% men compared to 17.3% women own a car or truck. Women showed their strengths as owners of good practices that enhance biosecurity, using alternatives to antibiotics and ensuring food safety.

The research suggests that it is necessary to improve law enforcement and administrative procedures on land entitlement to increase opportunities for women to access land and practice their land rights, especially to avoid risks when marriage breaks down. The interventions to enhance female status should recognize the reality of gender negotiation within the Vietnamese family. Supporting small-scale chicken businesses to have greater participation of women, higher levels of alternative medicine to antibiotics and drugs, better biosecurity and control of health risks could have dual results in terms of female empowerment and disease prevention and control. Better labour protection tools and improve the health status of actors working in the market; also regulating slaughtering conditions to ensure food safety and avoid the health risks for both men and women is necessary.

The Impact of COVID-19 and the subsequent economic crisis on the poultry development and distribution networks in western and north western provinces in Sri Lanka

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The poultry production network in Sri Lanka which consists of broiler, layer and backyard chickens plays an important role in providing financial and nutritional security to people. This sector underwent a crisis due to the restrictions that emerged with the COVID-19 pandemic and the ensuing economic crisis and this qualitative research was carried out to analyze the impact of the above variables on the poultry sector in Sri Lanka.

Seventy two participants (59 males and 13 females) from Western and North Western provinces where poultry density is the highest were interviewed using a pre-determined set of questions. The recorded responses were transcribed and analyzed using critical content analysis.

Data analysis showed that the poultry sector which went into an initial crisis with the onset of the COVID-19 pandemic managed to lift its operations. Later, the ensuing economic crisis which was exacerbated due to Covid-19 caused a depreciation in the Sri Lankan rupee and very low foreign reserves. As a result, the poultry industry faced economic difficulties. The significant effects were due to high cost of imported raw materials to formulate feed and sourcing of day-old chicks. The lack of fuel disrupted transportation networks and the lack of electricity impacted the production network adversely. The local industries became import dependent on feed resources and vulnerable. As recommendations, it is suggested that efforts to produce major poultry feed ingredients such as maize/ corn and soya locally is essential to mitigate such food shortage in the future for the economic sustainability of the poultry industry.

Gender Dimensions in the Backyard Poultry Sector in Sri Lanka

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Many households in Sri Lanka depend on backyard poultry production for both income and nutrition. This study aimed to explore gender roles associated with backyard poultry farming with the specific objectives of to document the key practices which influence health risks and their association with gender, region and ethnicity. The study employed secondary data and primary data collected through ethnographic methods such as semi-structured interviews and field observations. Households raising backyard chicken in Northern (N), North Western (NW), and Eastern (E) provinces were selected using the snowball sampling method. Fifteen women and fifteen men from 27 farms were interviewed for the purpose of this study. Data was analysed using thematic analysis on the MAXQDA software.

The study found that backyard farms exhibit a significant gender-based division of labour in North Western and Eastern Provinces. Men perform the heavy work, while women performed care-giving tasks and tasks that require less physical strength. However, in the Northern Province women not only performed strenuous tasks but they were also more independent in decision-making and financial management. The study found laxity towards health and hygiene in all provinces regardless of gender or ethnicity. There was considerable reliance on traditional treatment methods, informal purchasing of medicine over the counter and consulting of fellow farmers in the case of illness of birds.

The study concluded that gendered division of labour is practised among Sinhala and Muslim communities whereas Tamil women were independent in many senses due to their preexposure to war and hardship. Hygienic practices were poor regardless of gender or ethnicity in all three provinces.

Food safety threats of poultry products: linking production to consumption in India

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In India, the rapidly evolving poultry production systems and the surging demand for poultry products have highlighted critical concerns regarding food safety and infectious diseases emerging from poultry birds. Among the most pressing challenges facing the poultry industry in India are outbreaks of avian influenza and the widespread misuse of antimicrobials. Despite significant concerns and the industry's claimed adherence to international food safety standards, the Indian poultry sector grapples with these critical issues. While Indian consumers are increasingly conscious of the potential health hazards associated with intesively produced poultry, there exists a gap in the understanding of how these health risks are perceived and how they shape change toward practice for safer production/consumption.

This research investigates the linkages between consumers and producers to understand practices for disease transmission. Using quantitative and qualitative data, including 15 stakeholder interviews, consumer focus group discussions (11 male, 11 female) and 600 intrahousehold surveys in Chennai, Tamil Nadu, the research introduces an innovative methodology, highlighting diverse factors influencing poultry health risks transmission.

The study unveils the intricate interplay between poultry biological factors, inadequate rearing and distribution practices, and food health risk messages within poultry food chains, highlighting the precarious dynamics of poultry production and consumption and relative health threat outcomes. This innovative approach enhances the understanding of disease tramision dynamics to consumers within poultry systems and provides a valuable tool to guide development of effective interventions aimed at safeguarding both food secuirty and safety.

Food safety threats of poultry products: linking production to consumption in India

Urban intensive poultry provisioning creates convenient consumption

Increased consumption

Perceived health impacts of poultry provisioning influence diverse consumer choice

Poultry safety and quality

Traditional perceptions adjust to changes in intensive poultry provisioning

Intensive poultry provisioning gives rise to new health risk perceptions

Provisioning and social dynamics result in cautious consumption patterns across different groups

Unequal consumption (safety & quantity)

Global Capital and Big Poultry in South Asia

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The flow of global capital administered by corporate regimes and international financial institutions has created asymmetries of power and increased corporate concentration, shifting the way animal-sourced foods are produced and consumed, giving rise to "meatification of diets". This paper takes the example of poultry as a livestock commodity to show case how global capital flows have transformed the poultry industry globally, followed by the case of India and Bangladesh. The paper aims to answer the question, "Is there any thread leading from financial markets and/or neoliberal policies to the stomach?".

We do so by exploring (1) the role of international finance and liberalisation in the rise of industrial poultry production, and (2) how the global poultry industry facilitates corporatisation of the Indian and Bangladeshi poultry sector. We theoretically and empirically articulate the power laden financial linkages and disjuncture between global capital and South Asian rise in chicken production as being enabled by a set of variegated and incoherent neoliberalising processes of state absence and policy restructuring, co-constituted between national and state governments and corporate poultry agribusiness giants.

We argue that industrial chicken production systems are justified and propagated by both private and public sector stakeholders around what we call two key "do good" narratives, first of solving malnutrition and second of improving rural livelihoods.

We find that the gains from increasing productivity per unit through industrial chicken production practices are insufficient in addressing the stated "do good" objectives as externalised costs of labour exploitation, disease risk and food insecurity created from the industrialised production process, in turn disproportionately impacts the very populations facing malnutrition and livelihood insecurity.



Value extraction dynamics and zoonotic disease risks: Roles, practices, and relations along poultry production and distribution networks in Bangladesh

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Epidemiological research can identify configurations of poultry production and distribution networks (PDNs) that pose increased zoonotic risks. Changing these configurations through interventions, however, requires understanding PDN actors' practices within their broader social and economic contexts.

We carried out ~200 in-depth qualitative interviews with farmers, feed dealers, veterinarians, live bird traders, and retailers in Bangladesh to better understand their roles, practices, and relations to other actors (all male excepting 3 female veterinarians and 2 female backyard farmers). Hub researchers monitored traders' movements and their poultry transactions during 87 trips and conducted gross margin analysis associated with 60 batch production cycles.

The study reveals how actors' practices are constrained through specific interdependencies and limited capital of those handling poultry. Many farmers rely on production inputs (day-oldchicks, poultry feed, medication) to be provided on credit by feed dealers – who have specialised in supplying these. Some poultry companies started to work directly with farmers, advancing production inputs through contract-farming schemes and offering more stable poultry prices than the volatile market rates. Farmers' limited capital and access to inputs on credit, for example, favours medicine use over structural and operational biosecurity investments. Poultry wholesale traders and market retailers, in turn, often operate through delayed payments, paying upstream suppliers of poultry after its downstream sale. Besides limited capital, delayed payment is linked to price renegotiation in case of pre-symptomatic diseases causing losses as poultry moves downstream.

These results illustrate the importance of a systemic understanding of PDNs and their ongoing evolution to assess disease risk generation and design targeted surveillance and mitigation interventions.



Animal health expenditures and losses in poultry farms in Chattogram, Bangladesh

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Bangladesh's poultry industry confronts numerous challenges, such as a lack of performance data and a significant disease burden. This study evaluated the economic performance of broiler and Sonali chicken farms in Bangladesh and quantified the animal health-related expenditures and losses.

A longitudinal study encompassed 60 poultry farms: 30 contract-based broiler farms (CB), 15 independent broiler farms (IB), and 15 independent Sonali farms (IS). Sixty farmers (all male) were followed throughout one production cycle, collecting data on production and economic parameters. Farm economic performance was determined through the development of production functions, price analysis and gross margin analysis (GMA).

Preliminary results unveiled substantial variations in production performance among farmers. The mean cycle length was 34.3 days for CB, 32.3 days for IB and 59.4 days for IS. Average daily growth gains were 53.9 g for CB, 48.8g for IB and 18.5g for IS. Average feed conversion ratio was 1.78 for CB, 1.54 for IB and 2.11 for IS. Price analysis revealed that CB and IS benefited from a 21.4% and 39.2% price discount on day old chicks, and a 12% and 2% price discount on feed compared to IB respectively. CB and IB sold their finished birds at 18% and 21% higher prices than IS, respectively. GMA showed that a consistent 40% of CB, IB and IS experienced negative returns, primarily due mainly to disease outbreaks.

The study illustrates farmers' challenges regarding financial sustainability in Bangladesh, with CB currently facing the highest risk. The derived models can serve as valuable tools for interventions and training.



Movements of commercial poultry traders and their impact on Avian Influenza Virus transmission in Bangladesh

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Movements of poultry traders will likely impact the spread of avian influenza virus (AIV).

A total of 87 daily trading movements of 16 poultry traders (all men) from Chattogram, Bangladesh, were recorded using a phone App with survey questions incorporated into the App capturing details of transactions. Oro-pharyngeal swabs were collected from chickens at farms and transport endpoints. All samples were screened for AIV.

Traders visited up to 3 farms, but in 88% (n =77) of trips, chickens were collected from a single farm. Traders transported a mean of 820 chickens per truck load. On average, a single trip consisted of 8.5 stops to sell birds. Traders sold chickens to live bird markets (LBMs) (60%, n=384), roadside trading sites (38.4%, n=246) and storage facilities (1.6%, n=10). The mean duration of a trip was 4 hrs 47 mins, and the average distance between stops was 9.3 km (1.7-39.1 km), while the average total distance of a single trip was 68.6 km (6.8-198.3 km).

Chickens were distributed to 1-8 vendors per LBM, with a median of 100 chickens (20-1300) sold to a single vendor. At roadside trading sites, a median of 44 chickens (9-387) were sold to 1-2 vendors. Traders kept on average 167 (15-870) chickens at storage facilities.

Birds tested AIV positive on 11 trips, with 10 of these trips being longer than an average trip. Between Oct-Jan, AIV bird-level prevalence was 1.5% on farms and 6.5% at sale endpoints. Between Feb-Aug, AIV prevalence was 6.4% on farms and 7.2% and 9.4%, when chickens were, after reaching sale endpoints, kept for an additional 12h and 24h, respectively.

Infection of susceptible birds most likely occurred during transport, although 82.8% of trucks were clean without visible faeces. Enhanced biosafety during delivery of chickens at the frequent stopping points is recommended to reduce the likelihood of AIV infection.





A mobility study among Chennai-based traders to understand the spatial data of procurement and delivery of broiler chickens from farm to market

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The Indian Poultry sector was valued at Rs.80,000 crore during 2015-16. (National Action Plan for Egg & Poultry-2022) and the reason attributed is affordable cost, easy availability and market acceptance of products. This mobility study aimed to understand the trading movement of broiler chickens from farms to markets from the perspective of the traders who are mainly playing in the middle of PDN. The study focuses on Chennai-based traders, the patterns of sourcing broilers, the variations in geographical areas they source broilers from, and how and where they distribute them.

Primary data was collected by recording procurement trips using the poultry hub tracking App and through qualitative interviews with participants and their firm officials. Daily local BCC farm gate prices and CPWA market prices were collated to be correlated with procurement locations. Three traders were selected and two transporters (all male) were recruited from each. The study was conducted from 31.07.2023 to 12.11.2023 for 15 weeks to include different seasons and to observe differences in business modalities. Over this period, 116 procuring trips were recorded. All traders' procurement locations were mainly based on the availability of required weight and price of broilers, although each of them has a different strategy for reducing expenses by minimizing transportation costs and sourcing from areas with lower farmgate prices. The study results will be most useful when correlated with farm and delivery destinations, as well as purchase and sales data, and the pattern of sourcing, especially location, distance, frequency of visits and price fluctuations.



Predictive gravity models of live poultry trade networks in Bangladesh

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Exploring the dynamics of live poultry trade is crucial for understanding and managing public health risks, especially those related to the spread of avian influenza viruses.

Gravity models, commonly used in economic trade analysis, and machine learning techniques were used to predict poultry trade flows in Bangladesh. The approach is grounded in analysis of data from a cross-sectional study conducted in 2015-2016, which provides the quantity, species, and origins of poultry traded in Dhaka and Chittagong. Key variables such as driving distance, travel time, poultry population, and competitiveness indices were used to train the models; these are crucial for understanding their correlation with the volume of chicken exports to major cities, thereby offering predictive insights into trade flow dynamics.

The most effective model was Gradient Boosting Regression, which displayed high predictive accuracy. Driving distance and travel time were negatively correlated to broiler exports to major cities, underscoring proximity as a vital factor in poultry trade. Regions with larger poultry populations and higher farm densities showed positive correlation with exports, especially to Chattogram. Consumption patterns emerged as a notable influence on trade flows. The model allows us to predict the origins of chickens sold in specific cities (Comilla, Jashore and Bogura) based on comparison to data collected through Round 1 Hub field studies.



The predictive power of these models allows us to identify likely catchment areas of cities that were not surveyed, and to inform the H9N2 phylogeography analyses.

Predictive modelling maps illustrating the origins of live chickens (exotic broiler) sold in several cities (Dhaka, Chattogram, Cumilla, Jashore and Bogura), Bangladesh, using various regression techniques (linear regression, random forest regression and gradient boosting regression) compared to field data (LT R1).

Programme 2: Host-pathogen dynamics

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Programme 2 is focused on key zoonotic pathogens of poultry and antimicrobial resistance in sentinel and whole enteric bacterial populations, set against a background of varied antimicrobial use, host genetic and production system diversity.

Cross-linking to the production and distribution networks (PDNs) mapped in Programme 1, the occurrence of avian influenza virus (AIV), *Campylobacter* species, and non-typhoidal *Salmonella* has been assessed in a range of commercial settings in chickens and/or humans. Isolates collected during these surveys are being subjected to whole genome sequencing to assess diversity, evolution, and the presence of specific risks such as antimicrobial resistance genes (ARGs) and transmissible plasmids.

Escherichia coli has been used as an easily accessible sentinel of ARG carriage in parallel to an Illumina Ampliseq survey of ARG occurrence in enteric microbiomes. Context provided by detection of antimicrobial residues in feathers and breast meat, indicative of lifelong and recent antimicrobial exposure, provide a reliable measure of selective pressure towards antimicrobial resistance (AMR) and the risk of consumer exposure. Comparison with producer reported antimicrobial usage (AMU) is offering an interesting counterpoint, illustrating community awareness. The host (chicken) genetic contribution to variation in measures associated with viral and bacterial pathogens is being interpreted in combination with microbiome enterotypes to improve productivity, welfare, and public health.

Data generated in Programme 2 is being used to inform mechanistic and phylodynamic models for AIV transmission and evolution, with possible future applications to ARGs and plasmids, and combined with knowledge from Programme 1 in a range of modelling strategies.



Overview of biological field studies (avian) in farms and markets in Bangladesh

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Avian influenza is endemic in Bangladesh. It is challenging to control the disease without having information on its prevalence across the poultry value chain, transmission dynamics and risk factors. To get such information, several studies were conducted including a cross-sectional survey in chickens on farms and in live bird markets (LBM), an investigation on chickens on farms at their different production stages, screening clinically sick/dead birds submitted, and longitudinally screening chickens and ducks at nearer proximity to wetland habitats of migratory birds.

Oropharyngeal/cloacal swabs were collected from the birds sampled, and viral RNA was extracted and tested for the presence of the M gene of AIV followed by two subtypes H5 and H9, by rRT-PCR.

The results revealed that 28.9% birds in the LBM were positive for AIV, 27% for H9 but only 0.6% for H5. None of the birds on farms tested positive for H5 and only 0.75% was positive with H9. The broiler farm-level prevalence was 0%, 5.7% and 8.6% for AIV, 0%, 2.9%, and 4.3% for H9, and 0%, 4.3% and 4.3% for H5, respectively, in phase 1, 3 and 4 session of sampling. A similar low farm-level prevalence in Sonali birds was also observed. Regardless of bird types, the prevalence estimate was 20% for AIV, 12% for H9 and 7.6% for H5 in clinically sick or dead birds tested. Based on the preliminary results obtained so far, the prevalence of AIV, H5 and H9 in the birds at poultry-migratory birds interface sites was 26%, 8.5%, and 10.5%, respectively, with a higher proportion of H5 positivity in ducks compared with other species.

The overall results indicated that the prevalence of AIV, H5 and H9 are quite low in healthy birds on farms, moderate in sick/dead birds, but consistently very high in birds sampled from LBM with variable prevalence estimates for H5. The high presence of H5 in ducks as seen in duck-migratory bird interfaces is worrisome because ducks could act as silent carriers of this HPAI subtype and pose a persistent threat to cause clinical infection and mortality in chickens.

Prevalence of Avian Influenza A(H5) and A(H9) Viruses in broiler endpoints and farms in northern Việtnam

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Over the last 20 years Việtnam has experienced numerous outbreaks of avian influenza virus (AIV) causing significant losses to the domestic poultry industry. Research is needed to understand AIV prevalence during poultry production.

Using a cross-sectional study, we estimated the prevalence of avian influenza A(H5) and A(H9) viruses in hybrid and exotic broilers in farms and sales endpoints in four provinces in northern Việtnam. Bayesian hierarchical logistical regression models were fitted to rRT-PCR test results. Disease cluster analysis was performed to detect whether contaminated endpoints and farms were spatiotemporally clustered. Bayesian phylodynamic analysis was used to examine pathogen flow between regions and endpoints.

A(H5) was detected in a single hybrid broiler in a slaughter point. We detected A(H9) in most retail markets and slaughter points, in fewer than half of wholesale markets and in fewer industrial slaughterhouses. The lowest prevalence of A(H9) was in farms, suggesting viral amplification along the production and distribution network (Fig.1A). There was a high level of mixing of virus genetic diversity between endpoints and regions. Prevalence of A(H9) was higher at slaughter points than in retail and wholesale markets, and higher in endpoints supplied by farms in Bac Giang and Quang Ninh than in Hanoi (Fig.1B). A single spatiotemporal cluster of A(H9) positive farms was identified in Hanoi in January 2022. Phylogenetic analysis showed all but one of the clustered farms were genetically very closely related suggesting a common source of infection or a chain of transmission between those farms. Part of Việtnam's AIV control strategy has been to promote the use of industrial slaughterhouses over traditional slaughter points. As slaughter points are highly contaminated by A(H9), research is necessary to understand why these remain a preferred endpoint for processing poultry and why industrial slaughterhouses remain underutilised.



A. Odds ratios of endpoint type and farm catchment area compared to base variables slaughter point and Ha Noi. **B**.Overall bird level prevalence across different sites

The genetic and antigenic diversities of H9N2 avian influenza viruses infecting poultry in Bangladesh

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H9N2 avian influenza viruses (AIVs) of the G1 lineage are endemic in Bangladesh, persistently circulating within poultry populations and causing substantial production losses, including eggs and meat. Vaccination is crucial for inducing protective immunity and mitigating disease impacts. However, the genetic and antigenic dynamics of contemporary H9N2 AIVs in Bangladeshi poultry are poorly understood, hindering accurate antigenic matching of vaccine seed strains to the circulating viruses.

Large-scale poultry sampling across Bangladesh yielded multiple positive AIV isolates, which were whole genome sequenced. The immunodominant antigen, haemagglutinin (HA), displayed a diverse range of H9N2 virus variants. Phylogenetic analysis identified three distinct virus subgroups, one geographically localised and while the others were widely distributed. Additionally, some sites isolated each subgroup, co-infecting poultry simultaneously.

To identify genetic markers influencing antigenicity between subgroups, heterogenic amino acid residues in the HA antigen were integrated into reverse genetics (RG)-based candidate H9N2 vaccine viruses. Each mutation's impact was assessed through haemagglutination inhibition (HI) assays using sera propagated to the non-mutated HA RG virus. While some residues had no effect on antigenicity, several were directly linked to antigenic variability, leading to a significant reduction in HI titres. This poses a challenge to vaccine effectiveness in flocks co-infected with different antigenic variants.

These findings significantly advance our understanding of H9N2 AIVs in Bangladesh, offering valuable insights for optimizing vaccination strategies, AIV control, and disease mitigation in poultry populations.

Transmission dynamics of H9N2 Avian Influenza virus in a Live-Bird market in Chattogram, Bangladesh

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Multiple subtypes of Avian Influenza viruses (AIVs) are endemic in Bangladesh. Prevalence of H9N2 AIV is high in live-bird markets (LBMs), causing concerns about risk of reassortment and cross-species spillover events. Yet, AIV transmission dynamics remains poorly understood.

In this work, we analysed a novel dataset from a field experiment set in an LBM. Using Bayesian methods, we fitted a mechanistic model of AIV transmission in an LBM and assessed the impact of potential veterinary public health interventions to reduce AIV transmission.

Susceptible chickens become infected within one day spent at the market, while a high proportion of chickens were estimated to have been already exposed to H9N2 AIV when entering the market - 14 percent (7-35% 95% C.I.) of exotic broiler and 25 percent (18-39% 95% C.I.) of indigenous backyard chickens. We also estimated short latent periods of H9N2 AIV, ranging from 5.4 hours (2.2-7.9 hours 95% C.I.) for exotic broilers to 1 day (0.8-1.2 days 95% C.I.) for backyard chickens. Non-pharmaceutical interventions had a limited effect on transmission when considered separately, while vaccinating 50 percent of chickens prevented 60 percent of new infections within the market.

Our results indicate that short latent times and the rapid turnover of chickens facilitate transmission and persistence of H9N2 AIV in LBMs. Currently, veterinary public health interventions tend to focus on LBMs. However, our analysis suggests that these should be complemented with measures targeting farms and upstream transportation such as widespread immunisation of poultry against H9N2 AIV.



Probability of a susceptible chicken remaining susceptible after a given time spent at the market. Red and teal lines correspond to different threshold cycle (Ct) values to define positivity of a sample. Lines and shades represent posterior means and 95% C.I.

Drivers of avian influenza virus movement within a city: preliminary insights from Dhaka, Bangladesh

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The lack of virus genomic sampling from live bird markets has impeded our understanding of avian influenza transmission along poultry trading networks, which is essential to design effective interventions.

To address this gap, we integrated phylodynamic analysis of H9N2 virus genomes generated from Round 1 sampling with key metadata from the poultry trading network in Bangladesh. At the city level, we have collated rich information about network connectivity, chicken trade, trader movement, geographic distance, and niche overlap between markets.

Early findings from Dhaka indicate that avian influenza virus movement is positively associated with virus prevalence, geographic distance, and negatively associated with network distance based on the number of middlemen. Specifically, these results suggest that virus transmission is greater between markets that are geographically proximate and linked by fewer middlemen and that virus is like to spread from markets with higher virus prevalence to markets with lower prevalence.

Although additional analyses are required to determine the robustness of these findings and whether similar patterns are also observed in Chattogram, these preliminary insights imply that avian influenza transmission is focal within a city, and therefore targeting markets with higher virus prevalence could be important factor in reducing virus spread.



Prevalence of antimicrobial residues in broiler meat in Vietnam, Bangladesh and India

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Over/misuse of Antimicrobial Drugs (AMD) in commercial poultry farming could represent a human health risk. The aim of this cross-sectional study was to report incidence of AMD residues in meat in the poultry production system of Vietnam, Bangladesh and India.

576 samples were collected from poultry farms (at end of production cycle) and endpoints (live bird market and slaughterhouses) in Bangladesh (n = 287, 39% on farms), India (n=158, 32% on farms) and Vietnam (n=131, 38% on farms). Chicken breast samples were frozen (-80°C) until screening for the presence AMD at Maximal Residue Level (MRL) using a LC/MS method (EU reference laboratory), validated for a panel of 70 AMD \geq MRL. A sample was positive when at least 1 AMD was detected.

Sixty-five of the 576 samples (11.3%) had AMD residue above MRL. There were 42 (14.6%), 11 (7.0%) and 12 (9.1%) positive samples in Bangladesh, India and Vietnam, respectively. Multiple AMD were detected in 18 samples (Bangladesh=14, India=3 and Vietnam=1).

In Bangladesh, the most frequent AMD detected in positive samples included Tilmicosin (n=14), Doxycycline (n=7), Oxytetracycline (n=7), Sulfadiazine (n=6), Ciprofloxacin (n=5), Trimethoprim (n=5). In Vietnam, the most detected AMD were Doxycycline (n=2), Tilmicosin (n=2), Sulfamonomethoxine (n=2). In India, the most detected AMD were Enrofloxacin (n=3), Trimethoprim (n=3), Chlortetracycline (n=2), Tilmicosin (n=2), Sulfadiazine (n=2).

Centralised analysis highlights differences regarding country prevalence and nature of AMD detected and allows comparison with EU prevalence (0.05-0.1%) High prevalence of residue found suggests insufficient withdrawal times or undocumented administration.



Study on trend of antibiotic resistance of indicator bacteria *E. coli* during production cycle In Vietnam's chicken farms

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Antimicrobial resistance (AMR) in livestock, particularly in poultry, is a serious public health problem in Asian countries. This study aimed to get evidence of AMR in colour broiler chicken farms during the production cycle.

Sixty medium-size farms (2000-5000 chickens) in two Northern provinces of Vietnam were randomly selected. Across four sampling times (ST) of the production cycle (at 7-12, 30-40, 90-100 days, and one day before sale) a total of 237 cloacal swab samples from healthy chickens (one sample/farm/sampling time) were collected. Frome each swab, a single *E. coli* colony isolated on MacConkey agar, confirmed by PCR (*adk*- target gene), was selected for antimicrobial susceptibility testing. Disk diffusion was used for $3^{rd}/4^{th}$ generation cephalosporines, including cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg) and a fluoroquinolone (ciprofloxacin-5ug). Interpretation of inhibition zones and MIC was done according to EUCAST criteria and isolates showing intermediate resistance were categorized as susceptible to avoid overestimation. Proportion test was used to compare resistance proportions between sampling times.

E. coli were detected in 232/237 samples (97.9%) and 54/232 (23.3%) isolates at farm level were resistant to colistin (MIC 4-16 µg/ml). Interestingly, we found that prevalence of *E. coli* resistant to $3^{rd}/4^{th}$ generation cephalosporines and ciprofloxacin decreased significantly (p<0.05) during the production cycle (figure 1). For ciprofloxacin at 1^{nd} , 2^{nd} , 3^{th} , and 4^{th} ST prevalence was 41/60 (68.33%), 33/60 (55%), 25/57 (43,9%), and 9/58 (15.5%) respectively; for cefotaxime it was 63.3%, 38.3%, 24.6%, and 12.1%; for ceftazidime it was 55%, 20%, 12.3%, and 1.8%; and for cefepime it was 46.7%, 28.3%, 19.3%, and 3.4%. We detected *E. coli* isolates that were co-resistant to colistin and a $3^{rd}/4^{th}$ generation cephalosporine in 21 farms (15 farms at 1^{st} ST, 7 farms at 2^{nd} ST, and 6 farms at 3^{rd} ST).

In recent years, science-based communication about the risk of drug resistance affecting public health has occurred regularly in Vietnam and may have changed awareness of households and local vets about AMU and reflected a reduction in resistance rate with important antibiotics of *E. coli* indicator bacteria during production runs in the study. More research is needed to evaluate the effectiveness in stages of the strategy to reduce antibiotic use in livestock production. Indeed, strict monitoring of AMU/AMR particularly of important antibiotic groups mentioned by WOAH and WHO is needed to ensure the export livestock production strategy of MARD.



Reducing trend of antibiotic resistance *E. coli* during production cycle in broiler farms

Campylobacter isolated from LMIC poultry production networks show reginal links to antimicrobial resistance and novel *C. coli* clusters

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A rise in demand for low-cost poultry products, particularly in low middle income countries (LMICs) is being met by rapid intensification of poultry farming practices, leading to an increase in food related bacterial gastroenteritis. The most common cause of bacterial gastroenteritis is associated to *Campylobacter* spp infections. *Campylobacter* spp. are a high priority pathogen list for the development for new antibiotics due to the rise in antimicrobial resistance. Monitoring the use of antimicrobial resistance (AMR) profiles is essential when it comes to *Campylobacter* spp. in LIMCs.

This study utilises whole genome sequencing (WGS), coupled with phenotypic experiments to identify AMR profiles of isolated *Campylobacter* spp. across three rapidly developing south/southeast asian countries (India, Bangladesh, Vietnam), and has identified variations in the AMR profiles between the countries.

The findings may help generate monitoring/intervention strategies to improve food security. The study also compares the phylogeny of newly sequenced *C. jejuni* and *C. coli* strains, identifying potentially new clonal complex for *C. coli* predominantly found in India and Bangladesh indicating potential evolutionary adaptations.



Phylogram of study isolates. Core-genome multilocus sequenced types (cgMLST) generated for sequenced *C. coli* and *C. jejuni* strains plus NCBI reference genomes, built using chewBBACA. Branches are colour coded based on clonal clusters. The areas coloured blue and red show *C. jejuni* and *C. coli* respectively.

Diarrheal disease in children in a rural Indian setting – incidence, causes and attributable risks from poultry using a One Health approach

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Childhood diarrhoea is an important public health problem and can lead to repeated enteric infections and malnutrition, with long term impacts such as impaired cognition, poor scholastic performance and economic potential. Little is known currently on transmission of *Campylobacter* and Non-typhoidal *Salmonella* (NTS) from poultry to humans in rural settings.

We followed 527 under-5 children from a rural block in Tamil Nadu from Mar to Nov 2023. Stool samples were collected whenever a diarrhoeal episode was reported by the care giver and caecal samples from poultry collected during the episode if the household or immediate neighbour had poultry. All samples were cultured in mCCDA and Campy-BAP media for selective isolation of Campylobacter, and on blood agar, MacConkey agar and XLD Agar for NTS. Isolates were identified by biochemical tests, MALDI-TOF, rapid slide agglutination and MLST. Incidence rates and attributable risks were calculated using STATA 15.

During 320 child-years (CY) of follow-up 37.0% (195/527) of children experienced at least one episode of diarrhoea and episodes were more common in children under 2 yrs (72.9% vs 27.1%). Overall incidence was 74 episodes per 100 CY (95%CI:64.3-82.3). Incidence of *Campylobacter jejuni* diarrhoea was 13.7 per 100 CY, *Campylobacter coli* was 1.9 per 100 CY, *Shigella* was 9.1 per 100 CY and NTS was 8.7 per 100 CY. In 74 households, 98 chicken caecal cultures showed prevalence of 25.5% *C. jejuni* and 16.3% *C. coli*. The incidence rate of *C. jejuni* diarrhoea in children exposed to chickens carrying *C. jejuni* was 47.2 per 100 CY compared to 11.9 per 100 CY for children unexposed to chickens carrying *C. jejuni* in chickens is 74.7 % (95%CI: 26.8-89.4%). The incidence rate of *C. coli* diarrhoea in children exposed to chickens with *C. coli* was 23.7 per 100 CY compared to 1.2 per 100 CY for children unexposed to chickens with *C. coli* and the AR is 94.8% (42.1-99.2).

This study provides data on incidence of diarrhoeal disease in early childhood in a rural Indian setting following rotavirus vaccination in the National Immunization Schedule and explores diarrhoeal disease transmission from poultry to humans. A high proportion of *Campylobacter* diarrhoea in children was attributed to contact with infected household poultry. Testing to determine antimicrobial resistance will be explored.



Incidence of enteric pathogens 100 Child Years of follow-up

Insights into comprehensive antimicrobial resistance pattern of poultry caeca in Gujarat, India

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Antimicrobial resistance (AMR) is a serious global public health threat in 21st century. Poultry guts act as important reservoirs of AMR genes with the potential for transmission to humans via the food chain. This cross-sectional study aimed to create a comprehensive profile of AMR genes in chicken caeca of Gujarat region.

Chicken caecal contents from 106 broiler and 44 desi (indigenous) birds were collected, extracted metagenomic DNA was used for sequencing followed by analysis to check the presence of AMR genes.

A total 103 resistance genes to 18 different antimicrobial classes were detected. The set of core AMR genes (antimicrobial class) were tetQ, tetW, tet32, tet40, tet44 (tetracycline); InuC (lincosamide); ermF, ermG (macrolide) and aadS, aadE, aph(2')-lb (aminoglycoside). A total of 26 significantly different AMR genes were found between broiler and desi chicken. Usually, AMR genes conferring resistance to same class of antimicrobials were positively correlated such as some macrolide resistance genes positively correlated with aminoglycoside resistance genes, while tetracycline resistance genes showed a negative correlation. In hierarchical clustering, samples from broilers tend to remain in one single cluster. The resistance against critically detection of genes important antimicrobial agents (fluoroquinolones, 3rd generation cephalosporins, macrolides, and vancomycin) used for humans, may pose a potential risk to human health.

Intensive chicken production has limited resources without proper biosafety and biosecurity measures may increase antimicrobial resistance. Monitoring and reducing resistance development patterns is crucial in developing effective strategies to combat its prevalence in developing countries worldwide.



Microbiome Diversity and Antibiotic Resistance Gene Distribution in Poultry and Vegetable Shop Workers: Tales from Gujarat and Tamil Nadu

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The interaction between humans, environments and animals is considered as one of the critical foci for addressing antimicrobial resistance (AMR). The aim of this study lies in investigating whether and how the gut microbiomes and antibiotic resistomes are influenced by exposure to intensively or non-intensively reared poultry within chicken shop as compared to the vegetable shop workers in Gujarat and Tamilnadu states of India.

A total of 165 (Gujarat) and 142 (Tamil Nadu) fecal samples were collected for the gut microbiome analysis. The fecal samples from vegetable shop workers were taken as control to study the link of spread of AMR genes from poultry environment to humans. The 16S amplicons sequencing was performed to analyze the microbiome diversity and AMR AmpliSeq analysis was performed to detect antibiotic resistance genes (ARGs) and data was compared between chicken and vegetable shop workers.

No significant differences were found in terms of overall microbial diversity and prevalence of ARGs between the two groups in both the states. This study therefore provides the critical insights into the distribution of microbiome and ARG's in individuals associated with poultry in India and elucidates that there is no significant risk of poultry exposure for individuals associated with this sector.



Genetic characterisation of indigenous chicken ecotypes from India and Bangladesh

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The increasing global human population has led to an increased demand for animal protein. Chickens have relatively low production costs, high food conversion efficiency and low environmental footprint. The present work focusses on the genetic characterisation of key indigenous chicken ecotypes from India and Bangladesh in relation to diversity among the commonly reared commercial chicken lines.

Chickens were genotyped using whole genome sequencing (n=41) or skim genome sequencing (n=191). Fixation index (Fst) and regions of homozygosity (ROH) analyses were used to identify regions under selection pressure and assess levels of inbreeding in each ecotype. Moreover, birds were phenotyped for *Escherichia coli* colonisation by culturing on MacConkey agar. We compared birds with and without *E. coli* to identify genomic regions driving resistance to *E. coli* colonisation. PCA analysis based on genomic relationships among the different bird types showed clear separation of each chicken ecotype and line.

In genomic regions selection sweeps between broilers and indigenous chickens identified genes involved in physiological and growth-related traits and linked to phenotypic appearance including feather coloration. Both broilers and Kadaknath, a black-meat breed from India, showed higher number of ROHs indicating high inbreeding with Kadaknath exhibiting longer ROHs indicating recent inbreeding. In regions with high genetic differences between *E. coli* positive and negative birds, candidate genes such as Integrin gene *ITGA6*, pro-inflammatory cytokine gene *IL1RAPL1* and multiple subunits of fibrinogen gene were detected.

Kadaknath-FADH	0.124	0.105	0.135	0.118	0.131	0.083	0.078	0.064	0.081	0.095	
Aseel-FADH	0.13	0.104	0.145	0.125	0.141	0.071	0.046	0.061	0.048		
Desi2-Gujarat	0.117	0.09	0.129	0.108	0.123	0.045	0.018	0.059			
Desi1-Gujarat	0.07	0.05	0.082	0.063	0.077	0.041	0.05				
Desi-Bangladesh	0.101	0.078	0.111	0.092	0.106	0.043					Fst
Sonali-Bangladesh	0.098	0.076	0.109	0.092	0.107						
roiler-Gujarat-FADH	0.074	0.065	0.014	0.025							
piler-Gujarat-Cobb2	0.054	0.05	0.017								
oiler-Gujarat-Cobb1	0.073	0.064									
iler-Gujarat-SunBro	0.039					Desi-Ban					

Population differentiation measured as Fixation index (Fst) between pairs of chicken populations calculated from 21 million genomic variants. Groups of commercial broiler populations and indigenous bird populations show lesser values among themselves while higher values were observed between themselves.

Policy Engagement: supporting improved policy design and implementation

Robyn Alders

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Policy engagement by researchers enables policymakers to improve public policy and its implementation through collaborative research that documents and assesses relevant evidence and experience. In relation to livestock policies, in many countries they are not necessarily lacking, but rather lack accompanying standards, guidelines and implementation plans. Hence the importance of assessing the policy environment and its implementation.

Globally, the poultry industry expanded significantly in the 20th century to make vital contributions to national and household food security and economies. In the 21st century, the challenge is to ensure that poultry production systems evolve in line with wider food system transformations designed to achieve outcomes including improved productivity, safety, nutritional value, climate resilience and biodiversity gains. The transformation of the poultry industry is taking place at a time of economic uncertainty with the budgets of many national governments and commercial enterprises facing difficult decisions associated with simultaneously addressing multiple challenges. It is, therefore, crucial that the policy approaches taken to transform poultry agrifood systems to reduce threats associated with avian influenza, foodborne pathogens and antimicrobial resistance, also complement wider food systems actions to achieve synergistic outcomes.

Employing a One Health approach is crucial to dealing effectively and efficiently with such complex policy issues. While each Hub country has prioritized policy issues of importance to the specific circumstances faced by the poultry production systems studied, they have all used the One Health approach to engage with policy makers across a range of sectors. Examples of policy issues explored by Hub researchers include: identifying options for improving One Health implementation, public private partnership and policy awareness among poultry farmers in Bangladesh; the impact of the COVID-19 pandemic on One Health implementation in India; mapping livestock-related policies and developing a draft poultry policy taking gender and equity across production systems into account in Sri Lanka; and bringing human health, agriculture, environment, industry and trade sectors together to reduce antimicrobial resistance in Viet Nam.

Programme 4a: Policy Engagement



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Supporting improved policy design and implementation

- Policy engagement:
 - To improve public policy and implementation built on evidence/experience
- Policies appropriate for the 21st century
 - Support evolution of poultry production systems in line with wider food system transformations
- Hub policy engagement
 - One Health approach crucial to deal with complex policy issues
 - Each Hub country has prioritised issues of importance to its specific circumstances and use the One Health approach to engage with policy makers across sectors

Animal slaughter, wet markets and food safety in the context of rapid economic transformation: how can Vietnam make animal slaughter safer?

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"Wet markets" – where fresh fruits, vegetables, and meat are sold to people – in cities in lowand middle-income countries (LMICs) are thought to be hotspots for disease emergence and spared. In wet markets across Asia, it is common to slaughter live animals, and this creates an ideal condition for diseases to spread rapidly. Because of such threat to public health, policy makers are trying to better control these unsafe trading and sales practices for animals. Vietnam is one such country where the government has been aiming to industrialise animal slaughter for over a decade. However, their effort has been met with challenges and wet markets remain important in providing healthy and nutritious food for many. This study explores why various policy efforts to modernise chicken slaughter in Vietnam have had limited impact, and what policy instruments are better suited to improve the safety of chicken slaughter practices in Vietnam.

We evaluated policy documents and conducted in-depth interviews with key stakeholders (44 men and 63 women) along chicken value chains – including slaughterers of different scales, policy makers, and retail outlets.

Our analysis finds that policies aiming to industrialise slaughter sectors are not aligned with secure land usage rights, preventing long-term physical investment to build industrial slaughterhouses. Moreover, industrial slaughterhouses predominantly supply exotic broilers, which are less important for ordinary consumers. Despite the higher prices of coloured chickens, Vietnamese people prefer to consume special breeds chickens that are supplied through small-scale slaughterers.

Thus, we recommend prioritising improvement of the safety and hygiene of practices by smallscale slaughterers.



Mapping existing animal industry-related policies and developing a poultry policy for Sri Lanka; towards a sustainable and competitive poultry industry

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In Sri Lanka, animal industry-related policies play a crucial role in shaping and regulating the poultry industry. They serve as guidelines, rules, and frameworks that help ensure the smooth functioning and growth of the sector. However, the relevance of these existing rules and regulations to poultry production practices, gender equity, disease prevention, control and surveillance and Antimicrobial Resistance (AMR) has not been analyzed.

To fill this gap, all publicly available Acts, policies, circulars, regulations and guidelines currently applicable to poultry industry were collected and a policy matrix was compiled. Policy mapping is being done considering gender perspectives.

Currently, a policy specific for poultry is not available in Sri Lanka. This gap was identified as a major constraint to sustainability of the poultry industry. Therefore, One Health Poultry Hub - Sri Lanka joined hands with the Livestock Department of the Ministry of Agriculture to develop a poultry policy. In order to achieve the objective, it was decided to adhere to the government accepted protocol for policy development. Accordingly, three stakeholder meetings were held to discuss key points to be identified in the policy document. A steering committee was appointed and the first draft was discussed. An amended policy document will be presented to three separate groups of stakeholders representing large-scale, medium-scale and family poultry sectors before publishing in local newspapers for public comments. After addressing all the comments received from stakeholder meetings and the public, a final draft of the policy document will be prepared for approval by the National Cabinet.
One Health, multiple impacts: a review of 10 years of One Health work in Bangladesh and the region with a focus on the poultry industry in Bangladesh

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Bangladesh emphasises zoonotic diseases in its "One Health" endeavour. Bangladesh's poultry sector is vital to its economy and food security. This study analyses the 10-year outcome and lessons from adopting One Health in Bangladesh and the region, recommending it to improve health security, notably in the poultry industry.

The scoping review protocol was set for Bangladesh and selected South Asian Association for Regional Cooperation (SAARC) and Association of Southeast Asian Nations (ASEAN) documents. A personalised and semi-structured interview with 32 One Health informants (25 male, 7 female) from 10 countries assessed the approach's effectiveness to address emerging diseases and antibiotic resistance.

Bangladesh institutionalised One Health better than other countries included in the review and has strengthened national and international One Health networking. However, One Health working bodies have not yet adapted to community-level activities and there is a lack of communication with the general public as well as between the various sectors. Typical challenges that emerged included inadequate collaboration among stakeholders, funding incompatibility, limited availability of evidence-based research, lack of integrated data management system and One Health practices. The investigation of disease outbreaks and integrated surveillance, as well as the monitoring and early detection of poultry zoonotic diseases, are not yet thoroughly established.

Thus, Bangladesh needs to establish local One Health teams and improve inter-sectoral communication and public awareness like other countries. To maximise the success of One Health initiatives in poultry sector, we require cohesive coordination systems to promote timely knowledge management about preventive practices among farmers.



Poultry development policies in Bangladesh: Stakeholders practices, challenges and way forward

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Bangladesh's poultry sector has experienced significant expansion in the past few decades. Certain policies have been created to offer instructions and directions to stakeholders. However, the policies are not well known to many stakeholders and many of those who are aware of the regulations are not adhering to them.

This study was carried out to assess key stakeholders' (medium-scale exotic broiler and Sonali farmers) knowledge and implementation of the important policies in seven Upazilas of Chattogram district. Firstly, a policy review was done on the Animal Disease Act-2005, Fish Feed and Animal Feed Act-2010, Animal Welfare Act-2019 and antimicrobial usage and antimicrobial resistance guidelines in National Drug Policy-2017. A total of 42 man-farmers and the respective Upazila Livestock Officers of the seven upazilas (six male, one female) were interviewed in person (four) and via a voice over internet platform (three). A policy awareness program was implemented in eight upazilas (113 male, nine female). Later, thematic data analysis was performed.

It was discovered that most farmers are unaware of farm registration. One-third of all farmers were aware of the Fish and Animal Feed Act. 80% of farmers were not aware about the Animal Welfare Act. Additionally, more than 50% of farmers were ignorant of the usage of antimicrobials and the development of antimicrobial resistance. In most cases, farmers only turn to veterinarians as a last resort. Increasing awareness of the stakeholders by means of different creative tools and standards and easy to understand guidelines is very important to improve policy compliance.



Figure 1: Brief overview of the policy awareness study in Chattogram

Contribution analysis of complex research programmes: the case of the OneHealth Poultry Hub

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The paper discusses challenges and approach to evaluate the policy impact of research programmes. Often, the contribution of research to changes in, for example, sector policies provides the rationale for funding, even when the time for new policies to mature exceeds the time of the funding period, and the research is only one of the contributing factors to these changes, and out of its sphere of direct influence. The monitoring and evaluation work associated with research programmes, therefore, needs to focus on intermediate outcomes that are good proxy-indicators of progress towards the ultimate outcome of policy change.

The paper presents the experiences of the One Health Poultry Hub in responding to the accountability requirement related to policy impact. Using archival data (email correspondence), stakeholder surveys, interviews with key informants, and participatory observation during Hub events, we show how the advocacy and policy impact work in the project is influenced by the history of hub collaboration within each country. We describe the first phase where the project developed an overall theory of change for the programme, which each hub refined into a (nested) context-specific theory of change that reflected their institutional embedding and national-specific policy priorities. A second phase consisted of the 'harvesting' of impact stories that indicated where and how the contributions by the OHPH came about. The third phase is the critical verification of the main contribution claims through surveys and interviews with the boundary partners related to these impact stories.



Figure 1: The overall theory of change at the start of the OneHealth Poultry Hub

Programme P4b - Training and Evaluation

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This session will concentrate on training and capacity-building initiatives for various stakeholders involved in biosecurity within the production and distribution networks (PDNs), as identified by P1. Additionally, it will encompass the development of researchers specialized in antimicrobial resistance (AMR) diagnostics. The training in biosecurity will emphasize both the structural and operational dimensions within farms and throughout the PDN. Training included raising awareness of antimicrobial resistance (AMR) and strategies to prevent AMR development. The overarching goal is to mitigate the risks of disease and AMR transmission within poultry and proactively prevent potential zoonotic diseases and AMR genes that could pose a threat to the human population.

The training model employed an evaluative framework that commenced by gauging the preexisting knowledge, awareness, and perceptions (KAP) of all trainees. This initial assessment was followed by evaluating the changes immediately after the training event and then again three to six months later. Employing a "learning by doing" pedagogical approach, the model sought to actively build capacity and motivation among participants. The ultimate goal was to increase profitability in poultry production and also to ensure production of poultry that is safer for human consumption. By incorporating these strategies, the training aimed to create a comprehensive and sustainable impact, fostering a more proficient and motivated workforce in the poultry industry.

The training for researchers in antimicrobial resistance (AMR) molecular diagnostics was comprehensive, involving capacity building within and beyond the poultry hub. The objective was to establish a network of researchers dedicated to educating and fostering research in AMR. The training model incorporated both theoretical and practical components, starting with an online course and followed by a 10-day practical training session in Gujarat.

Ensuring gender balance among both trainees and trainers was considered essential across all training programs.



Comparison Between Biosecurity Practices on Commercial Broiler and Layer Farms in Gujarat

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Poultry farming is crucial to India's economy, but global trade and the movement of people and animals have raised concerns about disease transmission and antimicrobial resistance. Implementing biosecurity measures on farms can control the spread of infectious diseases and antimicrobial resistance. This study aimed to compare the biosecurity practices of commercial broiler and layer farms in Gujarat, identify any gaps, and provide insights into the measures adopted by farmers in the region.

A cross-sectional study was conducted using survey questionnaires collected from 74 commercial poultry farmers including 39 broiler (all male) and 35 layer (34 male, 1 female) farmers. The cleaned data was analysed using descriptive statistics, factor analysis of mixed data, and hierarchical clustering.

Five clusters, with each cluster having a predominant farm type were identified. The biosecurity measures that significantly differentiated farms were: type of farm, rearing system, production cycles per year, scale of biosecurity knowledge, source of income, farm previously encountering disease outbreak, vaccination and mode of operation. Coli septicaemia, Ranikhet disease, infectious bursal disease and coccidiosis are major problems observed on farms. Layer farmers are comparatively well educated, experienced, have good biosecurity knowledge and follow strict biosecurity protocols more so than broiler farmers. Many farmers were not aware of antimicrobial resistance and some were not maintaining records. Most farmers agreed that lack of knowledge, skilled manpower and investment limit the implementation. The analysis findings can guide strategies to reinforce compliance with critical biosecurity practices by farm type.



Pre-post training evaluation analysis on biosecurity awareness of small-scale backyard poultry farmers in Sri Lanka

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This study aims to assess the impact of training workshops on small-scale backyard poultry farmers' awareness and understanding of biosecurity measures. Biosecurity is critical in mitigating the risks of disease outbreaks in poultry farms, thereby safeguarding animal health and ensuring food safety.

The research employs a two-phase approach, encompassing a pre-training evaluation to establish baseline knowledge levels and a post-training evaluation to measure the impact of the educational intervention. In both training phases, data were collected through a structured questionnaire.

Five workshops were conducted with the participation of 178 farmers who were selected using a convenient sampling method. The training workshops covered key aspects of biosecurity, including disease identification, prevention strategies, vaccination, and the importance of implementing biosecurity measures on the farm. Demographic data showed that 45.5% farmers were 18-40 years old with 58.4% with secondary level education with a male to female ratio of 44:56. Data analysis showed that average awareness of both poultry disease and vaccination were improved significantly (P<0.001) from 44.99 to 89.61 and .59.01 to 91.61 respectively after the training. The overall farmers' awareness of biosecurity also improved significantly (P<0.001) improved after the training from 45.69 to 89.61. The improvement in overall biosecurity awareness dependent on the training approach and a combination of practical sessions and discussions were better than formal lecture method (P<0.001).

The results show the impact of targeted biosecurity training programs on farmers' awareness and practices. Future educational initiatives should build on farmers existing knowledge and appropriate training methods.

Assessment of farm profiles, knowledge gain and adaptation behaviors of biosecurity practices in small and medium scale commercial poultry farms in Western agro-climatic zones of Tamil Nadu, India

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Biosecurity measures play a crucial role in the health and well-being of poultry flocks. This study aimed to investigate the assessment of farm profiles, knowledge gain and adaptation behaviors of biosecurity practices in commercial poultry farms in Tamil Nadu.

A total of 89 farmers participated from commercial desi (32), layer (30), and broiler (27) farms. The study included an evaluative framework with a 2-day training intervention and pre and post-survey questionnaires to explore knowledge before and after training. Further evaluation of adaptation behaviors were conducted 90 days after the training intervention. Questionnaire data were analyzed using paired sample-t-test and chi square analysis.

Results revealed that 89% of trainees were male, predominantly between 21-60 years old (90%), with good education level at secondary and above (88%). Experienced (5-20 years) farmers dominated (56.2%) and the major source of income is from poultry farming and agricultural practices (46.1%). Comparison of pre and post survey data showed that all the farmers had significant knowledge gain in farm fencing, antibiotic usage, and wild bird management (p<0.05) immediately after the training. Broiler farmers acquired more knowledge in dead bird disposal, and chick quality compared to layer farmers (p>0.05). Subsequently, adaptation behavior also significantly increased (p<0.05) except for rodent and pest control.

This comprehensive study provided valuable insights for stakeholders and policymakers to enhance biosecurity measures and sustainability in the poultry industry. Besides, the study concluded that a tailored training program should be developed to educate small-scale producers about biosecurity measures to prevent poultry food-borne diseases.

Biosecurity in poultry farms: How to change production behaviors

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In conditions where avian influenza and other poultry diseases persist and can break out at any time, the application of biosecurity measures is crucial to minimize disease outbreaks.

In 2022, the One Health Poultry Hub team in Vietnam organized two-day training courses for 32 local veterinarians (21 men, 11 women) and 46 poultry farmers (26 men, 20 women) in three northern provinces to improve knowledge about biosecurity and poultry disease control. Before the training sections, quick surveys were carried out to assess the participants' understanding of biosecurity and poultry farming practices at their farms. During the training courses, the participants had a chance to discuss, exchange, and present their opinions about biosecurity and disease management in poultry farming.

One year after training, 41 poultry facilities out of the 46 trained farmers were visited for biosecurity and post-training evaluation. Results indicated that most medium-sized farms are rated at a good level of biosecurity, and one farm is rated at excellent. Most small-scale farms and households are rated at a fair level of biosecurity. All the interviewed farmers said they learned the correct practices for cleaning and disinfecting sheds and preventing poultry diseases after training. However, control of people and vehicles entering poultry housing has not yet been strictly applied due to the pressure of marketing and traders.

To improve the situation, it is necessary to encourage poultry farmers to integrate with processing companies and strengthen training and promotion on biosecurity for all actors in the poultry production and distribution networks.



Sensitizing molecular researchers towards the threat of antimicrobial resistance (AMR) by increasing knowledge and skills through training programs

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The growing prevalence of antimicrobial resistance (AMR) is a global health challenge and developing researchers in practical laboratory skills to perform phenotypic testing and molecular diagnostics for identifying and monitoring AMR is urgent.

Therefore, a training programme was developed and conducted by combining 6-week online training (theory) followed by a 10-day laboratory (practical) course to enhance knowledge, skills and develop a network of researchers with advanced molecular diagnostics to detect AMR.

The online training programme has been conducted four times in 2021 and 2022 and the fifth course is currently ongoing. The application numbers have steadily increased from 432 (21 different countries) in 2021 to 1021 (47 different countries) in 2024. Participants for the online courses are selected based on pre-determined selection criteria and for each online course 25-35 participants with a male to female ratio approaching 50:50 are enrolled. Participants for the practical courses are selected from those who successfully complete the online course.

Three practical courses of 10-day duration with 10 participants in each course have been conducted in GBRC and AAU India. The gender ratio for the practical courses is also maintained at male to female ratio approaching 50:50. Course evaluations show that more than 90% participants were extremely satisfied with the course content, assessments, delivery modes, value for their current and future activities.

The collaborations and researcher networks resulting from the training programs has led to submission of research projects based on AMR and related research from the participants of the training programmes.

Evaluation of poultry farmers' knowledge, attitude, and practices regarding production and biosecurity in poultry farming in Bangladesh

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Many poultry farmers in Bangladesh start small- to medium-scale poultry production without any prior training, experience, or qualifications, owing to the industry's suitability and flexibility. Few studies in Bangladesh described the conventional training programmes for poultry farmers without assessing farmers' knowledge.

Hence, this study was conducted to evaluate the knowledge, attitude, and practices (KAP) of the poultry farmers before and after participating in a 2-day long intensive and interactive training programme organized by a collaborative interdisciplinary team in 12 sub-districts of Chattogram district. The same structured questionnaire was administered to collect farmers' responses before and after the training. During pre- and post-training surveys 183 (including 10 women) and 171 (including 9 women) farmers' responses were obtained, respectively. The data were analyzed (R-software) to explore the farmers' perceptions towards farm management, vaccination practices, biosecurity measures, antibiotics, and antimicrobial resistance.

The difference between pre-and post-training surveys revealed that farmers' knowledge and understanding increased regarding multiple qualities of day-old chick selection (58%), appropriate brooding temperature (19%), different poultry disease names (2%), poultry vaccines (11%), causes of vaccine failure (17%), biosecurity measures (21%), the exact function of antibiotic (42%), names of antibiotics (22%), and antimicrobial resistance (39%). In addition, 94% of farmers anticipated learning about poultry farming-related topics in the pre-training survey, while 95% acknowledged learning about poultry farming-related topics in the post-training survey.

The study, therefore, recommends poultry farmers' training to be focused on the topics to enhance learning in areas where farmers have lower KAP.

Evaluation of poultry farmers' knowledge, attitude, and practices regarding production and biosecurity in poultry farming in Bangladesh



Qualitative social sciences at the Hub: results and legacy for future research

Ivo Syndicus¹ and the One Health Poultry Hub social science teams

¹ Veterinary Epidemiology, Economics and Public Health Group, Royal Veterinary College

The Hub set out an innovative qualitative social science agenda for understanding how zoonotic disease transmission risks are generated through people's practices in poultry production and distribution networks (PDNs). Importantly, this includes how practices are embedded within broader social, cultural and economic circumstances.

We approached this agenda through empirical research with a focus on (1) the roles of (types of) actors and (2) the relations they have with other (types of) actors along poultry PDNs. Our aim was to achieve a systemic understanding not just how but also why poultry is produced and moved in certain ways, paying attention as well to the dynamics of change that actors experience as part of their roles and (business) relations.

We pursued research through semi-structured interviews, complemented through observation to varying extent. In some sites we conducted research more broadly along PDNs (Bangladesh, Vietnam), while in others we focused on specific actors (Sri Lanka, Tamil Nadu).

In this presentation, I will illustrate this approach and results through examples from our research in Bangladesh and Tamil Nadu. Insights allow us to characterise the opportunities and constraints actors face, which structure their relations to other actors and shape specific business strategies and practices.

While we gained important insights about the studied PDNs, the main potential of this research agenda arguably lies in its further advancement by building on and refining its approach, its interdisciplinary integration and implementation in the field, which may yield significant contributions to One Health research and policy.

Concurrent study of poultry enterotypes and resistomes may enable the detection of acquired antimicrobial resistance.

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Identifying poultry farming practices that decrease abundance of zoonotic pathogens and antimicrobial resistance genes (ARGs) is needed to reduce the threat of acquired resistance in food-borne pathogens. However, surveillance of AMR is notoriously difficult because i) ARGs can spread between species via horizontal gene transfer, and ii) the vast majority of ARGs in any resistome represent normal innate resistance of a microbial population making the detection of an acquired AMR signal very difficult against the innate AMR background.

Enterotypes represent distinct microbial community phenotypes and resistomes represent population AMR phenotypes, and both are influenced by environmental and farm factors. Variation in these phenotypes can have significant effects on chicken and public health risks. Using 16S rRNA amplicon sequencing to define community composition and AMR AmpliSeq for ARG composition, we identified a striking correlation between enterotype and resistome. This correlation likely reflects that the majority of ARGs in a resistome represent the innate AMR of the species of which the population is composed. Interestingly, we also identified outliers, where enterotype-resistome correlation was weak and these may represent samples in which resistance has been acquired by horizontal gene transfer.

We discuss potential strategies to confirm this acquired resistance hypothesis using data from shotgun sequencing, antimicrobial residues, whole genome sequencing and geographical, farm biosecurity and link-tracing metadata. The integration of datasets may suggest a surveillance method to detect acquired resistance, as well as ways to model future risk.



Risks associated with disease spillover from chickens to humans

Punam Mangtani

London School of Hygiene and Tropical Medicine

New diseases jumping from animals to humans pose a major threat to health and economies worldwide. These "spillovers" caused five major pandemics in the past century, including COVID-19, and highlight the need for a "One Health" approach that considers human, animal, and environmental factors to prevent future outbreaks.

Actions include training communities to detect transmission pathways for infections including silent transmission, protecting ecosystems, sustainable safe food production including responsible animal human interactions, and well-functioning partnerships for mutual learning and response.

Poultry farming livelihood strategies and their relevance for zoonotic disease management in Northern Viet Nam

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This presentation summarises the findings from a component of social science work conducted as part of the One Health Poultry Hub's exploration of actors and structures shaping the poultry sector in Viet Nam. Research sought to explore the financial and social factors shaping farming operations, through identification of the techniques and strategies farmers employ to manage their farms, associated costs, and the actors associated with how these farming activities and finances are managed.

Drawing on qualitative data gathered through 80 interviews with 91 participants (60 male, 31 female) and field observations in three provinces of Northern Viet Nam, this empirical work has allowed us to understand how local livelihood strategies inform disease management efforts on farms.

Thus, this presentation describes some of the key strategies poultry farmers in Viet Nam employ to sustain their businesses and why. Specifically, it speaks to how choice of breeds, seasons of operation, physical farm size and infrastructure and farming modality (contract or independent) inform how farms are run. In doing so, this presentation will discuss why farming households in Northern Viet Nam choose to (or feel they must) employ these common strategies and how these strategies come together to inform potential disease emergence/transmission.

Business models and dynamics of change among Chennai's broiler wholesalers: an ethnographic exploration of live bird distribution networks

<u>Ivo Syndicus¹</u>, Vigneshvaran Paramasivam², Ganesh Janarthanan², Eve Houghton¹, Pallavi Mishra³, Kavita Yadav³, Vimal Rajkumar², Rajib Dasgupta³, Tony Barnett¹, Kumaravel Papaiyan², Guillaume Fournie^{1,4}

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Compared to the practices in production and retail of broilers, the wholesale trade of broilers is relatively less well understood. With the increasing concern about zoonotic diseases with pandemic potential transmissible by poultry, it is important to understand how and through what kinds of business models and practices broilers are moved between production areas and cities. Such understanding can contribute to establish tailored surveillance and mitigation approaches.

We took an ethnographic approach to research with wholesale live bird broiler traders in the South Indian city of Chennai, visiting premises and conducting interviews with interlocutors of 13 broiler wholesale enterprises (1 female-led, 12 male-led) and some related upstream and downstream actors over a period of 18 months in 2021 and 2022. All interlocutors were male apart from a female wholesaler and a female butcher.

Our focus in this paper is on describing the business relations between broiler wholesalers and upstream actors in production whom traders source birds from, on one hand, and their downstream wholesale clients such as retailers, caterers, and restaurants on the other hand. We go on to discuss traders' business models and associated broiler procurement and distribution practices from a perspective of disease transmission risks. Further, we situate current configurations of broiler wholesale trade within the broader historical trajectory and anticipated future of broiler commodity chains from a Chennai-centric perspective, constituting important context towards dynamic approaches to evolving disease transmission risks in changing poultry production and distribution networks.

The Research Mobility Tracking App

Joerg Henning

School of Veterinary Science, The University of Queensland, Australia

Research of mobility patterns is conducted across multiple disciplines and is most prominent in human geography, sociology and transport research. The spread of pathogens of veterinary and public health importance is also often linked to movements of animals or people. We developed a mobile phone App, that can be used by researcher to conduct their own mobility research.

The methodology for the *Research Mobility Tracking App* was developed in the One Health Poultry Hub project to track the movement of poultry traders. The tool has since been enhanced to allow its adaption for other research purposes.

Developed for Android devices, the *Research Mobility Tracking App* captures movements as well as survey data in real-time. It allows, for example, to record the path of a trader from the place of purchasing goods to the endpoint of sale. At each location where goods are traded, several questions can be asked to explore trading details at that location, such as types and numbers of goods sold or purchased.

No mobile phone reception is required to collect the movement data. All information is stored on the phone and can be uploaded to an Open Data Kit database when internet access is available. Data can be downloaded by permitted users from anywhere in the world.

The development of the *Research Mobility Tracking App* is currently being finalised. Users can download it from <u>Google Play</u>. It provides a sustainable research tool that can been used in a variety of scenarios to record movement and associated data.

Research Mobility Tracking App

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Research Mobility Tracking App in Goole Play Store

SWOT analysis on the current status of family poultry vaccination program implemented in the western province of Sri Lanka

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The poultry industry in Sri Lanka plays a vital role in the country's economy, encompassing both commercial poultry enterprises and small-scale family poultry businesses. However, the poultry sector faces numerous challenges, particularly the prevalence of vaccine preventable poultry diseases such as Newcastle disease, infectious bronchitis, infectious bursal disease (IBD), Marek's disease, fowl pox, salmonellosis, and coccidiosis. While vaccination programs have shown promise in controlling these diseases, their effective implementation is hindered by various obstacles, leading to frequent disease outbreaks.

To address these challenges, this small research activity aimed to identify and overcome the major barriers to effective poultry disease control, specifically regarding vaccination programs. A strengths, weaknesses, opportunities, and threats (SWOT) analysis was conducted involving three participant categories: eleven veterinarians (Male=7, Female=4), nine livestock development inspectors (LDIs) (Male=7, Female=2) and nine family poultry farmers (Male=8, Female=1) representing all the veterinary ranges of the Western Province. The SWOTs related to vaccination against poultry diseases were assessed by these three participant groups.

The results revealed several common concerns among the participants. Veterinarians highlighted the lack of data on antibody titres for common poultry diseases in local flocks and the unavailability of vaccines tailored to specific poultry viral strains, for diseases such as IBD, prevalent in Sri Lanka. LDIs stated the difficulties in delivering vaccines from local veterinary office to the farmers. Lack of awareness about the importance of vaccination, and financial constraints were the issues raised by farmers.

Addressing these limitations and challenges will contribute in controlling disease outbreaks and minimizing future economic losses. Furthermore, it will pave the way to a more sustainable development and growth of the family poultry sector in Sri Lanka.

Spatial distribution of poultry farms using point pattern modelling: a methodology to address livestock environmental impacts and disease transmission risks

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Distribution of farm locations and size is paramount to characterize patterns of disease spread. In regions undergoing rapid intensification of livestock production, including increased clustering of farms in peri-urban areas, measuring changes in spatial distributions is crucial for effective interventions. However, in many countries such data are not available.

We developed a farm distribution model (FDM) that allows the prediction of locations and sizes of poultry farms in countries with scarce data. The model combines a Log-Gaussian Cox process model to simulate the farm distribution as a spatial Poisson point process, with a random forest model to simulate farm sizes (i.e. number of animals per farm). Spatial predictors were used to calibrate the FDM on intensive broiler and layer farm distributions in Bangladesh, Gujarat (Indian state) and Thailand.

The FDM yielded realistic farm distributions in terms of spatial clustering, farm locations and sizes, and provided insights on factors influencing these distributions. We illustrated the relevance of modelling realistic farm distributions in the context of epidemic spread by simulating pathogen transmission on an array of spatial distributions of farms. We found that farm distributions generated from the FDM yielded transmission patterns consistent with simulations using observed data, while random point patterns underestimated vulnerability to epidemics. Indeed, spatial clustering increases vulnerability to epidemics, highlighting the need for realistic spatial clustering and farm sizes to study epidemic spread.

The FDM maintains a realistic distribution of farms and their sizes thus can inform mathematical models of transmission for regions where data are not available.



Probability of large outbreak. Average probability of large outbreak (i.e. the proportion of simulations where the attack rate exceeds 100 farms) as a function of transmissibility for long- and short-range kernels calculated for Thailand (A,B), Gujarat (C, D) and Bangladesh (E, F). The curves shown include LGCP + RF models trained in THA (red), BGD (yellow) and IN.GJ (blue). The markers denote simulations using empirical farm locations with original (black) and homogeneous (grey) farm sizes. The pink line corresponds to random farm locations with RFS-generated farm sizes. We set $\alpha = 0.643$ for long-range kernel and $\alpha = 3$ for short-range kernel.

Exploring Motivations of Poultry Farmers for Antimicrobial Use in Bangladesh: A Qualitative Investigation

Abdullah Al Sattar¹, <u>Ivo Syndicus</u>², Nusrat Irin¹, Md. Helal Uddin¹, Shariful Islam¹, Fatema Jannat¹, Chandan Nath¹, <u>Eve Houghton</u>², Guillaume Fournie², Md. Ahasanul Hoque¹

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This qualitative study explores the motivations and practices of poultry farmers regarding antimicrobial usage in Chattogram District, Bangladesh.

Drawing from a subset of Broiler and Sonali poultry farmers (n = 12, all male) in a parallel farm-based longitudinal study, the research investigates farmers' antimicrobial decisions, advice sources, influences, and access dynamics through a semi-structured interview guide.

Findings indicate that while most farmers correctly identify antimicrobials used on their farms, some face challenges in accurate identification. Prophylactic antimicrobial use is prevalent, especially during the brooding stages of each batch. This practice extends into later rearing stages, particularly in Sonali chickens, with farmers attributing it to their own experience, guidance from feed dealers, and past advice from veterinarian. Seasonal adaptation of antimicrobial types, opting for stronger ones in the winter, is observed. Therapeutic antimicrobial application guided by a veterinarian is common, with satisfaction in their cooperation and treatment effectiveness. However, instances of switching consultants or altering the prescribed antimicrobial dosage and duration during therapy were noted. The study underscores the influence of dealers on the entry of routinely used prophylactic antimicrobials into credit-dependent farms. Most farmers purchase antimicrobials and other drugs on credit, facing challenges in accessing prescribed medicines locally, necessitating distant procurement and occasional reliance on alternatives. Some farmers engage in antimicrobial stockpiling, emphasizing the need for improved availability and accessibility.

This research provides insights into the complex dynamics influencing poultry farmers' antimicrobial decision-making processes, emphasizing the necessity for targeted interventions to enhance responsible antimicrobial use in poultry farming.



Selling and management process of sick birds: a qualitative investigation

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Highly pathogenic Avian Influenza is a global concern due to its potential risk of infecting humans and causing a pandemic. In Bangladesh, farmers often sell their sick birds to minimize their economic loss and this is a major risk factor for avian influenza transmission. In this study, we identify distinct ways for farmers to sell and manage sick birds.

A qualitative study was conducted in the Chattogram district from February 2023 to May 2023. We selected six participants (all male) whose farms had morbidity or mortality of 10% or more with avian influenza or any other infectious disease suspects according to specialised poultry veterinarians. A semi-structured interview guide was followed.

We found that farmers sell and manage their sick birds in different ways based on their conditions. They sold their apparently healthy birds to their respective middlemen at a slightly lower price than the actual market price. Two farmers were found slaughtering the sick birds and storing them in the refrigerator for their own consumption while one of them sold hundreds of sick birds to his neighborhood at a low price. One of the farmers supplied his apparently healthy to a marriage ceremony via middlemen. It was also documented that one of the farmers dumped their deceased birds into the ground, while others flung them into a nearby river or pond.

This trading of sick birds from farms poses the potential risk of exposing farmers, transporters, traders, slaughterers, and even consumers to potentially deadly pathogens. We recommend a strong surveillance of poultry farms and trading processes.



Spatial traceable maps and management of live bird transportation along chicken value chains

<u>Nguyen Van Dai¹</u>, Le Thi Thanh Huyen¹, Pham Thi Thanh Hoa², Han Anh Tuan¹, Do Van Duc¹, Dao Thi Binh An¹, Le Tien Dung¹, Duong Thi Oanh¹, Nguyen Thi Phuong Anh¹, Nguyen The Vinh¹, Vu Dinh Ngoan¹, Ta Van Can¹, Ivo Syndicus³, Eve Houghton³, Joerg Henning⁴, Fiona Tomley³ and Guillaume Fournie³.

¹National Institute of Animal Science, Viet Nam; ²CIRAD, Viet Nam; ³ Royal Vet College, UK ⁴University of Queensland, Australia

This study was implemented in Hanoi and Bac Giang provinces, Viet Nam. It aimed to evaluate management practices and to trace spatial maps of live bird transportation along the value chain to identify high risk routes and points for increased social-technical One Health interventions and policy recommendations.

The methods used included in-depth interviews and note taking with 26 traders (17 men and 9 women; 13 big traders and 13 small traders), note and spatial road mapping using the bespoke Hub Mobility App on cell phones.

The results showed that big traders in both provinces bought over >800 colour chickens each trip from farmers, integrated farms, using middle men from other provinces to store or sell birds. Small traders bought <200 chickens per trip from big traders. 75% of big traders transported chickens through the two provinces with accompanying chicken health certificates whilst small traders mainly transported chickens within a single province and without chicken health certificates. Vehicle were regularly cleaned and disinfected by traders and farm owners. 83% of big traders and 69% of small traders kept chickens for 1- 3 days in the store/shop and in their homes, respectively. Most big traders sold chickens to small traders, retailers, consumers, and slaughterhouses. Clients mainly come to big traders' storage to buy chickens, while 70% of small traders delivered chicken to clients' homes.

In conclusion, there is a close relationship between large and small traders, farms, intermediaries and consumers. Therefore, bio-security control is very important not only from farm and in chicken storage, but also during chicken transportation. It is also necessary to improve the awareness about disease safety for all stakeholders along the poultry supply chain.



H9N2 avian influenza Virus: Emergence of Immune Escape Mutant with No Haemagglutination Activity

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H9N2 avian influenza viruses pose a global threat to animal and human health carrying potential pandemic risk. Vaccination plays a crucial role in mitigating the impacts of these viruses, nevertheless, the emergence of immune-escape variants impedes vaccines' effectiveness. In this study, we identified an immune-escape variant that also contributes to the loss of agglutination of chicken erythrocytes and decodes the molecular mechanism of immune escape mutations that underpins the loss of agglutination of chicken erythrocytes, replication fitness and stability.

The immune-escape variant acquired an amino acid substitution at position 149, replacing glycine (G) with glutamic acid (E) in the H9HA protein (G149E) lost the ability to agglutinate chicken erythrocytes, while still maintaining replication comparable to the wild-type virus *invitro* and *in-ovo*. Further, it was demonstrated that the charge of amino acid at immune escape mutation determines their haemagglutination activity; the substitution of negatively charged amino acid found to be responsible in the loss of the agglutination activity. The additional residues at positions 215, 216, and 217 were also found to be responsible for modulating H9N2 virus haemagglutination activity.

These findings highlight that the H9N2 virus may be likely acquire the G149E mutation under immune pressure in nature, challenging the vaccination and surveillance efforts as it evades immune protection and is not easily detectable by conventional haemagglutination assays. This underscores the intricate interplay between antigenic variation and viral traits, emphasising the critical need for ongoing surveillance and research to effectively mitigate the risks associated with avian influenza H9N2 viruses.



Keywords: influenza, immune escape mutants, haemagglutinin, haemagglutination

Graphical presentation of the proposed model for loss of the ability to agglutinate chicken erythrocytes with negatively charged H9HA.

Non-Typhoidal Salmonella in Humans in India, Vietnam, Bangladesh and Sri Lanka: A Systematic Review

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Non-Typhoidal Salmonella (NTS) infections usually present as self-limiting diarrhoeal illness although ~5% of cases develop bacteraemia or invasive infection. Invasive NTS (iNTS) is associated with severe life-threatening complications such as sepsis and rising incidence of antimicrobial resistance (AMR) increases risk of mortality. We conducted a systematic review to estimate the proportion of NTS isolated, serovar burden, serovar-specific AMR, and case fatality rate (CFR) rate in the four hub countries. We identified 13936 articles, after screening 1717 were reduced to 73 eligible articles between 1980-2020. The studies were from India (57/73) Vietnam (9), Bangladesh (5) and Sri Lanka (2). The pooled estimate of the proportion of invasive NTS (iNTS) isolated was 0.3% (95% CI:0.1%-0.5%), whereas NTS was 2.1% (1.2-3.2%). The common serovars were S. Typhimurium, S. Enteritidis, S. Weltevreden and S. Worthington. Only S. Enteritidis, S. Paratyphi B Var Java, S. Typhimurium and S. Weltevreden were present in all four countries. MLST data were available but 809/958 isolates were from Vietnam. 98 different ST's were reported but only ST11 (S. Enteritidis) was present in all countries. The pooled estimate of proportion of NTS demonstrated that AMR was common in isolates however in iNTS isolates AMR was mainly present in Vietnamese isolates with Indian isolates being highly sensitive except to nalidixic acid. Multi-drug resistance was estimated at 30.2% (2.1%-68.2%) in India for NTS and 22.3% (0.0-66.8%) iNTS, whereas it was 41.9% (21.1%-64.3%) for NTS in Vietnam and 41.2% (33.6% -49.3%) in iNTS. NTS infection is a concern with the emergence of AMR especially in low- and middle-income countries.

Ion	Identification of studie	es via databases and registers	India					Vietna	m ^{* hid}	Testers'		Vaporen (935-Q
Identification	Records identified from: Databases and Registers (n = 13,936)	Duplicate records removed. (n = 6,118)	NTS			0000. 1030 pt 4000 pt	на, а. (1937) а. (1937) а. (1938) а. (1	Annorana Alicente SLIVII SLIVI	alia 2004 20 alia 2004 1 5 2004 2 insurie 2004 2 alia 2006-20 5 3 alia 2006-20 5 3 alia 2006-20 5 4 alia 2014-2006 40	+		25-1 (110.427) 51 1 (250.042) 001000259 251 (454.829 - 206.0550.900) 350 (216.409 550 (262.06)
	Records screened. (n = 7803)	Records excluded (n = 6086)	iNTS				n wate Mari Mari Mari Mari Mari Mari Mari Mari	numerics.epstyle	inne 200-20 i	1 × 1	0+1 ()	61.0 (21.0, 700) - 62.0 (20.7, 615) 66.2 (20.3, 700) 70.2 (20.4, 700)
Screening	Reports sought for retrieval. (n = 1717)	Reports not retrieved. (n = 0)	Top ten	Ampicillin Resistance						¹⁰ Pastoringe	12	Ъ.
	Reports assessed for	Reports excluded	Rank	1	2	3	4	5	6	7	8	9
	eligibility. (n = 1717)	(n=1644)	India	S. Typhimurium (898, 29.0%)	S. Weltevreden (322, 10.4%)	S. Worthington (010, 10.2%)	S. Bareilly (255, 8.2%)	S. Newport (151, 4.9%)	S. Enteritidis (145, 4.7%)	S. Infantis (132, 4.3%)	S. Cholerasuis (61, 2.0%)	S. Kentucky (46, 1.5%)
Included	(n = 72)	PRISMA 2020 flow	Vietnam	S. Typhimurium (299, 34.7%)	S. Enteritidis (93, 10,8%)	S. Weltevreden (80, 9.3%)	S. Stanley (75, 8.7%)	S. Newport (42, 4.9%)	S. Rissen (20, 2.3%)	S. Java (16, 1.9%)	S. Cholerasuis (15, 1.7%)	S. Kentucky (12, 1.4%)
		diagram	Bangladesh	Group C1 (693, 34.2%)	Group B (533, 26.3%)	Group C2 (254, 12.6%)	Group E (186, 9.2%)	Group D (119, 5.9%)	Group G (79, 3.9%)	S. Typhimurium (21, 1.0%)	S. Java (16, 0.8%)	S. Enteritidi: (6, 0.3%)
			Sri Lanka	S. Ententidis (21, 50.0%)	S. Java (4, 9.5%)	S. Weltevreden (4, 9.5%)	S. Corvallis (3.7.1%)	S. Chester (3, 7.1%)		S. Typhimurium (1, 2.4%)	S. Stanley (1, 2.4%)	S. Mbandaka (1, 2.4%)

Non-Typhoidal Salmonella in Humans in India, Vietnam, Bangladesh and Sri Lanka: A Systematic Review

A Description of the Disease-associated Morbidity and Mortality in Semi-intensive Chicken Farms in Bangladesh, India, and Vietnam

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The rapid expansion of semi-intensive poultry production in South and Southeast Asia presents challenges for monitoring due to the lack of data collection and surveillance systems. This study aimed to investigate reported disease-associated morbidity and mortality rates on farms in the region.

A cross-sectional study was conducted on 251 semi-intensive broiler chicken farms from Bangladesh, Vietnam, Tamil Nadu (India), and Gujarat (India) between April 2021 and June 2022. Farm characteristics were collected through site visits and interviews with all relevant personnel on the farm. Famers' reported morbidity and mortality rates were estimated and standardized per 1000 chicken-days, and statistical comparisons were carried out using student's *t*-test. Biological and environmental samples were collected to detect *Campylobacter spp.* (C. *coli* & C. *jejuni*), non-typhoidal Salmonella, and avian influenza virus (H5 & H9).

There was an average of 3,525 chickens on each farm (Range: 100 - 27,000) across all four sites. A wide range of morbidity and mortality rates was reported, with an overall average of 1.70 (per 1000 chicken-day; Interquartile Range (IQR): 3.17 - 13.75) and 3.95 (per 1000 chicken-day; IQR: 0.70 - 3.33), respectively. Among the sites, Bangladesh exhibited the highest morbidity rate, while Gujarat exhibited the highest mortality rate. Notably, Vietnam had the lowest morbidity and mortality rate across all four sites.

Despite potential survival bias related to highly pathogenic avian influenza, recall bias from farmers, and disparities in farm sizes, this study highlights the variation in disease-associated morbidity and mortality rates within and between all four sites in the region.

Longitudinal study of antimicrobial residue in broiler meat in Vietnamese and Bangladeshi farms

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Antimicrobial Drugs (AMD) are allowed in commercial poultry farming but withdrawal periods should be respected to limit customer exposure to residues. This longitudinal study reports on incidence of AMD residues in chicken meat during a production cycle in Vietnam and Bangladesh farms.

Meat samples from 558 healthy chickens were collected at 3-4 sessions on 100 Bangladesh farms (70 white chicken and 30 Sonali) and 60 Vietnam farms (coloured chicken). Collection times were 7-12 days (session 1), 30-35 days (session 2), end of production cycle (session 3) and end of trading (session 4). Chicken breast samples were frozen (-80°C) until screening for AMD at Maximal Residue Level (MRL) using LC/MS method (EU ref laboratory), validated for a panel of 70 AMD \geq MRL. A sample was positive when at least 1 AMD was detected.

In Bangladesh, 68 farms had a positive sample along the production cycle. Positives at session 4 were seen in 26/90 farms (29%) with the same AMD (tilmicosin) found in 9 farms at session 3. In Vietnam, 48 farms had a positive sample during production; at session 4 there were positives in 6/60 farms (10%). In no case was the same AMD found at session 3. Fewer session 4 samples were positive in Vietnam than Bangladesh (p=0.006, chi-square test)

Late administration could be responsible for high prevalence of residue at the end of trading, especially AMD with long withdrawal periods such as as tilmicosin (12 days).



RESULTS ≥ 1 MRL

	Bangladesh (100)	Vietnam (60)
Farms positive (any session)	68 /100	48 /60
Farms positive Session 04	26 /90 (29%)	6 /60 (10%)
Farms positive Sessions 03 and 04	18 /90	0 /60

Genotypic characterization of antimicrobial resistance in Non-Typhoidal Salmonella poultry isolates using Multilocus Sequence Typing

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Salmonella is one of the important pathogens affecting poultry which is considered a major source of human food-borne infections. Apart from food- borne infection, increasing antimicrobial resistance (AMR) among Salmonella species can be a potential threat to public health. Furthermore, the innocuous presence of non-typhoidal salmonella (NTS) in poultry, poses a threat as AMR may be a source for transfer to other members of the gut microbiome. In this study we have investigated the prevalence of Salmonella species among native chickens in the northern region of Tamil Nadu.

A total of 622 samples including cloacal swabs and environmental samples were collected from apparently health native chicken and poultry farms. NTS was isolated and characterized phenotypically and genotypically. Salmonella isolates were subjected to MLST to identify their relatedness to existing isolates followed by identifying their antibiotic resistance patterns.

A total of 26 NTS isolates were obtained and subjected to MLST which revealed 8 serovars including *S*. Enteritidis, *S*. Typhimurium and *S*. Kentucky to be the most prevalent serovars followed by Agona, Infantis, Saintpaul, Bareilly and Weltevreden. Serovar Saintpaul was reported in poultry for the first time in India. Our results reveal that MLST analysis differentiates serovars with similar antigenic structures which are indistinguishable by conventional serotyping. AMR profiling of Salmonella isolates revealed 100% resistance to 3rd generation cephalosporins, followed by fluoroquinolones (84.61-92.30%), with sensitivity (100) to chloramphenicol. Multiple drug resistance was observed in all isolates with *S*. Kentucky isolates resistant to more than 9 antibiotics. Genotypic characterization of AMR revealed the presence of *TetA*, *blaTEM*, *Sul1* and *aphA1*, *StrA/B*, encoding resistance to tetracycline, β-lactams, sulphonamide and aminoglycosides respectively.

The presence of multidrug resistant NTS in backyard poultry is a potential hazard to the farming community due to possible transmission of resistance genes among bacterial species. Isolation of S.Enteritidis in common house Gecko from poultry sheds could be of much epidemiological significance in transmitting to humans.

Occurrence and antimicrobial resistance in zoonotic food-borne bacterial pathogens isolated from broiler farms and live bird shops in Tamil Nadu, India

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Multidrug-resistant (MDR) food-borne pathogens that cause infections are typically zoonotic and possess a greater challenge to the supply and consumption of safe and healthy animal-sourced foods. This study aimed to assess the prevalence and the <u>antimicrobial susceptibility</u> (AST) profiles of bacterial pathogens isolated from commercial broiler farms and live bird shops (LBS) in Tamil Nadu state of India.

A total of 1050 samples consisting of 150 environmental boot shocks, 150 cloacal swabs and 750 caecal swabs were collected from commercial broiler farms, commercial broiler and desi chickens in LBS between October 2021 - May 2022. AST for all bacterial isolates were performed using the disk diffusion method with 17 commercially available antimicrobial drugs.

The occurrence of *E. coli*, Non–Typhoidal *Salmonella* (NTS) and *Campylobacter* was found to be 93.3%, 42.6% and 32% respectively. *E. coli* isolates showed high frequency of resistance to Clindamycin (95.7%), followed by Tetracycline (94.3%), Ciprofloxacin (90.7%), Nalidixic Acid (86.4%), Azithromycin (84.3%), Co-Trimoxazole (80.7%), Vancomycin (79.3%) and Ampicillin (52.9%). NTS showed high resistance to Clindamycin (100%), Vancomycin (98.4%), Tetracycline (87.5%), Ciprofloxacin (81.3%), Nalidixic Acid (73.4%) and Ampicillin (51.6%). In the case of *Campylobacter*, the resistance pattern was Ciprofloxacin (92.3%), Azithromycin (85.2%), Clindamycin (85.2%), Erythromycin (84.5%), Tetracycline (83.1%), Nalidixic Acid (80.3%) and Ampicillin (71.1%) respectively. The isolates of E. *coli* (100%), *NTS* (93.75%) and *Campylobacter* (95.7%) were not susceptible to at least 3 antimicrobial drugs.

Our results highlight that, irrational usage of antibiotics in farms leads to development of antimicrobial resistance (AMR). Further policy engagement strategies needed to prevent and control food-borne bacterial pathogens in poultry.

Genomic Profiling of Antimicrobial Resistance in Poultry Litter Bacterial Community

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Global demand for protein-rich food has intensified livestock production, particularly in developing nations, where antibiotics are extensively used to promote growth and prevent diseases. Poultry manure is commonly utilized as fertilizer in agro-ecosystems. Previous studies link potential adverse environmental effects during land applications due to the release of zoonotic bacteria and antimicrobial resistance (AMR) genes.

This research delves into comprehensive profiling of AMR genes from 11 bacterial poultry (broiler and layer) litter isolates, providing evidence of multidrug resistance (MDR). Antimicrobial Susceptibility Testing (AST) to discern the resistance patterns was carried out along with 16s rRNA sequencing and MALDI-ToF to aid rapid, accurate identification and phylogenetic insights into MDR microbial composition. Biofilm formation assays to gauge potential virulence and Biolog GEN III carbon utilization added a metabolic dimension to the analysis, elucidating adaptive strategies. The integration of whole-genome sequencing (WGS) enhanced precision, unravelling the genetic basis of resistance.

Chi-square test was used to compare percentages of resistant, intermediate and susceptible phenotypes in poultry litter isolates against all 19 antibiotics tested and found to be significant. The concordance correlation elucidates relationships between AMR phenotype and genotype by underscoring MDR identification. This investigation highlights the importance of a multifaceted approach to understanding antibiotic resistance, emphasizing the importance of genomics in discerning intricate resistance mechanisms.



Endophytic bacterial community reveals antimicrobial resistance in response to poultry-manure application

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Poultry manure is widely used as fertilizer in agriculture during the cultivation of crops. However, the persistent high-level use of antibiotics in poultry production has raised concerns about selecting reservoirs of antimicrobial resistance genes (ARGs). Previous studies have shown that the addition of poultry manure can increase the abundance of genes associated with resistance to tetracyclines, aminoglycosides, fluoroquinolones, sulfonamides, bacitracin, chloramphenicol, and macrolide-lincosamide-streptogramin in soil and plants. Understanding the microbial populations harbouring these ARGs is essential to identify microorganisms that could enter the human food chain. Here, we test the hypothesis that environmental exposure to poultry manure increases the occurrence of antimicrobial resistance (AMR) in plant endophytes using selective culture, phenotypic Antibiotic Susceptibility Testing (AST), phylogenetic analysis, and whole genome sequencing (WGS).

Endophytes from poultry manure treated *Sorghum bicolor* (L.) Moench plant root and stem samples showed increased phenotypic and genotypic resistance against multiple antibiotics compared to untreated controls. Comparison of AMR phenotype-to-genotype relationships highlighted the detection of multi-drug resistant (MDR) plant endophytes, demonstrating the value of genomic surveillance for emerging drug-resistant pathogens.

The increased occurrence of ARGs in poultry manure-exposed endophytes highlights the need for responsible antibiotic use in poultry and animal farming to reduce contamination of ecological niches and transgression into endophytic plant microbiome compartments. It also emphasizes the requirement for proper manure management practices and vigilance in monitoring and surveillance efforts to tackle the growing problem of antibiotic resistance and preserve the efficacy of antibiotics for human and veterinary medicine.



The prevalence of poultry-related foodborne pathogens along the farm-to-fork continuum in the poultry industry in Sri Lanka

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Foodborne pathogens are a concern to the global poultry industry due to their impact on food safety and public health. *Campylobacter* and *Salmonella* from chickens are considered foodborne pathogens, but their prevalence in Sri Lankan poultry production systems is not clear.

Cloacal, caecal, and environmental samples were collected in a cross-sectional study conducted in large-scale poultry processing plants (n=7, three batches per plant) and wet markets (n=13) from Oct to Dec 2023. Sites were selected based on their contribution to national poultry meat production. Conventional isolation techniques were used to detect *Campylobacter* species and non-typhoidal *Salmonella*, followed by specific biochemical tests for identification.

In processing plants, *Campylobacter* was recovered from 40.7% (17/42) of caecal samples and 23.8% (5/21) of environmental samples. *Salmonella* was detected in 66.6% (14/21) of environmental samples. In wet markets, *Campylobacter* was recovered from 73.1% (19/26) of caecal and 61.5% (8/13) of environmental samples, while 53.8% (7/13) of environmental samples were positive for *Salmonella*. Selective culture for *Escherichia coli* was also undertaken as a sentinel of antimicrobial resistance genes. *Escherichia coli* was recovered from 76.1% (16/21) of cloacal swabs from processing plants and 100.0% (13/13) swabs from wet markets.

The risk of poultry foodborne pathogen contamination in Sri Lankan food chains was highlighted in this study, recommending a national surveillance program for foodborne pathogens. Work to define the antimicrobial resistance profiles of bacteria isolated here is ongoing.



Prevalence of zoonotic food-borne pathogens across commercial broiler farms and live bird shops in South India

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Poultry meat and eggs are vital for supplying safe and nutritionally adequate food globally. However, the poultry food chain has the risk for transmission of food-borne pathogens such as Non Typhoidal Salmonella (NTS) and *Campylobacter*. Hence this study was planned to explore prevalence of food-borne pathogens (FBPs) from commercial broiler farms and live bird shops (LBS) in Tamil Nadu State of South India.

In this study a total of 50 exotic broiler farms and 50 live bird shops from five cities were selected based on the production and distribution data. In each farm, five exotic broilers, and in each LBS, five exotic broilers, as well as five commercial desi chickens, were randomly chosen for sampling. The samples consisted of cloacal swabs for *E. coli*, caecal contents for *Campylobacter* and environmental swabs for NTS. The collected samples were subjected to microbiological analysis and PCR confirmation tests for the above pathogens.

The overall prevalence of *E. coli*, NTS and *Campylobacter* in commercial broiler farms and LBS was 93.3%, 42.6% and 32%, respectively. Whereas the farm prevalence rates for *E. coli*, NTS, and *Campylobacter* were found to be 94%, 46%, and 23.2%, respectively, in LBS, *E. coli*, NTS, and Campylobacter had prevalence rate of 96%, 42% and 34.8%, in commercial broilers, and 90%, 40%, and 38% in commercial desi chicken, respectively.

These results denote the significant prevalence of food borne pathogens in the broiler food chain in Tamil Nadu state of South India.

Colonization of zoonotic bacterial pathogens and associated resistance in village chickens in Sri Lanka

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In Sri Lanka, village chickens play a significant role in the rural agricultural landscape. The traditional practices of raising these indigenous chickens contribute to the preservation of local poultry genetic diversity and offer sustainable alternatives for resource-poor farmers. Unlike commercial poultry breeds, village chickens exhibit resilience to diseases but the available literature to prove the fact is sparse. The occurrence of zoonotic foodborne bacterial pathogens with their resistance profiles have not been studied in Sri Lanka. Nevertheless, there is a growing demand for meat and eggs of village chickens. Hence, the current pilot study aims to identify the presence of the above pathogens and their phenotypic resistance to commonly utilized antimicrobials in village chickens.

As of December 2023, thirteen family poultry farms situated in the Western Province were visited to collect environmental faecal sample to detect non-typhoidal *Salmonella*. From each farm randomly picked village chicken was slaughtered to collect caeca and cloacal swabs for isolation of *Campylobacter* and *E*. coli respectively. All the tested cloacal swabs were positive for *E. coli*. The *Campylobacter* colonization was 8% (1/13). Three farms out of 13 (27%) were positive for non-typhoidal *Salmonella*.

Based on the results, village chickens are not free from tested pathogens. The resistance profiles of the isolated *Salmonella* and *E.coli* is being tested. Continuation of the study including a larger population may warrants drawing conclusions and comparisons with commercial chickens. Comparison with broiler colonization values obtaned under the WP7 activity is appropriate as the sampled farms are located in the same geographical area and same protocols were utilized. Hence, partial funding by OHPH-SL is greatly acknowledged.

Seroprevalence of SARS-CoV-2 in live poultry market workers in Dhaka, Bangladesh in 2022

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Live poultry markets (LPMs) are important in many settings including Bangladesh for fresh animal protein and other food items. The high population density, limited biosecurity and ventilation in live poultry markets in Dhaka, Bangladesh, can facilitate transmission of airborne infectious diseases including SARS-CoV-2.

During February and March 2022, we measured the prevalence of anti-SARS-CoV-2 antibodies in randomly selected poultry and vegetable market workers alongside a pilot cross-sectional survey of avian influenza risk in 15 LPMs, and we investigated its association to socio-demographic characteristics and COVID-19 vaccination history.

We recruited 291 participants (204 poultry workers and 87 vegetable sellers). COVID-19 vaccine uptake was similar in both groups (respectively 69% and 67%), with the most common vaccines being whole inactivated (SINOVAC or SINOPHARM; 44%). Nearly all (99%) participants were positive for anti-S antibodies, which can be elicited by natural infection in the distant past and vaccination, whereas the prevalence of anti-N antibodies, indicative of recent infection, was 59.4% (95%CI 54.7% to 64.0%). The latter was similar in poultry (59.8%; 95%CI: 55.7 to 63.7) and vegetable sellers (58.6%; 95%CI: 46.2 to 70.1) (p=0.890). There was no evidence that the anti-N antibody seroprevalence was associated with any of the participants' characteristics, except for COVID-19 vaccination (adjusted OR 0.49; 95%CI 0.23 to 1.02; p=0.093).

The near ubiquity of anti-S and high prevalence of anti-N antibodies are consistent with SARS-CoV-2 high transmission rate in our study population from the three waves prior to the survey. This underlines the potential of LPMs to amplify transmission of airborne pathogens like AIV, and their importance as hotspots to monitor closely. This study also provided a template and capacity building experience for surveys of the risk and extent of future new and emerging infections in wet markets.

The use of Nanopore sequencing to identify plasmid-borne and chromosomally integrated antimicrobial genes from chicken microbiomes in Gujarat.

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Antimicrobial resistance (AMR) poses an enormous threat to global public health. The poultry industry in Asia is at high risk for the emergence of AMR due to the widespread use of antibiotics and rapid rate of intensification. Bacteria acquire and spread AMR via the horizontal gene transfer (HGT) of antimicrobial resistance genes (ARGs) on mobile genetic elements. The attainment of AMR in pathogens of humans such as *Campylobacter, Salmonella* and *Escherichia coli* is a significant risk. However, because ARGs can be carried on plasmids or chromosomally integrated into bacterial genomes, identifying the bacterial hosts of ARGs is extremely difficult.

To investigate the plasmidome and bacterial hosts of ARGs in chickens, the caecal microbiomes of 15 chickens from farms and markets in Gujarat were sequenced using long read Oxford Nanopore sequencing of unamplified DNA. The reads were assembled and contigs were screened using the machine learning tool, Plasmer, to identify putative plasmids.

A total of 3881 putative plasmids were detected, of which 479 had hits to known plasmids in the NCBI Plasmid Database (PLSDB), suggesting significant plasmid novelty in these samples. The assembly was also binned to form metagenome assembled genomes (MAGs). The MAGs were taxonomically classified using the Genome Taxonomy Database toolkit (GTDB-tk) and screened for chromosomally integrated ARGs against the Comprehensive Antibiotic Resistance Database (CARD). MAGs identified as *E coli* and *Campylobacter* included several hits to ARGs.



Are there ARGS in plasmids or in zoonotic pathogens?

Exploring an Establishment Chronicles of Microbial Community and Antimicrobial Resistance Genes in Broilers through High-Throughput Sequencing

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This investigation was undertaken to delineate the taxonomic composition of the microbial consortium inhabiting the caecal contents of broiler chickens and simultaneous profiling the antimicrobial resistance (AMR) genes from 0 to 42 days of their life.

Six replicates, each comprising 30 broiler chickens, were reared with standardized feeding protocols, encompassing pre-starter, starter, and finisher feeds, until the market age of a 42-day period. Weekly collections of caecal content samples were systematically conducted throughout the experimental duration. Extracted metagenomic DNA was used for microbiome and AMR gene sequencing to facilitate microbiome analysis and AMR gene profiling.

Examination of the caecal microbiome unveiled a dynamic structure, marked by fluctuations in the relative abundance of diverse microbial taxa during the life span of broiler birds from 0 to 42 days. The microbiome predominantly featured Firmicutes, Bacteroidetes, Proteobacteria, and 19 distinct phyla, with noteworthy shifts in their proportional representation across different growth stages. Furthermore, AMR gene profiling disclosed the presence of diverse resistance genes within the caecal microbiota, including those imparting resistance to antibiotics such as tetracycline, macrolide, and aminoglycoside.

This study yields valuable insights into the dynamic characteristics of the caecal microbiome in broiler chickens, shedding light on its compositional intricacies and temporal dynamics. Additionally, the identification of AMR genes within the microbiota underscores the potential of poultry as reservoirs for these genes. These findings underscore the significance of continuous monitoring and implementation of management strategies to curb the dissemination of AMR in the poultry industry, with consequential implications for public health.

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	0 Day	7 Day	14 Day	21 Days	28 Days	35 Days	42 Days
100	Clostridium	Phocaeicola	Helicobacter_D	Helicobacter_D	Phocaeicola	Phocaeicola	Lachnospiraceae_X
Genus	Escherichia	Helicobacter_D	Alistipes	Alistipes	Lachnospiraceae_X	CAG-266	Caccocola
	Niameybacter	Bacteroides	Faecalibacterium	Bacteroides	CAG-266	Lachnospiraceae_X	Phocaeicola
and a	aph3prime-III	tet32	tet32	tet32	tet40	tet40	aph3
AMR	aphA3	tet40	aph3prime-III	tetW	tetQ	aph2prime-la	tetW
gene	tet32	tetA	aphA3	tet40	InuB	InuC	ant6prime-lb

Distribution of highly abundant microbial composition (Genus level) and AMR gene from 0 day to 42nd days of age in Broiler Chicken

Investigating the presence of stalk directed avian influenza antibodies in highly exposed occupational workers in live bird markets in Bangladesh

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Studies have shown repeated exposure to influenza induces broader cross-reactive antibodies, such as those targeting the stalk region of the binding glycoprotein haemagglutinin (HA) which may reduce the risk of infection. We hypothesise similarly that repeated exposure to avian influenza viruses (AIVs) induces such antibodies and those in persistent close proximity to poultry where H9N2 and H5N1 AIVs are endemic may have some cross-protective immunity.

Naso-pharyngeal swabs and serums were obtained from 204 poultry (higher AIV exposure) and 87 vegetable stall (lower AIV exposure) workers randomly selected in 15 live bird markets (LBM) in Dhaka, Bangladesh during a cross-sectional survey in February 2022. Each serum sample was examined by ELISA for strength of binding to the stalk in comparison to the full (head and stalk) HA using chimeric HA proteins, incorporating an H10 head and H5 or H9 stalk and swabs were PCR tested for AIV.

All participants had negative swab PCR. ELISA found greater binding to H5HA than H9HA, despite lower isolation levels of H5 in poultry across Bangladesh. This may be due to greater cross-reactivity of H1 to H5 HA. Additionally, there was higher binding to the chimeric H10/H9 than the full H9 HA, which may be due to possible exposure to H10 or cross-reactivity of other exposed subtypes to H10.

Further investigation is ongoing utilising chimeric proteins engineered with H13 head and stalk only proteins to better quantify stalk antibodies. Future efforts will also assess the relationship of these binding antibodies in vitro with microneutralisation assays.
Mapping antigenic diversity to investigate putative antigenic residues of H5 avian influenza viruses

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Avian influenza viruses (AIVs) continue to circulate within and between wild and domestic avian populations, with devastating socio-economic consequences. Highly pathogenic avian influenza (HPAI) H5 viruses, from the A/Goose/Guangdong/1/1996 lineage, have diverged into several (>30) clades and subclades. Among these, clade 2.3.4.4 has caused substantial epizootics and remains dominant in Europe. Vaccination is one of the key approaches for reducing AIVs impact in poultry. However, the rapid genetic evolution and consequential antigenic drift results in vaccine failure. Therefore, this study investigated the key residues of the haemagglutinin (HA) which contribute to the antigenic evolution of H5 AIVs.

Candidate vaccine viruses representing recent and contemporary HPAI H5 clades were propagated using reverse genetic (RG) techniques. Cross-reactivity of antisera raised in chickens, were evaluated by haemagglutination inhibition (HI), and visualised using antigenic cartography. Putative antigenic residues predicted using in *silico* methods were introduced site-directed mutagenesis to a candidate RG and serologically analysed.

The results demonstrated that different H5 clades carry significant antigenic heterogeneity. The current dominant H5 clade 2.3.4.4 was antigenically different from other genetically distinct clades 1 to 2.3.2.1a. The strains within the 2.3.4.4b subclade were all antigenically similar; however, they were antigenically different from other 2.3.4.4 subclades (a-h). We also observed a positive correlation between the putative antigenic epitopes and virus antigenicity.

Control of AIVs relies on vaccines that are matched to viruses currently circulating within bird populations. Our data will allow for the generation of vaccines that are better strain-matched, thus reducing the impact of AIVs in the poultry industry.

Campylobacter and Non-typhoidal *Salmonella* infection among chicken shop workers in Tamil Nadu, India

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There is paucity of data on *Campylobacter* and Non-typhoidal *Salmonella* (NTS) infection among poultry workers in India. This study ascertains prevalence and risk of infection among individuals employed in chicken shops in Tamil Nadu.

This cross-sectional study was conducted from Dec 2021 - Apr 2022 in 5 cities (Coimbatore, Namakkal, Trichy, Chennai, Tirunelveli). Ten chicken shops per city were randomly selected from previously geospatially mapped poultry distribution networks and five vegetable/fruit shops within 5 meters of a poultry shop were selected. Two workers, ≥18 years old were approached from each shop to achieve a sample size of 100 and 50 respectively. The piloted questionnaire was administered by trained field workers and stool samples collected within a day. Samples were processed on site intoCary Blair medium and Campylobacter Preston Enrichment broth before transporting CMC Vellore for selective culture on blood agar, xylose lysine deoxycholate agar and deoxycholate citrate agar (NTS) and modified charcoal cefaperazone deoxycholate agar/Campy-BAP (*Campylobacter*).

From 105 participants from chicken shops and 50 from vegetable shops (all men) there was comparable prevalence of NTS with 7.6% (8/105) and 6% (3/50) positivity rates suggesting similar risk profiles for workers in both types of shops. Three (2.9%) chicken shop and 3 (6%) vegetable shop workers were positive for *Aeromonas;* 1 (2%) vegetable/fruit shop worker for *Shigella flexneri* and none for *Campylobacter*. The prevalence of NTS is slightly higher to a pooled estimate (2.1%; 95% confidence interval: 1.2-3.2%) from 4 southeast Asian countries (India, Bangladesh, Sri Lanka and Vietnam). However, there was no significant difference in risk of infection between chicken shop workers and vegetable shop workers. Failure to detect *Campylobacter* may be due to its fastidious nature or the transport time between collection and laboratory processing; for future such studies, molecular methods may offer higher sensitivity for detecting *Campylobacter*



Estimating the prevalence of avian influenza in chickens in Bangladesh

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Pathogens such as avian influenza viruses (AIVs) can be common in poultry and challenge human as well as poultry health. The aim of this cross-sectional study is to assess occurrence of AIVs at source of origin i.e. farms, and terminal trading points i.e. live bird markets (LBM) as a prelude to genetic studies of pathogen flow and human exposure.

Chickens were sampled in Dhaka, Chattogram, Cumilla, Bogura, Jessore, and Joypurhat from Aug 2021 - Jan 2022. LBMs were selected randomly in each city and for each LBM two dealerbased, credit-dependent, farms raising either exotic broiler or Sonali (crossbred Fayoumi/Rhode Island Red) within the LBM's catchment area were selected based on link tracing. Oropharyngeal and cloacal swabs were collected into viral transport medium and tested by real-time reverse-transcription polymerase chain reaction (rRT-PCR). Positive samples were sequenced. Bayesian models were fitted to the data to estimate AIV prevalence and how it varied with the type of chickens and site.

Fifty LBMs were sampled for exotic broiler, Sonali and Deshi chickens as well as the 50 exotic broiler and 50 Sonali farms. In total 593 of 3677 birds (16%) were positive for AIV H9N2; 582 of these were from LBMs and 11 from farms. Prevalence of H9N2 was estimated at 23.1% (95% HDI: 19.4-26.9%) in LBMs and 0.7% in farms (95% HDI: 0.1-2.1%). In LBMs, Sonali chickens, chickens in Jashore markets, chickens in mixed markets, and chickens sampled in autumn had higher odds of testing positive. Fourteen birds were positive for H5N1;13 from LBMs and one from farms, and the estimated prevalence in LBMs was 0.6% (95% HD: 0.2-1.2%).

Considering the low prevalence of AIVs in farms, viral amplification occurs either during transport or during time spent at the LBMs. Factors associated with higher prevalence suggest that longer journeys from farms to markets are implicated in viral amplification. Detailed genetic analysis of viral flow through poultry production and distribution networks could shed more light on the amplification.



Conjugation mediated transfer of Extended Spectrum of β -Lactamases resistance among Poultry Enterobacteriaceae isolated from health native chicken

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Antimicrobials as feed additives could be a driving source for horizontal transfer of AMR among gut microbiome. The present study was proposed to assess horizontal transfer of ESBL plasmid among members of Enterobacteriaceae isolated from native chicken in the presence and absence of antibiotics.

E. coli, Salmonella and *Klebsiella* were isolated from chicken and environment and antimicrobial susceptibility testing was carried to assess the resistance pattern. ESBL phenotype was identified in 8.51%, 37.5% and 7.41% of isolates of *E. coli, Klebsiella* spp. and *Salmonella enterica* respectively. Genotypic characterization of ESBL isolates revealed that *blaTEM* is the most common gene present in the poultry ESBL isolates. Further among ESBL genotypes, *blaTEM, blaSHV* and *blaOXA* of *Klebsiella* spp. were present in plasmids. Whereas, 87.5% of *E. coli* isolates possessed chromosome encoded ESBL resistance. Conjugation mediated transfer of ESBL resistance was studied among field isolates of ESBL and non-ESBL isolates. *In vitro* conjugation studies revealed that *Salmonella enterica* Enteritidis more efficiently transferred ESBL resistance to *Klebsiella* spp. and *E. coli* and found that only *Salmonella enterica* Enteritidis carrying *blaTEM* was able to transfer ESBL plasmid to *E. coli* and *Klebsiella* spp. after exposure to sub-lethal concentration of ceftazidime.

In conclusion, isolates carrying transferrable ESBL resistance could transfer ESBL plasmid even after exposure to sub-lethal concentration of antibiotics.

Virulence profiling to differentiate avian pathogenic and avian fecal *Escherichia coli* in poultry

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Emergence of Avian Pathogenic *Escherichia coli* (APEC) among genetically diverse commensal populations could be driven by intensification of poultry farming. Hence, the present study was proposed to assess the various virulence genes in *E. coli* isolated from poultry.

A total of 73 APEC isolates and 15 Avian Fecal *E. coli* (AFEC) were isolated and confirmed by PCR targeting the *Adk* gene. Virulence gene profiling was carried out for 13 genes identified by genome wide association study *viz. wzzB, fimD, eygS, papD, wcaJ, gnd, gltS, hisB, hokA, hokC, ompT, wcaJ* and *gspO* followed by antimicrobial susceptibility testing. The virulence gene profiling by PCR showed that considerable differences were found in the prevalence of *ompT, gspO, fimD* and *hokA* among the isolates for APEC and AFEC obtained from different age groups for layers. Antimicrobial susceptibility tests revealed that all the isolates were resistant to two or more antimicrobials tested. Further, 23.9 % isolates showed Extended Spectrum Beta Lactamases (ESBL) phenotypes by double disc diffusion test.

In conclusion, certain virulence genes can be employed as markers to differentiate APEC and AFEC. Presence of multidrug resistance and ESBL isolates in poultry could be a potential threat from a One Health perspective.

Quantifying the use of antimicrobials across the poultry production system in South and Southeast Asia

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Current methods for analysing antimicrobial drug (AMD) residues provide a limited perspective of usage within the poultry production system. In this study, we employ novel lateral flow test (LFT) techniques in combination with declarative data to assess the historical use of AMDs by analysing chicken feathers, a crucial factor in understanding the connection between AMD use and antimicrobial resistance (AMR).

In total, 100 Bangladeshi, 50 Indian, and 49 Vietnamese farms were sampled. Declarative data on the timings, duration, and type of drug used was obtained. Two LFT panels analysed the feather samples collectively detecting sulfonamides, tetracyclines, beta-lactams, quinolones, and aminoglycosides.

Results showed that 88% of farms reported using at least one AMD during the production cycle. India primarily used them for prevention, Vietnam for treatment, and Bangladesh for both, with the latter also reporting use for growth promotion (Figure 1). Singular AMD administration was the most common (31%), with no significant differences in duration and timing between countries (p>0.05). Quinolones were the most detected residue by LFT in Bangladesh (81%) and India (57%), with sulfonamides being most frequently detected in Vietnam (75%) (Figure 2). Aminoglycosides were rarely detected (0-10%).

AMD use is widespread within the poultry sector across South and Southeast Asia. Emerging feather residue methods complement and fill gaps in use from declarative studies. Further validation studies are necessary to determine LFT sensitivity and specificity, followed by Bayesian modelling to analyse administration timing and residue presence in feathers.



The number of reported antimicrobial drug uses across chicken farm sites in Bangladesh, India, and Vietnam. The declared reason for use is represented by therapeutic (green), preventative (blue), and growth promotion (orange).

Air sample contamination with Avian Influenza (A/H5N1 and A/H9N2) in live poultry markets in Dhaka, Bangladesh, and its association with market and stall-level characteristics: an ecological analysis

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Live poultry markets (LPM) play an important societal role in Bangladesh, but also present a risk for the spill over of zoonotic pathogens like avian influenza viruses (AIVs) from poultry to humans, including through exposure to contaminated aerosols.

Between February and March 2023, we collected air samples from 30 randomly selected LPMs in Dhaka, Bangladesh, and data on hygiene and practices from 383 poultry vending stalls. The air samples were PCR-tested for AIVs, and statistical association to hygiene and stalls characteristics was investigated using logistic regression.

12/30 (40%) LPMs' air samples were positive for AIVs, respectively 2/30 (7%) with H5N1 only, 4/30 (13%) with H9N2 only, and 6 /30 (20%) with both viruses. Purchased birds were slaughtered on the spot in 94% stalls, with 58% using a dedicated slaughtering area, and most (80%) stalls had no drain. 184/383 (48%) stalls used a defeathering machine, with 170 (93%) using a cover and 175 (86%) reporting they boil birds before defeathering. Protective clothing was rarely used (2%). In adjusted analysis, AIV positive air sample was associated with using defeathering machines (adjusted OR (aOR): 2.36; 95%CI: 1.14 to 4.88). Higher odds (but wider confidence intervals) were also associated with selling ducks (aOR: 1.92; 95%CI: 0.67 to 5.59) and having a dedicated slaughtering area aOR: 2.18; 95%CI 0.86 to 5.59). We found no association with market size, presence of drain, cleanliness of bird cages or stall floor.

We found a high prevalence of air contamination with AIVs in Dhaka's LPMs, and some suggestion that selling ducks and the use of defeathering machines may contribute respectively to pathogens' introduction and aerosolization.

No machine	1	1.00 (1.00, 1.00)	
Defeathering machine use		2.36 (1.14, 4.88)	0.022
No ducks sold	-	1.00 (1.00, 1.00)	
Selling ducks		1.93 (0.67, 5.59)	0.216
None	+	1.00 (1.00, 1.00)	
Dedicated slaughtering area	+	2.19 (0.86, 5.89)	0.098
Less than 20 poultry stalls		1.00 (1.00, 1.00)	
20 or more poultry stalls	+	1.56 (0.31, 7.99)	0.579
No drain	+	1.00 (1.00, 1.00)	
Drains present	+	1.08 (0.32, 3.64)	0.898

OR and 95%CI from logistic regression with robust standard error, adjusting for market clustering, and all variables presented in the plot

Forest plot of the association between AIV air contamination and stalls/market-level characteristics in Dhaka, Bangladesh

Prevalence of Campylobacter and non-typhoidal Salmonella in Chicken in Bangladesh

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This cross-sectional study aimed to assess the prevalence of *Campylobacter* and *Salmonella* in farmed and marketed chickens in Bangladesh. Across the country, 50 live bird markets (LBMs) were randomly selected, as well as 100 commercial farms supplying them. Caecal samples of chickens were collected and tested for *Campylobacter*, and environmental samples for non-typhoidal *Salmonella* (NTS).

The prevalence of *Campylobacter coli* and *C jejuni* in marketed chickens was 10.7% and 0.5%, respectively. The overall *Campylobacter* prevalence in deshi (*C coli*: 8.9%; *C jejuni*: 0%) and broiler chickens (8%; 1.2%) was lower than in sonali (15%; 0.4%). The prevalence was higher in farmed chickens, reaching 15% for *C coli* and 2.6% for *C jejuni*. Similarly to marketed chickens, the prevalence was higher in sonali (*C coli*: 22.8%; *C jejuni*: 3.2%) than in broiler (*C coli*: 7.2%; *C jejuni*: 2%).

The prevalence of *Campylobacter* also varied across the country, peaking in Chattogram and Bogura 22.7%.

Non-typhoidal *Salmonella* (NTS) was detected in the environment of 32% of LBMs, including in 54.5% of Chattogram's LBMs. NTS prevalence was lower on farms, with 8% and 2% of broiler and sonali farms' environment being respectively contaminated.

The frequent occurrence of these pathogens in farmed and marketed chicken populations raise food safety concerns about the consumption of raw and undercooked meat in the country.

Spatial and Temporal Distribution Patterns of Avian Influenza in Live Bird Markets near migratory and non-migratory birds population in Bangladesh

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Avian influenza (AI) is a disease caused by influenza A viruses that infect birds, especially wild waterfowl. Avian influenza can have severe impacts on the economy, public health, and food security of countries with large poultry industries, such as Bangladesh. Avian influenza is prevalent in Bangladesh in both high pathogenic (HPAI) and low pathogenic (LPAI) forms, with H5N1 and H9N2 being the most common subtypes. The study aimed to assess whether the diversity of avian influenza viruses (AIVs) and the prevalence of H5 subtypes in poultry was influenced by the presence of migratory bird populations.

A longitudinal study was conducted using a repeated cross-sectional approach across perirural/urban areas of Bangladesh to investigate the spatio-temporal distribution of Avian Influenza Viruses (AIVs) in live bird markets (LBMs) from December 2022 to July 2023. We utilized eBird prediction modeling (statistical and machine learning techniques) to select study areas with high and low migratory bird density, as well as high human population density. We used a random sampling method in LBMs, to ensure the selection of a representative sample for each poultry type. We collected oral and cloacal swab samples from ducks, exotic broilers, sonali, and deshi chickens.

The preliminary results of the study suggest pervasive detection of A (H5) and A(H9) viruses across nearly all surveyed LBMs. Live bird markets with multiple wholesalers exhibited higher A(H5) or A (H9) virus contamination rates than retail-only LBMs. A (H5) virus prevalence was higher in waterfowl, while A (H9) virus prevalence was higher in chickens, particularly in broilers compared to crossbred Sonali or indigenous deshi.

The study will soon proceed with data analysis to elucidate the relationship between AIV dynamics and migratory/non-migratory bird populations, contributing to a deeper understanding of their role in shaping virus prevalence and distribution within poultry trading networks as well as it to make significant contributions to understanding and mitigating the risk of AI outbreaks in Bangladesh.

A longitudinal epidemiological investigation on poultry pathogens and farm biosecurity standard in broiler and Sonali chicken production in Chattogram, Bangladesh

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Infectious diseases have emerged as a significant challenge for the poultry sector, posing a severe threat to the growth and sustainability of the sector. This study aims to assess the risk of Avian Influenza Virus (AIV) and *Campylobacter* sp. at various stages of a production cycle of broiler and Sonali (Rhode Island Red x Fayoumi) farms.

The research was conducted in randomly selected 60 poultry farms with different production modalities and breeds: 30 contract-based exotic broiler farms, and 15 exotic broiler and 15 Sonali credit farms operating through informal production input credit by feed dealers. The acquisition of data and samples (via oropharyngeal swab and fecal matter) was carried out over four consecutive sessions. The initial visit took place within a duration of 7 to 15 days, while the second visit was scheduled for a range of 30-35 days specifically for Sonali. The third visit occurred one week prior to the trading period, which spans from 25 to 35 days for broiler and 45-60 days for Sonali. Lastly, the final visit was conducted during the trading period. The specimens underwent assessment for avian influenza virus (AIV) and its subtypes through reverse transcription polymerase chain reaction (RT-PCR) and polymerase chain reaction (PCR) techniques, as well as for *Campylobacter* via both culture and PCR methodologies.

Credit Sonali farms had higher prevalence of *Campylobacter* (C) *coli* (80%) and *C. jejuni* (53.33%) than that of credit broiler farms (60% and 13.3%, respectively) or contracted broiler farms (6.7% and 46.7%, respectively). *C. coli* was more common during the trading period start irrespective of farm types (50% to 60%), while the prevalence of *C. jejuni* was higher before trading started in credit broiler (13.3%) and Sonali farms (20.0%) and during the trading period in contracted broiler farms (40%) compared to other production stages. The farm-level prevalence of AIV subtype- H5 and H9, respectively, were 13.3% and 6.7% in credit broiler farms. The trading period posed a high-risk entry point for H5 in all three types of farms (13.3% credit broiler, 6.7% contracted broiler, and 6.7% Sonali).

Addressing the risk of infectious diseases requires a comprehensive understanding of the prevalence dynamics, identification of high-risk entry points, targeted interventions, and continuous monitoring. Implementing robust and tailored biosecurity measures, along with collaborative efforts, is imperative for effectively controlling and preventing the spread of significant poultry pathogens.

Investigation of risk factor for avian influenza virus contamination of Live bird markets in Chattogram, Bangladesh

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Live bird markets (LBMs) are recognized as a network 'Hub' and potential location for emergence of infectious diseases. Although LBM play a critical role in the value chain, they can be a public health threat through emergence, transmission and dissemination of avian influenza viruses (AIV). Despite frequent detection of AIV in LBM, the risk factors related to AIV contamination within LBM of Bangladesh are poorly understood. A cross sectional study was conducted between August 2021 and January 2022 with random sampling of shops/stalls selling commercial broiler, Sonali and deshi chickens. A total of 4354 oropharyngeal and cloacal swab samples were collected from apparently healthy chickens and a questionnaire on biosecurity and management practices completed by 344 vendors (all male).

Of 4354 samples tested by RT-PCR, 644 (14.7%) were positive for AIV M-gene with 14 of these (0.32%) positive for H5, 615 (14.1%) positive for H9, 8 (0.18%) positive for both H5 and H9, and 15 (0.34%) remaining untyped. At stall level AIV H9 prevalence was 18.2% (273/1500) in Sonali,12.1 % (169/1384) in deshi and 11.7% (173/1470) in exotic broilers. At market level, H5 AIV were found in 14% (7/50) and H9 in 98% (49/50). Sick and dead bird management, cleanliness and access of water supply, slaughter and dressing of chickens at the stall were tested as potential risk factors by multivariate logistic regression analysis.

There is high prevalence of AIV H9 compare to H5 across stalls of the LBM. Effective and protective measures and mitigation strategies are needed to reduce the burden of AIV H9 in the LBM



Understanding prevalence of key food borne pathogens in selected Poultry Distribution Networks from Gujarat-India

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According to FAO, poultry meat is expected to represent 41% of all edible protein from meat sources by 2030. While addressing food security, maintaining a safer food chain is critical. Under 'One Health Poultry Hub' this study investigated the prevalence of food borne pathogenic bacteria in poultry value chain across eight cities in Gujarat, focusing on *Escherichia coli*, and *Campylobacter* spp.

We collected a total of 750 chicken ceca, 150 chicken cloacae (broilers only) in retail shops (broilers and desi) across eight cities of Gujarat-India. Samples were enriched and plated on selective media, followed by confirmation using genus/species-specific PCR assays. All the isolates were subjected to AST profiling by disc diffusion assay and whole genome analysis for selected isolates and phenotype-genotype concordance was evaluated.

The study identified 51.33, and 10.26 prevalence of *E. coli* and *Campylobacter* spp. *Campylobacter* prevalence was higher in shops than on broiler farms. All *E. coli* isolates were resistant to vancomycin and for *Campylobacter* there was high resistance to piperacillin/tazobactam followed by ampicillin/sulbactam. Whole-genome sequencing (WGS) of six *E. coli* isolates confirmed 95% concordance between phenotype and genotype data and 5% discordance because of partial gene sequences. For 18 WGS of *Campylobacter* concordance was 76.39% with 23.61% discordance due to six AMR genes, two of which were truncated whilst the rest carried mutations. For example, *macB* had (F) phenylalanine to (L) leucine substitution, and *gyrA* had (T) threonine to (I) isoleucine substitution. Additionally, missense mutations were found in *tet-O*, *cmeA*, and *cmeABC* genes.

This study emphasizes the critical need to understanding antimicrobial resistance (AMR) in PDNs, for public health and food safety in a "farm to consumer" One Health approach.



Antimicrobial Use Patterns in Broiler & Sonali Poultry Production in Chattogram, Bangladesh.

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The poultry industry in Bangladesh is a significant contributor to the economy, providing a cheap and readily available source of animal protein to the country's growing population. However, with the increasing challenges posed by infectious poultry diseases, the misuse of antimicrobials in commercial farming is becoming a growing concern. Our aim was to assess antimicrobial usage (AMU) patterns in chicken farms.

A study was conducted from December 2022 to July 2023 in 45 Broiler and 15 Sonali chicken farms having at least 500 birds in Mirsharai and Chandonaish districts in Chattogram. Data on AMU were collected using questionnaires, log books and daily phone calls.

About 133 treatment courses (median 4; IQR 5.6-4.4) were observed across the farms, revealing diverse patterns of antibiotic administration for prophylactic (48.1%), therapeutic (44.2%), and growth-promoting (0.7%) purposes. Notably, despite existing government regulations, antibiotics were suggested and used without veterinary consultation, primarily by dealers and farmers with previous experience. According to the descriptive analysis, fluoroquinolones are the most commonly used antibiotics group (28.6%), followed by amoxicillin (18.8%) and oxytetracyclines (7.8%). The preliminary quantitative metrics, including total antimicrobial usage (mg/PCU) and the number of defined daily doses per population correction unit (nDDD/PCU), have revealed concerning trends.

Although the detailed data analysis is still ongoing, these preliminary findings indicate an urgent need for intervention to reduce antibiotic usage in broiler farms. Strict adherence to regulations in antibiotic trade, continuous research for improved stewardship, and targeted awareness programs for farmers and stakeholders are essential components of comprehensive strategies to address indiscriminate antimicrobial usage and combat antimicrobial resistance effectively.

Opening the black-box of multisector partnerships: A Theory of Action for implementing One Health at country level

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The complex nature and social-rootedness of EIDs & AMR have prompted calls for multisector governance in the form of One Health approach. However, many have found it difficult to operationalise at national and sub-national levels. In this study, we describe the process of developing a practical framework for assessment and characterisation of One Health which will programme managers to adapt national One Health mechanisms to their respective contexts.

We use a review of academic and programmatic literature to develop a Theory of Action for multisector One Health partnerships that can nest into the Short-term Outcomes identified in the Theory of Change for One Health developed by the Quadripartite. This comprises of five elements: Characteristics; Starting conditions; Collaborative process; Outputs; and Responsiveness. We develop additional attributes to undertake a detailed characterisation of different 'levels' of One Health partnerships. In addition, this Theory of Action allows for multiple outcomes of interest to be recognised and addressed.

We then use the Theory of Action to develop a reflection tool to help country programme managers identify the specific characterises of their respective One Health partnerships; recognise the differences in capacities and expectations of different partners; and use these insights to identify specific ways to strengthen the collaborative process.

To our knowledge this is the first time a detailed characterisation of One Health partnerships based upon programmatic attributes has been developed. Such a conceptualisation of One Health adds to the existing guidance released by the One Health Joint Plan of Action and can help design, implement and assess locally-relevant multisector partnerships.

Exploring export potential: Can Sri Lanka export chicken and related products

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The poultry industry is well-developed in Sri Lanka and was self-sufficient in providing chicken meat and eggs before the COVID-19 pandemic. Sri Lanka also exported eggs for hatching, table eggs, broiler meat and other poultry-related products to Maldives, Nepal, Gulf region *etc.* before 2020. Unfortunately, the COVID-19 pandemic and economic downturn in the country heavily affected the poultry industry with a drastic loss of production. By the end of 2023 the poultry industry regained pre-Covid production levels and is now looking for avenues for expansion. The highly pathogenic avian influenza-free status has given a huge advantage for exploring potential export markets.

One Health Poultry Hub partnered with Sri Lanka Veterinary Association to facilitate poultry exports. The partnership's objectives were to establish and develop a common forum to discuss the gaps and barriers in the international poultry market and to facilitate the provision of necessary technical knowledge to local producers to bridge the foreign market requirement. The potential markets and their requirements (i.e. the Gulf region, the European Union etc.) will be focused. Two stakeholder meetings and a SWOT analysis session were conducted with potential exporters to achieve the objectives. The SWOT analysis revealed major gaps in communication, networking and collaboration between government departments and commercial poultry producers. A Third workshop was conducted to make a working group representing all stakeholders required for export facilitation in the poultry sector. The progress of a working committee to document recommendations for policymakers is in progress.

Mind the gap: An assessment of scope for collaboration between public and private veterinary services for effective disease management in Bangladesh's poultry sector

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Bangladesh's poultry sector is crucial for national protein and micronutrient requirements and employment opportunities. Despite the positive contribution of the sector, its growth also increases demand for veterinary support and disease management from public and private veterinarians. To date, no known research has been conducted to investigate gaps in veterinary care or explore collaboration between public and private veterinarians. This qualitative study, therefore, aimed to identify gaps between private and public veterinarian services and identify potential opportunities and obstacles in establishing public-private partnerships in Bangladesh's poultry sector.

Data was collected from June 2021 to July 2023 through semi-structured interviews with 62 veterinarians including 4 female veterinarians from Bangladesh, transcribed in Microsoft Word 10, and analysed using MAXQDA software. The study's methodology involved thematic analysis—developing and refining codes. The findings were organised into three major themes: a) affiliations and key differences between veterinarians, b) the role of non-veterinarians in veterinary provision, and c) opportunities and challenges for public and private sector collaboration.

The study reveals that the affiliations of veterinarians from specific companies lead to disparities in poultry veterinary service. Both public and private veterinarians face issues including staff shortages, excessive workloads, lack of recognition, and regulatory bodies. Communication breakdowns also contribute to dissatisfaction due to different service purposes, skills, and freedom.

The study suggests public-private partnerships will help to improve veterinary provision, data sharing, surveillance activities, laboratory facilities, and medicine and vaccine supply and emphasises a common association to initiate public-private partnership for benefitting the country and its marginal farmers financially.



Mind the gap: An assessment of scope for collaboration between public and private veterinary services for effective disease management in Bangladesh's poultry sector

Capacity Enhancement on Biosecurity in Poultry Farming for Line Supervisors in Contractual Commercial Broiler Farming across Gujarat

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Poultry farming confronts threats from disease and antimicrobial resistance, where the pivotal role of biosecurity measures, based on principles of isolation, traffic control, and sanitation, lies in preventing the entry and transmission of pathogens. This research aimed to enhance the capacity of line supervisors in contractual commercial broiler farming across Gujarat through comprehensive training, focusing on biosecurity measures.

A one-day training program was conducted with 33 line supervisors (all men) from various poultry feed and breeding companies operating in Gujarat. Pre-training and post-training questionnaires were administered to assess socio-economic characteristics, biosecurity practices, and training effectiveness. Data analysis was conducted using Microsoft Excel and the study revealed that the majority (54.5%) of participants fell into the young age group, with 39.4% completed education at the higher secondary level, with 66.7% married, 39.4% have over 10 years of high professional experience visiting 11-21 farms, with the mean of 17-18, indicating diverse experience. Correlation analysis revealed positive associations between education and ease of adopting biosecurity measures, a negative correlation with experience, and a subtle positive correlation with age. ANOVA analysis showed significant differences (p = 0.0000517) in Likert scale ratings for various biosecurity measures, with high ratings for "Disposal of Carcass," "Vaccination," and "Proper Manure Disposal." Training effectiveness assessment indicated strong agreement on its utility (90.9%) and knowledge improvement (75.8%).



Thus, this study concludes that training enhances line supervisors' biosecurity knowledge, reinforcing frontline capacity significantly.

Community involved biosecurity model: A new approach to reduce infectious diseases including avian influenza in small-scale poultry farming

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Biosecurity practice is an important tool to prevent the introduction and spread of infectious diseases, including avian influenza. The traditional biosecurity approach cannot satisfactorily reduce the rate of outbreaks due to complex farming systems in Bangladesh. To reduce the load of infectious diseases aiming for avian influenza, a community biosecurity model was piloted in a village in Bangladesh from 2020 to 2022.

A village was selected where at least one small-scale commercial layer farm was present with a record of avian influenza outbreaks. The major activities were assessing the poultry population with the prevalence of endemic diseases, providing health care services (cleaning, disinfection, disposal of dead birds, etc.), vaccinating all village chickens against Newcastle disease (ND) and Fowl pox, monitoring the antibody level after vaccination against ND, and identifying the causes of sickness and mortality. When any bird died with clinical signs of avian influenza, all surviving birds of the flock were purchased and all of that household poultry culled immediately providing compensation from the community fund.

The results showed that village chicken mortality due to infectious disease was significantly reduced and no mortality was recorded due to ND and fowl pox. The poultry population of that area increased two-fold within two years. There were 3 cases of village chickens confirmed with highly pathogenic avian influenza by rRT-PCR and no positive case was confirmed on the commercial layer farm.

From the study, it was concluded that community-involved biosecurity has a positive role in reducing infectious diseases including Avian Influenza in commercial poultry farming.

Flock Fortress: Bio-security Practices by Backyard Poultry Keepers

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Backyard poultry biosecurity is crucial for protecting flock health, preserving the economy, ensuring safe food, and promoting the sustainability of poultry farming. This study aimed to evaluate existing knowledge and practices regarding biosecurity and assess the effect of a training intervention on knowledge gain. Information collected during interviews revealed gaps in biosecurity measures being practiced.

A total of 27 farmers (26 male, 1 female) participated in the study, which included one-day training and surveys using pre- and post- training questionnaires. Data analysis revealed that farmers were relatively young (average age 35 years), and 44% were graduates. The average experience with backyard poultry was 3.48 years. It was discovered that 74 of farmers have additional species such as goats, cattle, and buffalo. Among participants, 59 followed a vaccination program. Fowl pox (18.75%), Ranikhet (23.75%), and Coli septicaemia infection (13.75%) exhibit higher prevalence in backyard poultry compared to other poultry diseases, emphasizing their significant impact. Likert scale analysis shows that 82% of backyard farmers strongly agreed that the listed bio-security measures should be followed, and 64% consider that the low number of birds and lack of awareness are major limiting factors in implementing bio-security measures. T

The study effectively explored the farmer profile and flock characteristics of backyard poultry farms in the Gujarat region, providing insights into their understanding and awareness of biosecurity measures.



Investigating Biosecurity protocols in commercial broiler poultry farms in Gujarat

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Biosecurity in commercial broiler farming is crucial to prevent the spread of diseases among poultry farms and to help in upscale of food safety and health of poultry.

Key informant Interviews, a link tracing study and biological sampling revealed that there is a gap in information on biosecurity being followed by poultry farmers in Gujarat. A one-day training program, including 39 broiler farmers, all male, was conducted in Anand, Gujarat, which mainly focused on biosecurity in poultry. Questionnaires were administered before and after training.

The data show the average age of participants to be 37.8 ± 1.5 years with more than 50% of farmers having only secondary education. Of the total, 21% of participants had small (< 5000 birds), 41% medium (5000 – 10,000 birds) and 33% large size (> 10,000 birds) commercial farms. Colisepticaemia (92.3%), avian influenza (64.1%) and infectious bursal disease (48.7%) are the major diseases observed. Factor analysis of mixed data indicated that dimension 1 contributed 13% of total variance and dimension 2 contributed 11.5% of total variance. The number of birds, number of labor staff, documentation and record keeping, and scale of biosecurity knowledge were variables that contributed more than 70% to variation distribution Likert scale analysis showed that 88% of participants believe the listed biosecurity measures were easy to implement, and 60% strongly agreed that lack of knowledge, lack of control and lack of awareness were limiting factors for implementing biosecurity measures. The study recommends enhanced information dissemination to improve the uptake of biosecurity measures

0.5- Documentation and record keeping				
Action during disease outbreak Action during disease outbreak Action during disease outbreak Action during disease outbreak		variab	Correlation between variables and principle dimension	
	Scale of biosecurity knowledge			
Presence of sanitizing facility for vehicles				
Good nutrition Restriction on vehicle entry				
	Education			
Awareness about AMR	Source of income			
Restriction on visitor entry				
Age (in years)				
	er poultry farm (in meters)			
Place where dead birds are k	ept Storage of poultry manu	e		
Experience of poultry farming (in years) Dead bird disposal method Annual fincome (in Rs.) Quarantine of new birds	No. of sheds of store room	Labour staff	No. of bird	
Vaccination No. of family members Isolation of sick	birds Presence of foot bath	MS) Clada d'anomhan		
Rearing Addition of pest Cleaning and disinfection	of phattry pates in busiy encountered disease outb	reak		
0.0 0.2	0.4 Dim1 (13%)		0.6	

Variables - FAMD

Effectiveness of on-site training on Good Management practices for poultry transporters in hygienic handling and transport of poultry

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In intensified broiler production, the poultry birds are transported by road over a long distance to consumer outlets and processing plants. The transportation of poultry places enormous stress on birds because they have limited space during transport. Further, unethical handling of birds in the farm while loading and unloading results in injuries to the healthy birds. Poultry welfare is one of the responsibilities of persons involved in handling and transportation of poultry birds.

In this context, an on-site training with lecture and demonstrations on good transport management practices was conducted for 50 randomly selected poultry transporters (all men) in Chennai for one day duration. The knowledge level of respondents was assessed through a questionnaire covering areas namely proper bird handling during loading and unloading, disinfection of vehicles and cages, personal hygiene, dead bird disposal etc before and after three months of conduct of training. The paired t-test was performed to analyse the significant difference between the scores of pre and post-evaluation scores.

The mean score before the training on 0th day and 90 days after the training was observed to be 2.73 ± 1.53 and 5.51 ± 3.33 , respectively. The results revealed that that there was a significant knowledge gain due to the on-site training for the poultry transporters, Further, the training has created an attitudinal change among the transporters not only in humane handling of birds during transport but in personal hygiene, public health issues and disease spread. This type of on-site training is the need of the hour for poultry bird transporters.

S.No	Test	Mean Score before the training (0 day)	Mean Score after the training (90 days)	d ± SD	"t" value
1	Biosecurity (B)	5.04	9.88	4.84±1.36	6.74**
2	Transport Hygiene (TH)	3.26	8.72	5.46±1.11	6.86**
3	Cage Hygiene (CH)	1.22	2.00	0.78±0.42	6.18**
4	Personal Hygiene (PH)	1.56	2.00	0.44±0.50	4.64**
5	Loading and Unloading (LU)	4.42	7.66	3.24±1.19	6.58**
6	Bird Handling (BH)	2.26	5.64	3.38±1.06	6.68**
7	Dead Bird Disposal (DBD)	1.36	2.70	1.34±0.79	6.03**
	Overall	2.73±1.53	5.51±3.33	2.78±1.98	6.24**

Knowledge gain through On-site biosecurity training programme among the stakehole involved in live poultry birds transport		
	(n = 50)	

** significant at one per cent level

Impact assessment of poultry farmer's training regarding poultry rearing and farm biosecurity measures in Bangladesh

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Small- and medium-scale poultry farms in Bangladesh, despite ongoing disease outbreaks due to inadequate farming knowledge of the farmers, supply affordable proteins. Few studies from Bangladesh broach the impact of training programmes for poultry farmers focusing on farming and farm biosecurity measures.

Therefore, this study was conducted to appraise the longer-term impact of a 2-day long training programme on 88 farms (a subset of willing farmers, all men from 183 training participants including 6 women) from 12 sub-districts of Chattogram district. After the training exposure (including interactive sessions on poultry farming), 88 farms were visited and observations on farming practices and biosecurity measures were gathered and scored. Data were collected using structured pre- and post-questionnaires, immediately before and after the 2-day training and at least 4 months after the training programme. The same researcher-led questionnaire was used at each data collection point. Data were analysed using descriptive and paired t-test as appropriate.

The t-test revealed an overall increase in mean value (from 12.3 to 15.5) of the farming practices and biosecurity measures with significant improvement after training regardless of production type, investment type, and education level of farmers. The descriptive analysis suggested that overall, a good percentage of farmers improved operational biosecurity practices by introducing disinfectants (26%), separating sandals and clothes (31%), improving vaccination (27%), and vaccine transport (26%), and increasing shed (9%), and other farm equipment cleanliness (28%).

The findings explicitly suggest that training interventions can improve poultry farming and operational biosecurity practices.



Examining the Influence of Training on Biosecurity: A Comparative Analysis of Biosecurity Scoring between Commercial Layer and Broiler Farms in Gujarat, India

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Poultry farm biosecurity stands as the forefront safeguard, preventing the introduction and transmission of diseases and infections. The aim of this study was to examine the influence of training on biosecurity in layer and broiler poultry farms located in a densely populated poultry area of Gujarat.

A total of 35-layer (34 male and 1 female) and 39 broiler (all male) poultry farmers actively participated in the training and pre- and post-training biosecurity levels were evaluated using a scoring system.

Layer farms exhibited the highest compliance with biosecurity measures. Following training, scores increased from 80.26 to 82.66 (\uparrow 2.4%) for broiler farms and from 81.86 to 85.76 (\uparrow 3.9%) for layer farms out of total 100 score. Category-wise shifts in biosecurity scores were seen for broiler and layer farms when comparing knowledge before and after training, with changes in Average (broiler 8.60% to 5.70%, layer 4.80% to 0.0%), Very Good (broiler 28.60% to 17.10%, layer 38.10% to 23.80%), and Outstanding (broiler 54.30% to 68.60%, layer 52.40% to 71.40%). Farmers with an educational background and substantial investment demonstrated rigorous adherence to biosecurity protocols on their farms. Disinfectant foot bath, farm hygiene lock, infrastructure condition and restricted entry were the biosecurity measures most compiled with layer farms compared to broiler farms. Some deficiencies were observed in the disposal of dead birds and foot bath in broiler farms.

In conclusion, poultry farmers possessed a high baseline knowledge of biosecurity, the training intervention did not lead to significant changes. Further investigation is needed to identify factors contributing to the limited impact and areas for improvement in future training initiatives.

Key words: Biosecurity, Training, Broiler, Layer, Biosecurity score card



Figure 1 section wise improvement in biosecurity scores of broiler and layer farms before and after training.

Training on Food safety and hygienic meat practices as a tool for empowering poultry meat handling butchers in Chennai

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Chicken meat is the second most popular animal flesh consumed after fish in Chennai, Tamil Nadu. In this context, poultry-meat handlers play a vital role in food safety and prevention of meat borne illnesses. It was observed that there is a dearth of knowledge on food safety among the meat handlers and to assess the same, a survey was conducted to test the level of knowledge of poultry meat handlers about food safety and hygiene practices from November 2022 to July 2023.

A total of 375 organised poultry meat shop butchers (all men) were surveyed in all zones in Chennai city. Majority (93%) of butchers interviewed revealed that they had not attended any kind of biosecurity/food safety training programme. It was interesting to note that 7% of poultry meat handlers had attended the biosecurity training program conducted by TANUVAS. In the hygiene practices section, 56% of them reported rodent problems in their retail outlets, as well as processing money while handling meat. Among the butchers interviewed, 57% knew about poultry diseases while the rest were unaware (43%). With regard to profile of butchers, majority of them were in the age group of 20 to 30 years (62%), had undergone primary education (62%) and had 15 years of poultry meat handling experience (65%). Therefore, they did not have a thorough knowledge of poultry diseases and food-borne pathogens.

It could be concluded that regular training of meat handlers would improve the knowledge in safe meat handling, personal hygiene and prevention of meat borne illness.



HYGIENIC PRACTICES OF THE ORGANISED POULTRY RETAIL SHOP BUTCHERS IN CHENNAI CITY (n=375)

Abstract #89

Biosecurity Approaches in Commercial Layer Poultry Farming: A Gujarat Perspective

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The poultry industry in Gujarat has become a significant contributor to global chicken meat and egg production, fostering notable growth. However, challenges such as disease transmission, antimicrobial resistance (AMR), and foodborne pathogens necessitate robust biosecurity measures. This study assesses the state of biosecurity protocols in commercial layer poultry farms in Gujarat, aiming to enhance the existing practices.

Two training programs conducted in Anand and Mahuva involved 47 farmers, with 35 participants (34 male and 1 female) responding to pre- and post-training questionnaires. Descriptive statistical analysis highlights that 48.6% of farmers are middle-aged, with 65% relying solely on the poultry industry for income. A substantial 94.3% operate on their land, underscoring a strong connection to their agricultural pursuits. The Likert scale reveals that limitations while implementing biosecurity measures are mostly because of a lack of awareness, knowledge, and manpower. Farmers also identified different epidemiological points during training, covering farms, transport, and markets that contribute to disease transmission in the poultry industry. Factor analysis of mixed data (FAMD) revealed that the variables contributing to most variations were knowledge of biosecurity, number of sheds, and experience in poultry farming.

In conclusion, this study provides a nuanced exploration of biosecurity practices in commercial layer poultry farms, emphasizing demographic insights, economic reliance, and the impact of training. The findings contribute to fortifying biosecurity measures, ensuring the resilience and efficacy of the poultry industry in Gujarat.

