UNIVERSITY OF CAPE TOWN

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A research report submitted to the School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town in partial fulfilment of the requirement for the award of the degree of Master of Medicine (MMed) in Occupational Medicine

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DECLARATION

Allergic sensitization and work related asthma among poultry workers in South Africa

I, Dorothy Ngajilo, hereby submit my dissertation for the degree of Master of Medicine (MMed) in Occupational Medicine. I declare that this is my original work (except where acknowledgements indicate otherwise) and that neither the whole work, nor any part of it, has been, is being, or is to be submitted for another degree in this or any other university.

Signed by candidate Signature removed

Dr Dorothy Ngajilo 6th April 2016

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DEDICATION

To God almighty for His blessings, strength and good health that enabled me to complete this dissertation.

To my loving parents Neema and Amaleck

To my sisters Irene, Amelile, Tunu and Gloria

To my nieces Godglory and Keren-Blessing

To all my friends.

PUBLICATION AND PRESENTATIONS

Parts of this dissertation has been published and presented in conferences as follows:

Publication

- **Ngajilo D**. Respiratory health effects in poultry workers. Curr Allergy Clin Immunol. 2014;27(116-124).

Conference oral presentations

- **Ngajilo D,** Singh TS, Ratshikhopha E, Baatjies, and Jeebhay MF. Risk factors associated with asthma phenotypes in poultry farm workers: preliminary results. UCT School of Public Health and Family Medicine Research day. 17 September 2015, Cape Town, South Africa.
- **Ngajilo D,** Singh TS, Ratshikhopha E, Baatjies, and Jeebhay MF. Risk factors associated with asthma phenotypes in poultry farm workers: preliminary results. PHASA conference. 8-10 October 2015, Durban, South Africa

ABSTRACT

Objective: The aim of the study was to determine the risk factors associated with allergic sensitisation and various asthma phenotypes in poultry workers.

Methods: A cross-sectional study of 230 currently employed poultry workers used a modified ECRHS questionnaire, spirometry, fractional exhaled nitric oxide, Phadiatop and ImmunoCAP for specific IgE to poultry farming associated allergens (chicken meat, feathers, serum protein, droppings, sunflower seeds, storage mite) and mould mix to investigate work-related asthma.

Results: The mean dust particulate concentration (geometric mean (GM) =11.04mg/m^3) was highest in the rearing department while mean (1-3)-ß-D-glucan (GM=148 ng/ m^3) and endotoxin levels (GM=2298 EU/m^3) were highest in the catching department. Worker's mean age was 37±9 years, 68% were male, 43% current smokers, 34% atopic and 5% casual workers. The prevalence of non-atopic asthma (NAA=10%) was higher than atopic asthma (AA=6%) or probable occupational asthma (OA=5%). Sensitisation to at least one poultry work related allergen was 24%, with sunflower seed and storage mite sensitisation being the most prevalent (13%). In multivariate adjusted models, workers sensitised to chicken specific allergens were more likely to be atopic (OR=20.9, 95% CI: 4.7-93.2) or have casual job status (OR=6.0, 95% CI: 1.1-35.9). Workers with work-related chest symptoms were more likely to work in the rearing department (OR=3.2, 95% CI: 1.2-8.3) and to report episodes of high gas/dust/fume exposures causing asthma symptoms (OR=4.8, 95%CI: 2.4-9.6). Reversible obstructive airway obstruction was more strongly associated with employment in small broiler farms (OR=11.6, 95% CI: 1.0-129.0) as well as casual work (OR=6.4, 95% CI: 1.4-28.0). Furthermore, NAA was strongly associated with casual work (OR=5.0, 95% CI: 1.3-19.0) as well as working in the automated egg laying system (OR=8.0, 95% CI: 1.0-68.6).

Further analysis revealed that the proportion of workers sensitised to at least one poultry work related allergen declined with increasing years of service (chi-square trend p=0.023), with workers having <2 years employment demonstrating a higher risk compared to those with >6 years employment (OR=4.0, 95% CI: 1.2-13.8).

Conclusion: Non-atopic asthma is the most common asthma phenotype among poultry workers with work in the rearing department demonstrating an increased respiratory risk. The increased risk of reversible airway obstruction among workers in small broiler farms and the declining prevalence of sensitisation with increasing employment duration suggests a healthy worker effect. Preventive measures, including appropriate training, are recommended to reduce respiratory health risks, particularly in novice workers.

ABBREVIATIONS AND ACROMYMS

AA:	Atopic Asthma
ACGIH:	American Conference of Governmental Industrial Hygienists
ALLSA:	Allergy Society of South Africa
ATS:	American Thoracic Society
BDL:	Below Detection Limit
CFU:	Colony Forming Units
CSIR:	Council of Scientific & Industrial Research
CV:	Co-efficient of Variation
DECOS:	Dutch Expert Committee on Occupational Standards
ECRHS:	European Community Respiratory Health survey
ERS:	European Respiratory Society
EU:	Endotoxin Units
FEF:	Forced Expiratory Flow
FeNO:	Fractional exhaled Nitric Oxide
FEV:	Forced Expiratory Volume
FVC:	Forced Vital Capacity
HP:	Hypersensitivity Pneumonitis
HPC:	Heterotrophic Plate Count
HSE:	Health and Safety Executive
HSL:	Health & Safety Laboratory
ISO:	International Organization for Standardization
LAL:	Limulus Amoebocyte Lysate test
LOD:	Limit of Detection
LOQ:	Limit of Quantification

LRTS:	Lower Respiratory Tract Symptoms
MAS:	Microbiological Air Sampler
MDHS:	Methods for the Determination of Hazardous Substances
MRC:	Medical Research Council of South Africa
NAA:	Non Atopic Asthma
ND:	Not Done
NIOH:	National Institute for Occupational Health
NIOSH:	National Institute for Occupational Safety and Health
NS:	Not Specified
NWS:	Non Water Soluble
OA:	Occupational Asthma
ODTS:	Organic Dust Toxic Syndrome
OR:	Odds Ratio
PCR:	Polymerase Chain Reaction
Ppb:	Parts Per Billion
Ppm:	Parts Per Million
SANAS:	South African National Accreditation System
SD:	Standard Deviation
SOB:	Shortness Of Breath
UK:	United Kingdom
URTS:	Upper Respiratory Tract Symptoms
USA:	United States of America
WS:	Water Soluble

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SECTION A: PROTOCOL

INTRODUCTION

The South African poultry industry dominates the agricultural sector with more poultry products being consumed yearly compared to all other sources of animal-protein combined [1]. The poultry industry produces 65.5% of locally consumed animal protein. According to the South African Poultry Association, the poultry industry in 2012 contributed 22.3% of agricultural income. Being an important contributor to job creation and formal employment opportunities, the poultry sector employs about 10% of all workers in agriculture [2].

Agricultural production is associated with a variety of occupational illnesses [3]. Individuals involved in animal rearing, particularly poultry workers, have been shown to have a higher prevalence of respiratory disease compared to other farmers or rural residents [4]. This is due to exposure to a large spectrum of potentially hazardous airborne agents including chemical pollutants, dust particulate, allergens, endotoxins, micro-organisms and organic gases [5]. Work-related acute and chronic respiratory symptoms have been demonstrated in poultry workers exposed to these pollutants [6,7].

There are various other adverse health effects associated with this particular industrial activity. With poultry work involving activities requiring repetitive motion, heavy physical work load and excessive body motion, poultry workers have an increased risk of developing work related back, neck, shoulder and upper limbs disorders [8]. Furthermore, cases of occupational contact dermatitis have been reported commonly secondary to antibiotics and preservatives added to poultry feed [9,10]. Workers may also be at increased risk of infection following scratches by bird claws [11].

Justification

Despite an association between poultry work and respiratory illnesses, very few studies have documented the extent to which occupational exposure to various allergens constitute a risk to the health of poultry workers.

While most studies report on the prevalence of asthma based on symptoms and occasionally using spirometry, further research needs to focus on describing the various asthma phenotypes and their prevalence in poultry workers. Furthermore, very few studies have documented the extent to which occupational exposure to various allergens constitute a risk to the health of poultry workers.

A previous study conducted by the NIOH looking at work-related respiratory symptoms in poultry workers found a very high prevalence of respiratory symptoms associated with organic dust exposure [6]. However the study was unable to find specific causative agents which are important in the diagnosis and management of symptomatic workers. Additionally this study did not conduct objective exposure assessments and was therefore unable to demonstrate an association between various exposures and respiratory symptoms. From 2002-2006 the NIOH allergy unit tested several workers from poultry farming with respiratory symptoms and asthma. While some workers (43%) were positive to common aeroallergens and poultry specific allergens (chickens feathers, poultry feed, shavings and poultry litter), a larger proportion (57%) did not react to these allergens. This is consistent with the finding from previous studies suggesting that at most, 50% of asthma cases are attributable to “allergic asthma”[12]. These findings suggested the need for further research investigating other potential agents such as (1-3)-ß-D-glucan, causing non-IgE mediated inflammatory reactions.

This study aimed at exploring the association between environmental exposures and host risk factors in causing respiratory diseases as to enable the development of improved preventive strategies in reducing the adverse respiratory health impacts associated with poultry work.

Purpose and benefits

There is limited information available on occupational respiratory diseases in South African poultry industry. This study will enable:

a) Characterization of exposures to various biological agents among poultry workers

b) Description of the various asthma phenotypes present in poultry workers

c) Exploration of the association between environmental exposure to different biological agents and asthma phenotypes

d) Description of the extent to which host risk factors modify the relationships between asthma phenotypes and environmental exposures.

Results from this study will be beneficial in:

a) Providing recommendations to the poultry industry so that control measures can be implemented to protect the health of workers.

b) Designing appropriate medical surveillance programmes related to poultry work.

c) Development of guidelines for preventative measures which include administrative measures (e.g. use of personal protective equipment), environmental (e.g. engineering controls) and education (e.g. training of workers and management) so as to reduce occupational health risks.

Research questions

a) Which asthma phenotypes exist among poultry workers?

b) What is the prevalence of asthma related symptoms reported?

c) Is occupational exposure to different biological agents present in the poultry dust, associated with respiratory symptoms and asthma among poultry farm workers?

d) What is the relationship between environmental exposures and host risk factors in causing respiratory disease among poultry farm workers?

Hypothesis

Multiple biological agents and their metabolites present in the poultry dust (e.g. endotoxin and (1-3) β-D-glucan) produced in various high risk processes, can cause respiratory symptoms including asthma among poultry workers. These respiratory outcomes are mediated by both allergic and/or non-allergic mechanisms.

Aim

The aim of the study was to determine the risk factors associated with allergic sensitisation and various asthma phenotypes in poultry workers.

Objectives

a) To identify tasks with high risk of exposure to poultry dust among poultry workers.

b) To determine the prevalence of allergic sensitisation and work-related asthma and related constitutional symptoms associated with exposure to organic dusts in poultry workers in order to better characterise various asthma phenotypes.

c) To investigate the contribution of other host risk factors such as atopy, smoking status, age, gender, history of allergy, employment duration and job category in developing sensitisation and allergic respiratory disease among workers in the poultry industry.

METHODOLOGY

Study design

This study involved the analysis of data that was collected from 2011 to 2012 in an analytical cross-sectional study of 230 poultry workers from a commercial poultry farm in the North West Province, South Africa. The study was conducted in two phases.

Phase I: Mapping of the poultry industry.

During this phase a commercial poultry farm producing broilers and eggs was identified in the North West Province. The farm had 15 different sites with processes such as rearing, laying (manual and automated), hatchery (old and new), broiler (housed – large/small and free range – large/small) and catching crew. Between 40 000 to 750 000 chickens were kept in the farm depending on the number of houses that are filled at any one time. Broiler houses were classified based on the number of chickens they housed. Broiler houses with ≤ 20 000 chickens per house were classified as small broilers whereas large broilers were classified as those that housed > 20 000 chickens. The study did not include areas in the factory where slaughtering is conducted as the exposure was different and the processes included wet work which was not within the scope of this study.

Phase II: Environmental exposure and health assessment and analysis.

During this phase a cross sectional survey of workers employed in the commercial poultry farm in the North West province was conducted.

Population and sampling

Study population

The study population comprised poultry workers from a commercial poultry farm in North West Province. All workers from different employment/job categories, including administration departments, were included. All workers completed a consent form prior to participating in the study.

The total number of employees at the farm was approximately 1315 which included permanent (90%) and casual labourers (10%). Among these, 639 worked in the factory, 596 in the farms and 80 were administrative staff. Of the 596 farm workers, 523 were permanent and 73 were casual workers[13].

Sampling strategy

The sampling strategy was based on the Similar Exposure Group (SEG) approach [14–17]. The workers were categorised into exposure groups based on the site inspections. The exposure groups were categorised based on type of poultry process and potential level of exposure (hatchery rearing, laying, broiler and catching). A stratified random sample of all workers was chosen based on the size of the farms (i.e. broiler large/small farm, hatchery old/new and laying manual/automated farm). Small farms housed ≤ 20 000 chickens whereas large farms housed > 20 000 chickens. Hatchery was regarded as a low exposure group, rearing and laying were regarded as medium exposure groups whereas broiler and catching were regarded as high exposure groups.

Power calculations for the sample size

Power calculations were computed for a collected sample size of 230 poultry workers. The power associated with detecting differences in prevalence of asthma among the baseline adult population group relative to the occupationally-exposed group were calculated. This was calculated with α=0.05, using the background prevalence of asthma in the South African adult population of 3.8% [18] and the prevalence of occupational asthma among poultry workers of 7% [19]. These power calculations assume a binomial distribution with no covariate adjustment. Statistical calculations indicated that a sample size of 230 obtained in the study had a power of 66.8%. This suggested that the study would be slightly under power for testing the hypotheses of interest.

Measurements

Study instruments

Exposure assessment

a) Environmental monitoring

Organic dust samples were collected using personal sampling pumps and 37mm, 1μm pore size sure sealed preloaded weigh checked glass fibre filters at a flow rate of 2L/min [20] for a minimum of 4 to 6 hours[20,21]. The sampling pumps were placed in each participants' breathing zone during their work activity. Field blanks and laboratory blanks were included on each day of measurement. Pumps were calibrated before and after sampling to ensure variance was within 5%. Sampling was conducted during the entire shift. Workers in the departments were categorised into exposure groups based on the initial walk through inspections. A stratified random sample of all workers was chosen from each group. Repeated sampling

was conducted on a subgroup of workers (20%) on at least two further occasions to assess the intra-individual variability. In total, 298 samples were collected.

b) Laboratory Analysis

i) Measurement of total particulate, endotoxin and (1-3) β-D-glucan

Dust measurements: Filters were weighed prior and after sampling using an OHAUS analytical balance in accordance with the MDHS14-3 2000 (HSL, HSE, United Kingdom). The airborne dust concentration was calculated using the flow rate, sampling time and total dust. The average weight gained was calculated by subtracting the average weight of the blank from the average weight of the sample.

Extraction: After gravimetric analysis, filters were then extracted at room temperature in 5 ml pyrogen free water with 0.05% Tween 20. Extracts were aliquoted for endotoxin and (1-3)-ß-D-glucan tests separately and stored at -20 °C for later use.

Endotoxin analysis: Concentration of endotoxin in the filter extracts was determined by using endpoint chromogenic Limulus amoebocyte lysate test (LAL) assay for endotoxin (Lonza, Walkersville, USA) as recommended by the manufacturer. The assay has been previously used for quantifying endotoxin in poultry houses (Rylander and Carvalheiro, 2006) and is already set up in the Immunology bioaerosol laboratory at the NIOH. Using the calculated volume of air sampled and endotoxin concentrations, the results were expressed as (EU/m^3) using the formula: [EU/ml **X** sample volume (ml)] / [time (min) **X** (rate (L/min) **X** $1m^3$/1000L] (BioWhittaker 2001). Unknown concentrations were calculated from the standard curve.

Measurement of (1-3)-ß-D-glucan detection: The aliquot was diluted two-fold with pyrogen free water with 0.05% Tween 20 and heat treated by autoclaving at 120°C for 1h before testing. (1-3)-ß-D-glucan present in extract was measured using Glucatell assay (Associates of Cape Cod, East Falmouth, MA, USA). The detection limit for (1-3) β-D-glucan was 20 pg/ml (Rylander and Carvalheiro, 2006).

Quality control: The limit of detection (LOD), which is the lowest level of detecting the agent, was computed by calculating the mean of the field blanks. The limit of quantification (LOQ), which is the limit above which there is confidence of the agent measured, was then calculated by adding the mean plus 3 times the standard deviation of the field blanks. For statistical analyses, values for exposure measurements below the LOD were censored using $L/\sqrt{2}$ (Finkelstein and Verma 2001). The co-efficient of variation (CV) between two measurements of ≤ 10 and $R^2 = 0.98$ for all assay runs was accepted. Quality control was applied to the measurements done by CSIR (accredited by SANAS: ISO 17025).

Health outcome assessment

a) Respiratory questionnaire

The study questionnaire was modified from previous questionnaires used for investigating asthma in the European Community Respiratory Health survey (ECRHS II) and seafood processing workers study [22].

The interviewer administered questionnaire collected information on important demographic factors, work related skin and respiratory symptoms, smoking status, history of previous illnesses, employment history and level of exposure to bioaerosols. The modified questionnaire was administered in English and in the language of the worker where necessary.

b) Serum tests

Blood samples were taken from each of the participants for immunological assessments. Sera were tested to determine for the presence of atopy and specific IgE to commercially available poultry work related allergens viz. storage mite (*Lepidoglyphus destructor)*, sunflower seed (*Helianthus annuus*) and mould mix (containing *Penicillium chrysogenum, C. herbarum, A. fumigatus, C. Albicans, A. alternata, S. rostrata*) using the UniCap Phadia system. The sera were also tested for specific IgE to chicken specific allergens (meat, feathers, serum protein, and droppings). Chicken allergens from the farms were prepared in-house. Allergens were extracted from these samples according to LABSOP01v1 (Occupational and Environmental health group, Netherlands) and then labelled with biotin. The biotin protein labelled extracts were loaded onto the streptavidin immunoCAP and quantified for allergens specific to this group using the serum of participants.

c) Lung function testing

Lung function tests (pre and post-bronchodilator tests) were conducted using a computerised portable spirometer (EasyOne™ Diagnosic Spirometer). A three-litre syringe was used to calibrate spirometers at least twice a day under controlled temperature and humidity. The procedure was done according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines for spirometry by an experienced technologist who was blinded to the exposure status of each worker. Reproducibility criteria was the two best tracings for both FEV_1 and FVC varying by no more than 150 ml or 5%, whichever was greater. The best FEV_1 and FVC were used irrespective of whether they belonged to the same tracing. An increase in FEV1≥12% and ≥200 ml after inhalation of a short-acting $ß_2$ agonist salbutamol (400 mg) was regarded as a significant airway reversibility suggestive of asthma.

d) Fractional exhaled nitric oxide (FeNO) determination

A portable hand-held sampling device (NIOX MINO) was used to determine FeNO during the work shift as per the American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations [23,24]. Testing of workers occurred throughout the working week and work shift, with no particular variation with regard to time of testing in the different departments. Two technically adequate FeNO efforts were measured and an average was determined. Special instructions were given to workers to refrain from smoking, eating or drinking (at least 1 hour) before the test. This was confirmed prior to testing, and those who did not follow the instructions were tested at a later stage after establishing their full compliance with these instructions. Those with recent chest infections were tested at a later stage after resolution of their symptoms. A value >50ppb was considered to be indicative of allergic airway inflammation [24].

List and definition of variables

Exposure variables

The following exposure variables of interest were identified:

(a) Total dust particulate
(b) Endotoxin
(c) (1-3)-β-D-glucan
(d) Independently measured specific IgE to occupational allergens related to poultry work namely, chicken meat, chicken feathers, chicken serum protein, chicken droppings, *Lepidoglyphus destructor*, sunflower seed and mould mix.

These are all continuous variables but could be converted to categorical variables if necessary.

Outcome variables

Outcome variables of interest for this study included:

(a) Doctor diagnosed asthma

(b) Current asthma: Yes to any of these 2 questions: "Have you had an attack of asthma in the last 12 months?" or "Are you currently taking any medicines including inhalers, aerosols or tablets for asthma?"

(c) Adult-onset asthma: "doctor-diagnosed asthma and having had the first asthma attack at the age of 16 years or later."

Operational definitions of asthma phenotypes

(a) Atopic asthma was defined as either having an asthma attack or use of asthma medication in the past 12 months or having a positive bronchodilator test (increase in $FEV_1 \geq 12\%$ and ≥ 200ml increase compared to baseline); AND presence of atopy or FeNO > 50ppb.

(b) Non-atopic asthma was defined as either having an asthma attack or use of asthma medication in the past 12 months or having a positive bronchodilator test (increase in $FEV_1 \geq 12\%$ and ≥ 200ml increase compared to baseline); AND absence of atopy.

(c) Probable Occupational Asthma was defined as either having an asthma attack or use of asthma medication in the past 12 months or having a positive bronchodilator test (increase in $FEV_1 \geq 12\%$ and ≥ 200ml increase compared to baseline); AND sensitisation to poultry work related allergens.

Covariates

Potential confounders that were considered included smoking (categorical variable), atopy (binary variable based on Phadiotop test), gender (binary variable), and age (continuous variable).

DATA MANAGEMENT AND ANALYSIS PLAN

Data from health questionnaires, lung functions, immunological testing, exhaled FeNo and environmental monitoring were analysed using STATA V.12 computer software (StataCorp, College Station, Texas, USA). The development of exposure metrics was based on individually measured exposures and mean levels within each exposure group. Descriptive statistics were used to summarise the distribution of each measured variable.

The main associations of interest were between exposure variables (e.g. duration of employment, employment status (casual versus permanent), area of work and history of an episode of high gas, dust or fume (GDF) exposure causing asthma symptoms) and important clinical endpoints (e.g. work related ocular-nasal and chest symptoms, airway inflammation (elevated FeNO > 50ppb), reversible airway obstruction (FEV_1 increase ≥12% and ≥200 ml post-bronchodilator), allergic sensitisation (positive ImmunoCAP test for antigen-specific circulating IgE antibodies in human serum to poultry work related allergens), and asthma phenotypes (atopic asthma, non-atopic asthma, probable occupational asthma). These associations were first explored using bivariate analyses and then saturated multivariate logistic regression models were developed. Models were chosen on a priori grounds based on existing information. Only atopy and gender were shown to be potential confounders, hence they were adjusted for in multivariate analyses. Odds ratios (OR) were used as the measure of effect.

ETHICS AND COMMUNICATION

The original study received ethical approval from the University of Witwatersrand Human Research Ethics Committee (Clearance certificate number – M10646, Appendix I). This study involved the analysis of a sub-set of the collected data, with no new data being collected. Therefore, there was no additional risk to the individuals who participated in the original study. This study followed ethical principles as stipulated in the World Medical Association Declaration of Helsinki [25].

Autonomy

Informed consent was sought from workers before embarking on the project. Participation was voluntary at no cost to the worker and the employer.

Confidentiality

Results of the tests were only discussed with the participants. All data was coded and workers and company confidentiality was maintained. All data were held in strict confidentiality in the School of Public Health and Family Medicine at University of Cape Town. All files were kept in cabinets under lock and key. For public presentations summary data will be presented and no personal identifiers will be used.

Benefit

Study participants were informed of their tests results and workers with abnormal findings were provided with advice and if necessary they were referred to the respective doctors for further examination. Although this analysis may not have direct benefits for the participants, the study findings will provide more information about the extent of exposures to multiple biological agents present in the poultry dust and their relationship with various respiratory

health outcomes. This information could be used to develop various programmes which will help in improving the health of people working in poultry farms.

Non-maleficence

The original study was low risk to participants in that there were potentially low risks associated with performing lung function tests, fractional exhaled nitric oxide (FeNO) determination and drawing blood. Study participants may have suffered discomfort from the needle prick during the collection of blood samples. All participants gave informed consent and records of the results indicate that no adverse events were reported. Since the current analysis did not require their participation, there was no additional risk.

Justice

The increased risk of respiratory health problems in poultry workers reported in literature justifies research to be done in this group.

Dissemination of research results

A full report will be sent to the respective company participating in the study. The results of the study will also be disseminated in the form of:

a) MMed dissertation
b) Research forum, research day, local/international conference
c) Publications in scientific journal (local and international)
d) Report to the company management (oral briefings).

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SECTION B: LITERATURE REVIEW

RESPIRATORY HEALTH EFFECTS IN POULTRY WORKERS - A REVIEW OF LITERATURE

LITERATURE SEARCH STRATEGY

This review focuses on respiratory diseases associated with poultry work as they continue to be the most commonly reported adverse health effects. Google Scholar and MEDLINE were searched to identify relevant articles on adverse respiratory outcomes in poultry workers from 1980-2014. Review articles found in the process were examined for additional and earlier citations. The key words used included: poultry, dust, asthma, allergy, work related, occupational, poultry, chicken, broiler, bioaerosols, bacteria, fungi, virus, endotoxin, (1-3) β-D glucan, allergens, and mites. Reports from credible international occupational health and safety institutions such as the Health and Safety Executive (UK) and the National Institute for Occupational Safety and Health (USA) were also included.

THE POULTRY WORKING POPULATION AND WORK PRACTICES

Although exposure patterns related to the development of respiratory symptoms in the poultry industry are not clearly understood, the literature suggests that; the type of production, housing systems, and tasks performed have a significant influence on the level of exposure to environmental contaminants in workers [1].

Poultry facilities may operate by either housing birds in cages or on litter on the floor. These two types of production processes may affect the levels of different environmental contaminants from poultry dust [2]. In the cage based system, chickens are reared in cages mainly for the production of eggs while in the floor based system, they are kept on the floor mainly for meat production [3,4].

The two poultry operations differ in a number of ways including the time spent by workers in bird houses, the age of birds, the time spent by birds in the houses and the housing

management practices [3]. Workers in cage based systems usually spend less time in poultry houses and this is associated with a reduced duration of exposure which possibly reduces the risk of adverse health effects [5].

In a prospective evaluation of air quality and worker's health comparing the two poultry operations, the level of air contaminants were shown to vary with the type of poultry operation with dust, endotoxin and ammonia levels being reported to be higher in floor based systems for broilers than cage based operations for egg layers [4]. Other factors influencing these variations included flock age, size and activity as well as litter age [6,7].

Previous studies among poultry workers have shown that dust and bioaerosols exposure vary with specific jobs and tasks. Table 1 summarizes important tasks that are sources of potential airborne exposure to poultry dust in poultry workers. Some of the poultry farm jobs that appear to be associated with a higher risk of respiratory effects associated with exposure include; chicken catchers, turkey workers as well as broiler and layer workers [8]. Tasks such as poultry inspection and handling, vaccination, routine house maintenance and cleaning during growth or production periods, laying down litter, populating and depopulating of the birds, litter/manure removal, catching or removing birds are known to increase exposure to poultry dust [9]. However, tasks such as sweeping and brushing down surfaces and spreading litter have been associated with higher exposures [10].

Table 1: Tasks associated with sources of potential airborne exposure to poultry dust in poultry workers

Task	Description of duties	Source of exposure
Laying down litter/bedding	Spreading litter/bedding in poultry houses	Nature of material. Some contamination with fungal spores and inhalable dust.
Catching	Repopulating and depopulating chicks/chickens	Proteins from feather debris or dander, mites (dust mites, storage mites), faecal residues, dust particulate, airborne bacteria, endotoxin.
Litter/manure removal	Removing soiled litter	Inhalable dust, organic compounds released during degradation of litter and feed.
Cleaning	Routine cleaning	Exposure to feathers, mites, feed particulate.
Routine housework/flock management	• Adding fresh litter/bedding and feed • Routine checks (inspections, weighing, vaccinations)	Microbial degradation of material. Bedding contaminated with organic matter derived from poultry feed, faeces, dander, feathers, mites, microorganisms and their constituents.
Forklift drivers	Load boxes/cages of chicken into trucks for transportation to slaughter-houses	Inhalable poultry dust.

Modified and adapted from Singh and Matuka, 2010 [11]

CONSTITUENTS OF POULTRY DUST

'Poultry dust' refers to the dust comprising of biological agents that are produced from processes involved in poultry production [8]. The constituents of poultry dust may range from uncontaminated wood dust to a mixture of complex organic and inorganic materials containing poultry faeces, feathers, dander, bacteria, endotoxin, mycotoxin, fungi, fungal spores and mites [9,12,13]. In addition to particulates, the decomposition of biological

materials may produce gases such as ammonia, hydrogen sulphide and carbon dioxide that may also cause respiratory health problems [14–16].

Particles in poultry dust vary in size from 0.5 to 50 microns. This range includes respirable particles (<5 microns), which are capable of penetrating in the gas exchange region of the lung [8].

Constituents of poultry dust have been reported to vary according to the type of birds, the point in the growing cycle and the work activity in the poultry farm [8]. A study of exposure to bioaerosols in poultry houses reported a significant increase in bioaerosol levels during the poultry fattening period. The task of catching mature birds was associated with higher endotoxin concentration (6198 EU/m^3), levels which far exceed the proposed Dutch occupational recommended value (90 EU/m^3) (DECOS, 2010). Exposure to total bacteria and staphylococci was also reported to be high during this period [17].

EXPOSURE LEVELS OF DUST AND BIOAEROSOLS IN POULTRY HOUSES

Various studies have been conducted to evaluate different airborne contaminants that poultry workers may be exposed to when working in poultry houses. Table 2 summarizes these various environmental exposure assessment studies of poultry houses.

Poultry dust

The reported mean concentration of total dust from poultry operations ranged between 0.12mg/m^3 to 26mg/m^3 [18,19]. These dust particles may either be in the respirable range (<5microns), with the ability to penetrate the lungs' gas-exchange region or in the non-respirable range with much larger particle sizes impacting in the larger airways [8]. The inhalable dust concentration in poultry operations has been shown to range from 0.02 to

81.33 mg/m^3 and that of respirable dust from 0.01 to 6.5mg/m^3 [20]. The type of poultry operation has great influence on the concentration of dust with higher concentrations having been reported in floor based operations than in cage based operations [19–22].

Material used for poultry litter may also contribute to dust emissions. Commonly poultry litter is derived from pine shavings, sawdust or eucalyptus, or other various types of wood. Use of chopped straw (plain or treated with mould-inhibitor), shredded paper, oilseed rape haulm, rice hulls, peanut, coffee, sugar cane, hay and grass, has been reported in other operations [9,23].

Microbes

Bacteria

In the poultry dust, bacteria maybe derived from soil, bird's faeces, skin micro flora and zoonotic agents, as well as feed and bedding [8]. The determination of bacterial concentrations is usually done by culturing air or surface dust samples on agar media followed by counting the number of viable bacterial cells capable of growing into colonies expressed as colony-forming units CFU/m^3 of air [11]. Alternatively, the use of real time polymerase chain reaction (PCR) [17] and epifluorescence microscopy [24] has been reported in other studies [17]. The use of these methods makes it possible to detect dead or uncultivable bacterial strains. In these methods bacterial concentrations may be expressed as cells/m^3 of air.

The average concentration of viable bacteria from different studies ranged between $3.86x10^5$ [25] to $4.66x10^5$CFU/m^3 [18]. Using epifluorescence microscopy, the median total bacteria (viable and non-viable) was $4.7x10^9$ cells/m^3 with a range between $2.7x10^7$ and $4.2x10^9$ cells/m^3 [24].

The concentration and kind of bacteria in poultry houses depend on factors such as the type of the housing system, number of birds, type of ventilation systems, dust concentration and microclimatic conditions (humidity, temperature), concentration of gases and lighting [26]. Other factors include improper management practices and poor hygienic conditions.

Some of the bacterial species commonly found in poultry houses include, *Salmonella* ssp, *Staphylococci, Streptococci, Enterococci, E.coli, C.perfringes, Pseudomonas* and *Acinetobacter* [8,18,24,27–31].

In a microbiological evaluation of air contamination in different types of housing systems for laying hens, high levels of airborne bacteria were reported in non-cage compared to cage systems [27]. The highest airborne concentration of bacteria was reported by a UK study of poultry houses during laying litter $2.0x10^7$ followed by repopulating ($8.4x10^6$) and the lowest ($1.2x10^6$) during routine house work such as checking feed and maintaining water supply equipment [9].

Fungi

Similar to bacteria, fungi in poultry dust are derived from feed, soil and bedding and lesser amounts from the birds themselves. In bedding materials, fungi usually grow and multiply in moist conditions [8].

Previous research on poultry workers' exposure to fungi reports the concentration of airborne fungi ranging from 1800 CFU/m^3 [29] to levels reaching $1.1x10^7$ CFU/m^3 [24]. A study that measured fungal levels with respect to specific tasks done in poultry houses reported a range from 1120 CFU/m^3 during depopulating (catching) task to $4.0x10^6$ CFU/m^3 during laying of bedding or litter. The latter involved handling mould-damaged litter in which the major proportion of fungal spores detected were derived from *Asp. Fumigatus* [9]. Using

epifluorescence microscopy, the median fungal concentration was $2.0x10^7$cells/m^3, ranging from below detection limit to $1.1x10^9$cells/m^3 [24].

Other fungal species present in poultry dust include, *Aspergillus* including *Asp. fumigatus* and *Eurotium (Asp. glaucus group), Cladosporium, Basidiospores, Acremonium, Aurobasidium, Drechslera, Pithomyces, Penicillium, Crysosporium, Scopulariopsis, Fusarium, Epicoccum, Mucor, Geomyces, Alternaria, Ulocladium,* and *Rhizomucor*. Many of these species are known allergens as well [8].

Toxins: endotoxin and mycotoxins (glucans)

Endotoxins

Endotoxins are common air contaminants in agricultural setting including poultry operations [3]. High endotoxin concentrations have been reported in the poultry industry compared to the swine, animal feed and grain industries [32]. These agents have been shown to be present in poultry dust samples collected throughout the poultry production cycle [8].

Endotoxins are structural components of the outer lipopolysaccharide containing membrane of gram negative bacteria [22]. Studies of exposure to poultry farms have reported concentrations ranging from below detection limit to 83640 EU/m^3 for total endotoxin, 7.1 to 704 EU/m^3 for airborne respirable endotoxin and 1000 to 8600 EU/m^3 for non-respirable endotoxin [6,22,29,33].

Similar to dust particulate, personal endotoxin exposure concentrations may be affected by poultry housing type, age and number of birds, type of litter and feed, ventilation, amount of time spent by workers in poultry houses, manure management system and general housekeeping tasks [3]. These levels were reported to be similar or higher in cage based as compared to floor based operations [2,5,15]. A study of environmental exposure levels in poultry operations reported higher endotoxin levels with significantly lower total dust, in

cage-housed as opposed to floor-housed poultry operations [2]. Similar results have also been reported in a recent study in which the respirable fraction of endotoxin in cage based operations was found to account for a greater percentage of the total endotoxin concentrations compared to floor based operations [22].

(1-3) β-D-glucan

(1-3) β-D-glucans are non-allergenic cell wall components of most fungi, some bacteria and plants and can be found in organic dust. They are usually used as surrogate markers of fungal exposure [11,34]. A study that measured the levels of (1-3) β-D-glucan reported values of 20 (0.01–70) ng/m^3 for water soluble and 270 (4–870) ng/m^3 for non-water soluble (1-3) β-D-glucan [35]. Another study reported values ranging from 0.8 to 6886 ng/m^3[29].

Allergens

Allergen exposure in poultry industry may occur from contact with mites, poultry dust containing grain or animal feed protein allergens (wheat allergens), and allergenic fungi (mostly from *Aspergillus spp*) [8,36]. Other sources of allergens include bird feathers, dander, serum and droppings which poultry workers may be exposed to during close handling of birds [8].

A study evaluating exposure to moulds and house dust mite *Dermatophagoides pteronyssinus* allergen in poultry farms reported levels ranging from <0.1 to 3.3 ug/g [37]. Moreover, a previous study conducted in Croatian poultry farms reported levels of 17.9 (3.8-72.4) ng/g for Asp f 1, which is the major allergen of *Aspergillus fumigatus* [36].

Chemicals and pollutant gases

Livestock buildings have been reported to be one of the major anthropogenic sources of atmospheric pollutants, such as ammonia, methane, nitrous oxide and carbon dioxide as well as chemicals such as disinfectants and pesticides [15,31]. Among the studies that measured environmental levels of different gases in poultry houses, ammonia levels have been the most commonly reported ranging from below detection limits in active areas [24] to 170ppm in unused areas of poultry houses [6]. Carbon dioxide levels have been reported in the range of 600 to 4100 ppm [24] while methane and nitric oxide levels reported as being similar to general ambient levels [15].

Table 2: Environmental exposure assessment studies among poultry workers

Study	**Type of sample**	**Area sampled**	**Dust particulate concentration (mean ±SD/range)** [mg/m^3]	**Endotoxin concentration (Mean ±SD /range)** [EU/m^3 -airborne, EU/g -in the dust mass]	**Microbial concentration (Mean ±SD /range)** [CFU/m^3]	**Gaseous chemicals concentration (Mean ±SD/range)** [ppm]	**Mycotoxins (β- glucan) (Mean ±SD /range)** [ng/m^3]	**Allergen concentration** [ng/g]
Silvana P et al, 2013[27]	Area	Cage system	ND	ND	Mesophile: 3.88×10^5 Staphylococci: 1.66×10^5 Streptococci: 9.54×10^4 Gram-negatives: 2.85×10^3 Fungi: 4.09×10^3	ND	ND	ND
		Non cage system	ND	ND	Mesophile: 4.17×10^6 Staphylococci: 2.25×10^5 Streptococci: 8.48×10^5 gram-negatives: 3.82×10^4 Fungi: 5.19×10^4	ND	ND	ND
Lawniczek-Walczyk A et al 2013[29]	Area and personal	Poultry houses	ND	Total airborne : BDL-83640	Bacteria: 2.5×10^2-2.9×10^6 Fungi: 1.8×10^2-1.8×10^5	ND	0.8 -6,886	ND

Table 2 (Continued)

Study	**Type of sample**	**Area sampled**	**Dust particulate concentration (mean ±SD/range)** [mg/m^3]	**Endotoxin concentration (Mean ±SD /range)** [EU/m^3 -airborne, EU/g -in the dust mass]	**Microbial concentration (Mean ±SD /range)** [CFU/m^3]	**Gaseous chemicals concentration (Mean ±SD/range)** [ppm]	**Mycotoxins (β- glucan) (Mean ±SD /range)** [ng/m^3]	**Allergen concentration** [ng/g]
Wang-Li L et al, 2013[25]	Area	Layer barn	ND	ND	Bacteria: $3.86\times10^5\pm1.74\times10^5$ Fungi: $3.0\times10^3\pm4.45\times10^3$	ND	ND	ND
		Ambient surrounding area	ND	ND	Bacteria: 1.3×10^3 to 6.2×10^3 Fungi: 7.4×10^3 to 1.7×10^4	ND	ND	ND
Le Bouquin S et al, 2013[38]	Area	Floor system	Respirable: 0.37	ND	ND	ND	ND	ND
		Cage system	Respirable: 0.13	ND	ND	ND	ND	ND
Guillam MT et al, 2013[21]	Personal	Cage system	Respirable: 0.234	ND	ND	ND	ND	ND
		Floor system	Respirable: 0.297	ND	ND	ND	ND	ND
Sowiak M et al, 2012[39]	Area	Breeding facilities	ND	ND	Fungi: 1.60×10^5(1.22×10^3-5.87×10^3)	ND	ND	ND
Le Bouquin, S et al, 2011[19]	Area	Cage system	Respirable: 0.12	ND	ND	ND	ND	ND
		Floor system	Respirable: 0.46	ND	ND	ND	ND	ND

Table 2 (Continued)

Study	**Type of sample**	**Area sampled**	**Dust particulate concentration (mean ±SD/range)** [mg/m^3]	**Endotoxin concentration (Mean ±SD /range)** [EU/m^3 -airborne, EU/g -in the dust mass]	**Microbial concentration (Mean ±SD /range)** [CFU/m^3]	**Gaseous chemicals concentration (Mean ±SD/range)** [ppm]	**Mycotoxins (β- glucan) (Mean ±SD /range)** [ng/m^3]	**Allergen concentration** [ng/g]
Kirychuk SP et al, 2010[22]	Area	Cage system	Total: 1.69 Non -respirable: 1.32 Respirable: 0.48	*In dust mass* Total: 901.5 Non respirable: 983 Respirable: 704.0 *Airborne* Total: 1513.1 Non respirable 1121.6 Respirable 340.4	ND	ND	ND	ND
		Floor system	Total: 4.62 Non respirable: 3.80 Respirable: 0.80	*In dust mass* Total: 538.0 Non respirable: 585.6 Respirable: 3311.0 *Airborne* Total: 2504.1 Non respirable: 2216.1 Respirable: 272.3	ND	ND	ND	ND

Table 2 (Continued)

Study	**Type of sample**	**Area sampled**	**Dust particulate concentration (mean ±SD/range)** [mg/m^3]	**Endotoxin concentration (Mean ±SD /range)** [EU/m^3 -airborne, EU/g -in the dust mass]	**Microbial concentration (Mean ±SD /range)** [CFU/m^3]	**Gaseous chemicals concentration (Mean ±SD/range)** [ppm]	**Mycotoxins (β- glucan) (Mean ±SD /range)** [ng/m^3]	**Allergen concentration** [ng/g]
Brooks JP et al 2010[28]	Area and Surface	Poultry houses	ND	Total airborne: 170-23000	HPC Bacteria: 2.1×10^4 -3.0×10^6 Coliforms: BDL-5.3×10^1 C. perfringens:BDL- 1.7×10^2 Staphylococci: 1.5×10^4-8.65×10^5 Enterococci: 1.8×10^2 to 2.4×10^3	ND	ND	ND
Prester L et al, 2010[36]	Area	Poultry houses	ND	ND	ND	ND	ND	Asp f 1: 17.9 (3.8-72.4)
Rimac D et al, 2010[37]	Area	Poultry houses	ND	Total airborne: 230-284	Fungi: 4.9 x 10^3-6.8 x 10^4	ND	ND	Der p 1: <0100-3300
Oppliger A et al, 2009[17]	Personal	Poultry houses (catchers)	Non respirable: 26 ±1.9	Total airborne: 6198 ± 2.3	§Bacteria: 53 ±2.6x 10^7 §Staphylococcus Ssp: 62±1.9 x 10^6	ND	ND	ND
Matković K et al, 2009[40]	Area	Laying hen coop	ND	ND	Fungi: 6.89 x 10^4 - 1.13 x 10^5	ND	ND	ND
Crook B et al, 2008[9]	Area	Poultry houses	2.7-80	Total airborne: 30.7-16600	Bacteria: $2.5x10^6$-$2.0x10^8$ Fungi: $1.12x10^3$-$6.0x10^5$	ND	ND	ND

Table 2 (Continued)

Study	**Type of sample**	**Area sampled**	**Dust particulate concentration (mean ±SD/range)** [mg/m^3]	**Endotoxin concentration (Mean ±SD /range)** [EU/m^3 -airborne, EU/g -in the dust mass]	**Microbial concentration (Mean ±SD /range)** [CFU/m^3]	**Gaseous chemicals concentration (Mean ±SD/range)** [ppm]	**Mycotoxins (β- glucan) (Mean ±SD /range)** [ng/m^3]	**Allergen concentration** [ng/g]
S. P. Kirychuk et al, 2006[2]	Personal	Cage system	Total: 7.57±8.99	Total airborne: 1291.47±1349.74 In dust mass: 9544.02 ±14189.62	ND	Ammonia: 10.5±11.2	ND	ND
		Floor system	Total: 9.56±7.95	Total airborne: 1106 ±1420 In dust mass: 7484±9020	ND	Ammonia: 17.2 ±18.2	ND	ND
Rylander R et al, 2006[35]	Area	Poultry houses	ND	Total airborne: 4100 (100–10030)	ND	ND	WS: 20 (0.01–70) NWS: 270 (4–870)	ND
Bakutis B, et al 2004[18]	Area	Poultry houses	Total: 11.4 ± 1.01	Non respirable: 4000±2287	Bacteria: 466.08±38.14×10^3 Gram-negative: 12.35±1.97×10^3	ND	ND	ND
Borghetti C et al, 2002[30]	Area	Poultry houses	Total: 2.6 (1.8-4.6)	Total airborne: 1371 (586-2439)	Bacteria: 7.6X10^3 (9.07X10^2-2.78X10^4)	Ammonia: 7.5 (5-20)	ND	

Table 2 (Continued)

Study	**Type of sample**	**Area sampled**	**Dust particulate concentration (mean ±SD/range)** [mg/m^3]	**Endotoxin concentration (Mean ±SD /range)** [EU/m^3 -airborne, EU/g -in the dust mass]	**Microbial concentration (Mean ±SD /range)** [CFU/m^3]	**Gaseous chemicals concentration (Mean ±SD/range)** [ppm]	**Mycotoxins (β- glucan) (Mean ±SD /range)** [ng/m^3]	**Allergen concentration** [ng/g]
Radon K et al, 2001[24]	Area and personal	Poultry houses	Total: 7.0 (0.4–21.8)	Total airborne: 2576 (190–16348)	*Bacteria: $4.7x10^9$ ($2.7x10^7$-$4.2x10^{10}$) *Fungi: $2.0x10^7$ (<DL–$1.1x10^9$)	Ammonia: 12 (<DL–40) Co_2: 2100 (600–4100)	ND	ND
Donham KJ et al, 2000[41]	Area	Poultry houses	Total: 2.4 Respirable: 0.16	Total airborne: 614	ND	Ammonia: 12	ND	ND
Ellen HH et al 2000[20]	NS	Poultry houses	Non respirable: 0.02 - 81.33 Respirable: 0.01 - 6.5	ND	ND	ND	ND	ND
Hartung J, 1998[33]	Area	Poultry houses	Non respirable: 10 Respirable: 1.2	Non respirable: 8600	ND	ND	ND	ND
Wathes CM et al 1997[15]	Area	Poultry houses	Non respirable: 2 - 10 Respirable: 0.3 - 1.2	Non respirable: 1000	ND	Ammonia: 12.3 - 24.2	ND	ND

Table 2 (Continued)

Study	**Type of sample**	**Area sampled**	**Dust particulate concentration (mean ±SD/range)** [mg/m^3]	**Endotoxin concentration (Mean ±SD /range)** [EU/m^3 -airborne, EU/g -in the dust mass]	**Microbial concentration (Mean ±SD /range)** [CFU/m^3]	**Gaseous chemicals concentration (Mean ±SD/range)** [ppm]	**Mycotoxins (β- glucan) (Mean ±SD /range)** [ng/m^3]	**Allergen concentration** [ng/g]
Jones W et al, 1984[6]	Area	Confinement building	ND	Total airborne: 7.7-610 Respirable: 7.1 - 150	Bacteria 1.5 X 10^5 Fungi:1.0 X 10^4	Ammonia: Active areas: 25 Unused areas: 170	ND	ND

NS=not specified, ND=Not done BDL= below detection limit, WS=Water soluble, NWS= non water soluble, HPC= Heterotrophic Plate Count
§= measured in cells/m^3 using real time polymerase chain reaction; *= total bacteria measured in cells/m^3 using epifluorescence microscopy

UPPER AND LOWER AIRWAY RESPIRATORY OUTCOMES ASSOCIATED WITH POULTRY DUST EXPOSURE

Poultry workers are reported to have an increased incidence of upper and lower airway respiratory symptoms and deficits in lung function related to exposure to organic dust [2,37]. Poultry dust, similar to any other agricultural dusts, contains various potential respiratory sensitisers, irritants and other pro inflammatory agents capable of causing respiratory disease [8]. The severity of symptoms may be influenced by the airborne levels as well as the composition of the dust [42]. The extent to which these components cause disease will depend on the dose, the duration of exposure and possible worker's susceptibility [11]. A summary of published studies are presented in Table 3.

Rhinitis

The prevalence of rhinitis among poultry workers from various studies has been in the range of 19% to 51%, which is higher than that reported in unexposed controls [21,23,24,37,43–45]. A study by Rimac et al, reported a fourfold higher prevalence of rhinitis among poultry workers compared to controls [37]. Additionally, a higher prevalence was reported among workers in floor based operations when compared to cage based operations, in a study comparing the two housing systems [21]. Symptoms of rhinitis were reported in all workers employed for more than 11 years in a poultry farm, and the incidence of rhinitis was shown to have a bearing on the incidence of both cough and breathlessness [45].

Asthma

Studies conducted among poultry workers have documented the prevalence of asthma varying between 1% and 43% for self-reported asthma and 6% to 14% for asthma diagnosed using spirometry [4,6,30,37,44,52,53].

The prevalence reported in most studies has been that of general adult asthma, which is mostly higher in poultry workers than other occupations including other farm workers [43,44]. Similarly, studies have reported a higher prevalence of work-related symptoms that were consistent with asthma in poultry workers compared to non-poultry workers. A study by Barsela et al, reported work-related asthma like symptoms in 14 out of 16 poultry workers, one of whom tested positive to northern fowl mites [49]. In a South African study, asthma had been diagnosed in 4%, 13% and 11% in poultry workers with low, medium and high exposure to poultry dust respectively, compared to 3% in the controls [47]. A study conducted on Victoria broiler growers, reported that 10.7% workers had asthma compared to a prevalence of 5.7% among adults in South Australia [50]. On the other hand, Zuskin et al reported no significant difference in asthma symptoms between poultry workers and controls [48]. Similarly, Donham et al found little evidence of asthma in US poultry workers [51].

Chronic bronchitis

The prevalence of chronic bronchitis in various studies among poultry workers varies between 4.4% to 21% [32,47,48,52,53]. This prevalence in poultry workers has been reported to be higher when compared to unexposed controls. A study done to assess the respiratory symptoms and lung function of Norwegian farmers reported a two fold increase in odds of chronic bronchitis among poultry farmers compared to crop farmers (OR 2.2, CI 1.3-4.0) [52]. Similarly, a significantly increased risk of chronic bronchitis was reported in South African poultry workers with medium (OR=2.88, CI: 1.14 -7.28) and high dust exposure (OR=2.86, CI: 1.04-7.90) [47]. Furthermore, work related chronic bronchitis was most commonly found in poultry handlers (15.5%) compared to other workers exposed to organic dusts [32].

Hypersensitivity pneumonitis (poultry worker's lung)

Exposure to aerosolized avian proteins may result in hypersensitivity pneumonitis (HP)[54,55]. Although HP is commonly reported to be a result of pigeon exposure, a few cases have been shown among people exposed to poultry [54,56,57]. This has been attributed to chronic exposure to high levels of airborne fungal spores, chicken antigens, and litter [8,37,55–57]. In addition, a combination of (1-3) β-D-glucan and endotoxin have been shown to cause histological changes resembling HP in animal experiments [58]. Having exposure to all these agents, poultry workers could be expected to be at an increased risk of HP, however, the incidence of clinically recognizable HP among poultry workers is said to be very low [35]

In the Agricultural Health Study, working in poultry houses was associated with a non-statistically significant increased OR for self-reported hypersensitivity pneumonitis (OR 1.55, 95% CI 0.93-2.58) [59].

Contrary to an abundance of reported cases of HP among pigeon breeders, it is not clear why this disease is uncommon in chicken farmers who are often exposed to a larger number of birds. Further studies to quantify the burden of this disease and the immunogenicity of chicken antigens in causing HP may be important[57].

Other symptoms

Other acute and chronic work-related respiratory symptoms in poultry workers include, sneezing, cough, wheezing, shortness of breath, eye irritation, phlegm, flue like illness, chest tightness, dry throat, dry nose, nose bleeding, headache, and fever [1,2,24,48]. These symptoms are usually non-specific and may disappear during periods away from work [8]. This wide range of symptoms emphasizes the complexity of pathophysiological mechanisms by which poultry dust may cause respiratory disease.

Table 3: Epidemiological studies of work-related asthma and other respiratory symptoms among poultry workers

Study	**Subjects (n)**	**Asthma prevalence (%)**	**Other symptoms**	**Skin prick (% positive)**	**Other immunological evidence (% positive)**	**Tools used**
Guillam MT et al, 2013[21]	63	8% (ever had) 6%(clinically diagnosed)	Sneezing: 46% Rhinitis: 19% Cough: 43% SOB: 21% Fever: 3% Eczema: 24%	Common inhalant allergens: 25% Mite allergen: 21%	ND	Questionnaire, Spirometry
Viegas S et al, 2013[23]	47	7% (clinically diagnosed) 43% (self-reported)	Rhinitis: 51% Sneezing:13% Chest-tightness and wheezing:13%	ND	ND	Questionnaire, Spirometry
Rimac D et al, 2010[37]	41	39% (self-reported)	Rhinitis: 39% Eye: 34% Skin: 29%	Common allergens: 12% *D. pteronyssinus*: 7% *D. farinae*: 2% *L. destructor*: 2% *Mucor mucedo*: 5%	Total IgE[a]: 10% IgG to moulds: 63%	Questionnaire, Spirometry
Eduard W, et al 2009[52]	199	ND	Chronic bronchitis: 9% COPD: 10%	ND	ND	Questionnaire, Spirometry
Kirychuk SP et al, 2006[2]	303	ND	Cough: 13% Phlegm: 19% Wheeze: 16%	ND	ND	Questionnaire, Across shift spirometry

Table 3 (continued)

Study	Subjects (n)	Asthma prevalence (%)	Other symptoms	Skin prick (% positive)	Other immunological evidence (% positive)	Tools used
Kirychuk SP, et al 2003[1]	303	ND	*Cage based:* Cough: 19% Phlegm: 20% *Floor based:* Cough: 16% Phlegm: 17%	ND	ND	Questionnaire, Spirometry
Hoppin JA, et al 2003[46]	764	7% (self-reported)	Wheeze: 25%	ND	ND	Questionnaire
Borghetti C et al, 2002[30]	14	36% (self-reported) 14% (spirometry) 7% occupational asthma	ND	ND	ND	Questionnaire, Spirometry, Bronchial provocation test positive to storage mite *L. destructor*
Magarolas R, et al 2001[43]	808	39% (self-reported)	Rhinitis: 32%	ND	ND	Questionnaire
Radon K, et al 2001[24]	104	ND	Rhinitis: 20% Cough without phlegm: 15% Cough with phlegm: 9% SOB: 12% Wheeze: 10% Flu-like illness: 14%	ND	ND	Questionnaire

Table 3 (continued)

Study	Subjects (n)	Asthma prevalence (%)	Other symptoms	Skin prick (% positive)	Other immunological evidence (% positive)	Tools used
Kimbell-Dunn MR, et al 2001[53]	23	ND	SOB: 20% Chronic bronchitis: 4% Flu-like illness: 9%	ND	ND	Questionnaire
Singh AB, et al 1999[45]	144	ND	Rhinitis: 37% Cough: 35% SOB: 19% Wheeze: 8%	ND	ND	Questionnaire
Kimbell-Dunn M, et al 1999[44]	23	17% (self-reported)	Rhinitis: 26% SOB: 9% Wheeze: 44% Eczema/skin allergy: 35%	ND	ND	Questionnaire

Table 3 (continued)

Study	Subjects (n)	Asthma prevalence (%)	Other symptoms	Skin prick (% positive)	Other immunological evidence (% positive)	Tools used
Rees D, et al 1998[47]	134	*Low exposure:* 4% (self-reported) *Medium exposure:* 13% (self-reported) *High exposure:* 11% (self-reported)	Cough: 32% Severe Dyspnoea: 7% Chest tightness: 19% Wheezing: 23% Chronic bronchitis: 21%	Common allergens: 28.4% Chicken serum: 1% Faeces: 2% Feed:0.75% Feathers: 0.75%	**IgE:** Chicken serum: 0% Faeces: 7% Feed: 3% Feathers: 3% **IgG:** Chicken serum: 7% Faeces: 6% Feed: 4% Feathers: 3% **Immunodiffusion** Chicken serum: 24% Faeces: 14% Feed: 1% Feathers: 39%	Questionnaire
J. C. Simpson, et al 1998[32]	84	ND	URTS[a]: 45% LRTS[b]: 38% Chronic bronchitis: 16% Flu like illness: 6%	ND	ND	Questionnaire

Table 3 (continued)

Study	Subjects (n)	Asthma prevalence (%)	Other symptoms	Skin prick (% positive)	Other immunological evidence (% positive)	Tools used
Zuskin E, et al 1995[48]	343	1% (self-reported)	Chronic cough: 30% Chronic phlegm: 24% Severe dyspnoea: 7% Chest tightness: 19% Chronic bronchitis: 21% Others: Eye irritation, dry throat, dry nose, nose bleeding, rhinitis	ND	ND	Questionnaire, Spirometry
Tudor A, et al 1985[60]	93	ND	Cough and expectoration: 11% Severe dyspnoea: 7% Chest tightness: 19% Wheezing: 14% Chronic bronchitis: 21%		IfG to avian allergens: 76%	Questionnaire, Spirometry

ND=not done, NA= not applicable, SOB= shortness of breath, LRTS=lower respiratory symptoms, URTS=upper respiratory symptoms; [a] included eye and nasal irritation; [b] included cough, phlegm, shortness of breath, wheeze, and chest tightness;[c] Grass pollens, birch, hazel, *Ambrosia elatior*, Artemisia vulgaris, dog, cat, *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, *Alternaria alternate*, *Cladosporium herbarum*,

ENVIRONMENTAL RISK FACTORS AND EXPOSURE-RESPONSE RELATIONSHIPS FOR POULTRY DUST AND RESPIRATORY DISEASES

Environmental risk factors for development of respiratory disease among poultry workers depends on mode and route of exposure to a hazardous agent, the level of exposure to the agent, concomitant exposure to other agents in the workplaces, and the design and operation of the poultry facilities.

Mode and route of exposure

The mode and route of exposure to a sensitising agent has been shown to increase the risk of occupational asthma and other work related respiratory illnesses. By far, inhalation is the main route of exposure to poultry dust causing respiratory illnesses. Recently there have been concerns as to whether dermal exposure may be a source of sensitization and asthma development [61]. Regarding poultry workers, dermal exposure to poultry feed, preservatives and antibiotics has been reported to cause allergic contact dermatitis. A case of bronchial asthma and allergic dermatitis due to spiramycin has been reported in a non-atopic chick breeder who handled poultry feed containing this antibiotic [62].

Level of exposure

An association between the levels of exposure to poultry dust and respiratory illnesses among poultry workers has been reported. The odds of having cough, wheeze, dyspnoea, chronic bronchitis and asthma increased among poultry workers from low medium to high exposure groups [47].

Hoppin et al reported a dose response relationship between the number of poultry on the farm and wheeze [46]. A dose related decline in lung function with across shift decline in FEV_1 among poultry workers exposed to endotoxin and respirable dust has also been shown. Exposure levels associated with these significant pulmonary function derangements were 614

EU/m^3 endotoxin, 2.4 mg/m^3 total dust, 0.16 mg/m^3 respirable dust, and 12 ppm ammonia [41].

Length of time of exposure has also been shown to correlate with the risk of respiratory illnesses among poultry workers [23,63]. Poultry workers with more than ten years of exposure had a higher prevalence of symptoms and/or decreased lung function compared to those with fewer years of exposure [8,48,49]. Workers spending more than five hours at a time in confinement areas have been shown to be at increased risk of having rhinitis [64].

Concomitant exposures to chemical pollutants in the workplace

The risk of respiratory illnesses has been shown to increase with poultry workers exposed to a combination of particulates, bioaerosols, and gases [63,65]. A study of 257 poultry production workers reported synergistic effects between airborne dust and ammonia levels. This synergy explained up to 43% and 63% of the deterioration in FEV_1 and FEF_{25-75} respectively. Additionally, assessing the synergy index revealed that, in combination, the effects of dust and ammonia were 53% to 156% higher than their expected individual effects [16].

These findings emphasize the importance of considering the combined effects of air contaminants in causation of health problems during planning for control measures. There may be a need to redefine occupational exposure limits of airborne contaminants when workers are exposed to more than one agent known to have synergistic effects [66].

Design and operation of facilities

Ventilation of the poultry facility has a significant impact on the risk of respiratory disease [24]. Using wood shavings for bedding, dry feeds, disinfectants, and non-slatted floors has been shown to increase the risk of flu like illness (ODTS) [31]. The type of housing system,

viz. floor or cage based may also influence the development of respiratory symptoms among poultry workers. One study showed a greater preponderance of cough, wheeze and decreased lung function in workers from cage compared to floor-housed facilities [2].

In contrast, a study of naive subjects exposed to cage rearing system versus an alternative rearing system for layers reported a 3-hr exposure in confined egg production buildings to induce an acute upper airway inflammatory reaction and increased bronchial responsiveness in the latter group. Additionally, stronger reactions were demonstrated in the group exposed to facilities with loose housing for laying hens compared to those using the cage system [5].

HOST-ASSOCIATED RISK FACTORS FOR RESPIRATORY DISEASES ASSOCIATED WITH POULTRY DUST

Common host associated risk factors for respiratory disease include genetics, atopy, age, gender and smoking.

Genetics

The role of genetics in the development of asthma and other work related respiratory illnesses among poultry farmers has not been well documented. A study done on Indian farmers did not demonstrate any hereditary factors to influence the incidence of respiratory symptoms in examined poultry and other farm workers (except farm workers working in granaries) [45]. It would appear that the environment and other host related factors are more important [67].

Atopy

Atopy has been shown to have independent and additive effects on the occurrence of chronic bronchitis in dusty farm work [68]. A study done to assess the effects of animal exposures on wheeze among farmers, reported that the odds of wheeze among poultry workers were higher in atopic than non-atopic workers [46]. A South African study reported tentative evidence

that atopic workers may have been selected out of the poultry workforce, because the prevalence of workers with atopy declined with increasing years of service. In this study 38.5%, 30.8% and 21.4% of poultry workers with 0- 5 years, 6 - 10 years and > 10 years of service, respectively, were atopic[47].

Age

The risk of occupational asthma among farmers has been shown to increase with increasing age [69]. A study to assess the incidence of respiratory diseases in farmers reported a high incidence (31.6%) of breathlessness in poultry workers aged 30–44 years when compared to a 13.6% incidence among workers under 29 years [45].

Gender

Relatively few studies have compared the effect of poultry work on respiratory function by gender. A Brazilian study reported no association between gender and respiratory test results [70]. The study by Zuskin et al demonstrated that the prevalence of chronic bronchitis was higher in males than in females with similar asthma prevalence in males (1.2%) and females (1.1%). However this study had 5 times more males than females [48]. Gendered distribution of work has been attributed to differences in prevalence of occupational asthma in other settings [71]. Whether this has relevance to this setting needs to be explored further.

Smoking

Smoking has been reported to increase the risk of many respiratory illnesses including chronic bronchitis among farmers [68]. Zuskin et al reported male poultry workers who were smokers to have a higher prevalence of chronic cough, phlegm, and bronchitis than poultry workers who were non-smokers[48]. Past smokers had the highest odds (OR=2.88) followed by never smokers (OR=1.46) and then current smokers (OR=0.80) among poultry workers in

the Agricultural Health Study [46]. Smoking habit has also been shown to influence the incidence of cough in poultry farm workers in an Indian study but had no direct effect on breathlessness and rhinitis [45].

CONCLUSION

This review has demonstrated that poultry farm workers are at increased risk of developing adverse respiratory health outcomes that may be attributed to exposure to dust and other airborne contaminants in poultry house environments. Intervention programs aimed at reducing exposure to dust is likely to have a positive impact on the respiratory health of workers.

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SECTION C: JOURNAL ARTICLE MANUSCRIPT

This manuscript has been prepared to be submitted for publication in the journal, Occupational and Environmental Medicine. The format of the article follows the journal's guidelines for authors (Appendix 9)

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ALLERGIC SENSITIZATION AND WORK RELATED ASTHMA AMONG POULTRY WORKERS IN SOUTH AFRICA

Dorothy Ngajilo

ABSTRACT

Objective: The aim of the study was to determine the risk factors associated with allergic sensitisation and various asthma phenotypes in poultry workers.

Methods: A cross-sectional study of 230 currently employed poultry workers used a modified ECRHS questionnaire, spirometry, fractional exhaled nitric oxide, Phadiatop and ImmunoCAP for specific IgE to poultry farming associated allergens (chicken meat, feathers, serum protein, droppings, sunflower seeds, storage mite) and mould mix to investigate work-related asthma.

Results: The mean dust particulate concentration (geometric mean (GM) =11.04mg/m^3) was highest in the rearing department while mean (1-3)-ß-D-glucan (GM=148 ng/ m^3) and endotoxin levels (GM=2298 EU/m^3) were highest in the catching department. Worker's mean age was 37±9 years, 68% were male, 43% current smokers, 34% atopic and 5% casual workers. The prevalence of non-atopic asthma (NAA=10%) was higher than atopic asthma (AA=6%) or probable occupational asthma (OA=5%). Sensitisation to at least one poultry work related allergen was 24%, with sunflower seed and *storage mite* sensitisation being the most prevalent (13%). In multivariate adjusted models, workers sensitised to chicken specific allergens were more likely to be atopic (OR=20.9, 95% CI: 4.7-93.2) or have casual job status (OR=6.0, 95% CI: 1.1-35.9). Workers with work-related chest symptoms were more likely to work in the rearing department (OR=3.2, 95% CI: 1.2-8.3) and to report episodes of high gas/dust/fume exposures causing asthma symptoms (OR=4.8, 95%CI: 2.4-9.6).

Reversible obstructive airway obstruction was more strongly associated with employment in small broiler farms (OR=11.6, 95% CI: 1.0-129.0) as well as casual work (OR=6.4, 95% CI: 1.4-28.0). Furthermore, NAA was strongly associated with casual work (OR=5.0, 95% CI: 1.3-19.0) as well as working in the automated egg laying system (OR=8.0, 95% CI: 1.0-68.6). Further analysis revealed that the proportion of workers sensitised to at least one poultry work related allergen declined with increasing years of service (chi-square trend p=0.023), with workers having <2 years employment demonstrating a higher risk compared to those with >6 years employment (OR=4.0, 95% CI: 1.2-13.8).

Conclusion: Non-atopic asthma is the most common asthma phenotype among poultry workers with work in the rearing department demonstrating an increased respiratory risk. The increased risk of reversible airway obstruction among workers in small broiler farms and the declining prevalence of sensitisation with increasing employment duration suggests a healthy worker effect. Preventive measures, including appropriate training, are recommended to reduce respiratory health risks, particularly in novice workers.

Key words: poultry; work-related asthma; allergic sensitisation

WHAT THIS PAPER ADDS

1**.** Non-atopic asthma is the most common asthma phenotype among poultry workers

2. Working in the rearing department has higher exposure to poultry dust and an increased risk of reporting work related chest symptoms

3. Casual workers have an increased risk of allergic sensitization and respiratory disease compared to permanent workers

INTRODUCTION

Agricultural production is associated with a spectrum of different occupational diseases [1]. Workers involved in animal production, particularly poultry workers, have been shown to have a greater prevalence of respiratory symptoms than other farmers or rural residents [2]. This is due to exposure to diverse and potentially hazardous airborne agents including chemical pollutants, dust particulate, allergens, endotoxins, micro-organisms and organic gases [3].

Both acute and chronic work-related respiratory symptoms have been reported in poultry workers exposed to these pollutants [4,5]. The extent to which airborne agents cause rhinitis and asthma depends on various environmental risk factors such as the overall design and operation of the poultry facilities, the level of exposures and concomitant exposure to multiple respiratory sensitisers [2,4,6,7]. Furthermore, other host associated risk factors such as age, atopy, and smoking have also been associated with rhinitis and asthma in poultry workers [2,8].

Although an association between poultry work and work related respiratory diseases has been demonstrated, very few studies have reported on the extent to which occupational sensitization to specific allergens present in poultry dust constitutes a risk to the health of poultry workers. This study investigated the prevalence and risk factors associated with occupational allergic sensitisation and various asthma phenotypes in poultry workers.

MATERIALS AND METHODS

Study design and population

A cross-sectional epidemiological study of 230 currently employed poultry farm workers from a commercial poultry farm was conducted in the North West Province, South Africa. The study received ethical approval from the University of Witwatersrand and the University of Cape Town (South Africa).

Exposure assessment

Work Process

The poultry farming processes involved five major departments viz. rearing, laying, hatchery, broiler and catching. In rearing farms, chickens are procured and reared until they are 21 weeks old after which they are transferred to laying farms. In the laying farms, hens and cocks are mated and fertile hens lay their eggs until they are 60 weeks old after which they are sold. There are two systems for laying eggs viz. automatic and manual systems. The main task of workers is to collect eggs from nests five times per day. Workers spend more than half the day inside the chicken houses for manual collection whereas egg collectors spend only three hours inside the house in the automated collection system. In the hatchery, eggs are hatched under controlled temperature and humidity conditions, before being transferred to broiler farms. The day old chicks are raised to 32-35 days on the broiler farms before they are transferred to the factory for slaughtering. Broiler farms are categorised in two groups viz. small farms that house ≤ 20 000 chickens and large farms housing > 20 000 chickens. Transfer of chickens between the various processes are done by catchers who are outsourced to a service provider. This task is done at every growth cycle of the chickens.

Personal sample collection

Workers in the departments were categorised into exposure groups based on the initial walk through inspections. A stratified random sample of workers was chosen from each exposure group. Particulate dust samples were collected using personal sampling pumps with 37mm, 1μm glass fibre filters inserted in a PAS-6 sampling head. The pumps were operated at a flow rate of 2L/min (Gladding et al. 2003) for a minimum of 4 to 8 hours (Mandryk et al. 2000; Gladding et al. 2003). The sampling heads were placed in each participant's breathing zone during their work activity to measure the inhalable fraction of particulate. Field blanks and laboratory blanks were included on each day of measurement. Pumps were calibrated before and after sampling to ensure the variance of readings was within 5%. Sampling was conducted during the entire work shift. Repeated sampling was conducted on a subgroup of workers (20%) on at least two further occasions to assess the intra-individual variability. A total of 298 samples were collected.

Measurement of total particulate, endotoxin and (1-3) β-D-glucans

Filters were weighed prior to and after sampling using an OHAUS analytical balance in accordance with the MDHS14-3 2000 (HSL, HSE, United Kingdom). The airborne dust concentration was calculated using the flow rate, sampling time and total dust particulate mass.

After gravimetric analysis, filters were extracted at room temperature in 5 ml pyrogen free water with 0.05% Tween 20. Extracts were aliquoted for endotoxin and (1-3) β-D-glucan tests separately and stored at -20 °C before analysis. The aliquot was diluted two-fold with pyrogen free water with 0.05% Tween 20 and heat treated by autoclaving at 120°C for 1h before testing. (1-3) β-D-glucan present in extract was measured using Glucatell assay

(Associates of Cape Cod, East Falmouth, MA, USA). The detection limit for (1-3) β-D-glucan was 20 pg/ml (Rylander & Carvalheiro 2006).

The concentration of endotoxin in the filter extracts was determined using the endpoint chromogenic Limulus amoebocyte lysate test (LAL) assay for endotoxin (Lonza, Walkersville, USA) as recommended by the manufacturer. The assay has been previously used for quantifying endotoxin in poultry houses (Rylander & Carvalheiro 2006). Using the calculated volume of air sampled and endotoxin concentrations, the results were expressed as (EU/m^3) using the methods used previously (BioWhittaker 2001). Unknown concentrations were calculated from the standard curve.

The limit of detection (LOD) was computed by calculating the mean of the field blanks. The limit of quantification (LOQ) was then calculated by adding the mean plus 3 times the standard deviation of the field blanks. For statistical analyses, values for exposure measurements below the LOD were censored using $L/\sqrt{2}$ (Finkelstein and Verma 2001). The co-efficient of variation (CV) between two measurements was ≤ 10 and $R^2 = 0.98$ for all assay runs.

Health outcome assessment

Questionnaire

The study questionnaire was a modified version of the European Community Respiratory Health survey (ECRHS II) adapted for local conditions [9]. The interviewer administered questionnaire collected information on important demographic factors, work related respiratory and skin symptoms, history of previous illnesses, employment history, degree of exposure to bioaerosols and smoking status.

Immunological tests

Blood samples were taken from each of the participants for immunological analysis. Sera were tested to determine the presence of atopy and specific IgE to commercially available poultry associated allergens viz. storage mite (*Lepidoglyphus destructor)*, sunflower seed (*Helianthus annuus*) and mould mix (containing *Penicillium chrysogenum, C. herbarum, A. fumigatus, C. Albicans, A. alternata, S. rostrata*) using the UniCAP Phadia system. The sera were also tested for specific IgE to chicken specific allergens such as meat, feathers, serum protein and droppings. Allergen extracts from chicken related sources were collected from the farms and were prepared in-house. Allergens extracts were prepared according to LABSOP01v1 (Occupational and Environmental health group, Netherlands) and then labelled with biotin. The biotin protein labelled extracts were loaded onto the streptavidin ImmunoCAP and quantified for chicken specific allergens using the sera of participants. An ImmunoCAP value ≥0.35 kUA per litre was considered positive.

Spirometry

Lung function tests (pre and post-bronchodilator) were performed using a computerised portable spirometer (EasyOne™ Diagnosic Spirometer). Spirometers were calibrated at least twice a day with a three-litre syringe under controlled temperature and humidity. The procedure was performed according to the American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations for spirometry by an experienced technologist who was blinded to the exposure status of each worker [10]. Reproducibility criteria entailed the two best tracings for both FEV_1 and FVC varying by no more than 150 ml or 5%, whichever is greater. The best FEV_1 and FVC were used regardless of whether they belonged to the same tracing. An increase in FEV1≥12% and ≥200 ml after inhalation of a short-acting

ß$_2$ agonist salbutamol (400 mg) was regarded as a significant airway reversibility suggestive of asthma.

Fractional exhaled nitric oxide

A hand-held portable sampling device (NIOX MINO) was used to determine FeNO during the work shift according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations [11,12]. Testing of workers occurred throughout the working week and work shift, with no particular variation with regard to time of testing in the different departments. Two technically adequate FeNO efforts were performed on each worker and an average was determined. Special instructions were given to workers to refrain from smoking, eating or drinking (at least 1 hour) before the test. Those with recent chest infections were tested at a later stage after resolution of their symptoms. A value >50ppb was considered to be indicative of allergic airway inflammation.

Operational definitions of asthma phenotypes

Atopic asthma (AA) was defined as either having an asthma attack or use of asthma medication in the past 12 months or having a positive bronchodilator test (increase in $FEV_1 \geq$ 12% and ≥ 200ml increase compared to baseline); and presence of atopy or FeNO > 50ppb. Non-atopic asthma (NAA) was defined as either having an asthma attack or use of asthma medication in the past 12 months or having a positive bronchodilator test (increase in FEV_1 12% and ≥ 200ml increase compared to baseline); and absence of atopy. Probable occupational asthma (OA) was defined as either having an asthma attack or use of asthma medication in the past 12 months or having positive bronchodilator test (increase in $FEV_1 \geq$ 12% and ≥ 200ml increase compared to baseline); and sensitisation to poultry work related allergens viz. storage mite, sunflower seed, mould mix, chicken meat, feathers, serum protein, and droppings.

Statistical analysis

Data was analysed using STATA V.12 computer software (StataCorp, College Station, Texas, USA). The main associations of interest were between risk factor variables (host and environmental) and important clinical endpoints viz. work related ocular-nasal and chest symptoms; airway inflammation viz. FeNO > 50ppb; reversible airway obstruction (FEV_1 increase ≥12% and ≥200 ml post-bronchodilator); allergic sensitisation; and asthma phenotypes (atopic asthma, non-atopic asthma, probable occupational asthma). These were first explored using bivariate analysis and then using saturated multivariate logistic regression models, which were adjusted for atopy and gender so as to examine the relationship between the exposure variables and the important clinical endpoints.

RESULTS

Exposure assessment

The mean dust particulate levels (geometric mean GM =11.04mg/m^3) was highest in the rearing and hatchery department (Table 1). Poultry catchers had the highest mean (1-3) β-D-glucan (GM=148 ng/m^3) and endotoxin levels (GM=60x10^3 EU/m^3). Compared to the manual egg laying system, the automated system had the highest mean dust particulate, endotoxin and (1-3) β-D-glucan levels. In broiler farms, large broilers had the highest particulate and endotoxin concentration while small broilers had the highest (1-3) β-D-glucan levels.

Table 1. Personal inhalable dust particulate mass, β -glucan and endotoxin exposure levels in poultry workers

	Particulate mass concentration (mg/m^3)					β -glucan (ng/m^3)				Endotoxin (EU/m^3)			
Area of work	**n**	**AM**	**GM**	**GSD**	**Range**	**AM**	**GM**	**GSD**	**Range**	**AM**	**GM**	**GSD**	**Range**
Hatchery	67	65.81	36.25	3.46	0.83-252.68	37	24	2.51	3-210	14486	697	6.97	178-372 x10^3
Rearing	24	60.91	54.04	1.81	5.52-129.30	162	105	2.48	25-1069	25039	8015	3.34	568-319 x10^3
Laying	45	21.63	12.69	3.42	0.78-53.99	195	116	2.55	16-1373	3052	781	2.99	282-78 x10^3
- Manual	21	4.92	3.95	2.13	0.78 -9.42	121	97	2.08	21-255	1891	680	2.97	282-23 x10^3
- Automatic	24	36.26	35.23	1.27	25.37-53.99	260	136	2.94	16-1373	4067	880	3.04	323-78 x10^3
Broiler	120	6.42	4.17	2.31	1.16-57.52	532	290	3.58	15-2275	65708	2300	11.08	212-3081 x10^3
- Small	30	6.00	3.96	2.22	1.19-38.66	808	636	2.15	83-2275	39403	2037	12.17	215-566 x10^3
- Large	90	6.56	4.24	2.35	1.16-57.52	440	223	3.70	15-1700	74477	2395	10.87	212-3081 x10^3
Catching	42	11.84	9.28	1.78	1.78-112.31	773	644	1.89	126-2059	196809	23902	9.64	409-4534 x10^3
Overall	298	27.22	11.04	3.87	0.78-252.68	374	149	4.62	3-2275	59932	2298	10.56	178-4534 x10^3

AM: arithmetic mean; GM: geometric mean; GSD: geometric SD; n: number of workers

Demographic characteristics

The demographic characteristics of the study population are outlined in Table 2. Subjects were predominantly male (68%), the mean age was 37±9 years, majority were Sotho speakers (60%), 44% were current smokers having an average 5 pack year smoking history, with a significantly greater proportion of male than female smokers (Pearson chi square $p < 0.001$). A significant but modest correlation (Spearman rho 0.40, $p < 0.001$) existed between age and employment duration. A family history of atopy was reported in 13% of the workers. All workers reported habitual consumption of chicken products regularly with 4% reporting allergic reactions following ingestion of poultry products viz. chicken canned food, chicken feet, livers, tripe, skin, eggs and spice.

Occupational history

Most participants worked in the laying department (36%), broiler (30%), and hatchery (23%) (Table 2). The mean duration of employment in the farm was 5.3 years and was very similar to the 4.9 years employed in the current job. A high proportion of workers (95%) worked day shift. About 5% of workers were employed on a casual basis and 25% of workers had less than two years of employment. A large proportion (97%) of workers reported their current job to produce dust, with 72% reporting very high levels. Most workers (89%) reported wearing respiratory protective equipment for an average of 4 years duration. Other personal protective equipment used by workers included aprons (68%), goggles (80%) and gloves (78%). Only 68% of workers reported receiving safety training on the risks associated with poultry farming.

Table 2: Demographic and employment characteristics of poultry workers.

Demographic characteristics (n = 230)	
Age (yrs)	37±9
Gender (%F:M)	68:32
Home language:	
- Sotho	137 (60)
- Tswana	42 (18)
- Xhosa	31 (13)
- Zulu	9 (4)
- English	5 (2)
- Afrikaans	3 (1)
- Pedi	3 (1)
Obesity (BMI >30 kg/m^2)	39 (17)
Smoking status:	
- Never-smokers	129 (56)
- Current smokers	98 (43)
- Ex-smokers	3 (1)
Pack-year history of current smokers (pack-years)	4.6±3.8
Area of work:	
- Hatchery	52 (23)
- Rearing	22 (10)
- Laying	83 (36)
- Broiler	68 (30)
- Catching	5 (2)
Employment status	
- Duration of employment in poultry farm (yrs)	5.3±6.2
- Duration of employment in current job (yrs)	4.9±6.0
- Permanent job status	218 (95)
- No. < 2 years employment	59 (25)
Past history of lung disease (self-reported):	
- Previous treatment for tuberculosis	9 (4)
- Repeated childhood chest infections	7 (3)
- Previous treatment chronic bronchitis	3 (1)
Family history of atopy	30 (13)
Self-reported adverse reactions to:	
- Chicken products†	6 (2.61)
- Eggs	2 (0.87)
- Chicken spice	1 (0.43)

Continuous variables, mean±SD; categorical variables, number (%). †Chicken canned food, chicken feet, livers, tripe and skin.

Respiratory symptoms

Chest symptoms (any one of tight chest wakening, wheeze, shortness of breath, shortness of breath wakening) were reported by 7 – 15% of workers, with 23% having work related symptoms (Table 3). About 5% reported symptoms suggestive of chronic bronchitis (defined as “cough and phlegm on most days/nights for 3 months or more per year during the last 2

years"). Workers with symptoms suggestive of chronic bronchitis were more likely to be current smokers (OR=7.36, 95% CI: 1.21-44.80). The prevalence of work related ocular-nasal symptoms (46%) was twice that of work related chest symptoms (23%). About 48% of workers reported an episode of high exposure to gas, dust and fumes (GDF) causing tight chest, wheeze or cough. The most common work related agents suspected to cause respiratory symptoms reported by subjects (n=53) included poultry dust (74%), chicken feathers (47%), bedding (9%) and chicken feed (8%). Job change due to work-related chest symptoms was reported by 8 workers (3%) all of whom reported GDF causing respiratory symptoms.

Table 3: Prevalence of upper and lower respiratory symptoms among poultry farm workers

Symptom	Prevalence (%) n=230
Upper airway symptoms	
Work-related ocular-nasal symptoms	105 (46)
Asthma history	
Doctor diagnosed asthma	2 (1)
- < 17 years	1 (0.5)
- ≥ 17 years	1 (0.5)
Wheezing or whistling in chest in the past year	35 (15)
Woken up with a tight chest in the past year	17 (7)
Asthma attack in the past year	2 (1)
Work-related asthma symptoms	
Work-related chest symptoms	54 (23)
Episode of high exposure to GDF causing tight chest, wheeze or cough	110 (48)
Job change due to work-related chest symptoms	8 (3)

GDF; Gas, dust and fumes

Patterns of allergic sensitization

The overall prevalence of atopy in this population was 34%, with atopic workers having a higher prevalence of sensitization to poultry work related allergens (Table 4). The prevalence of sensitisation to at least one poultry work related allergen was 24%, with sensitization to

poultry associated allergens such as sunflower seeds (13.0%) and storage mites (13.0%) being most prevalent. The prevalence of sensitization to at least one chicken specific allergen was lower (8%), with chicken droppings being the most common sensitiser (6%). A statistically significant correlation was found between sensitization to chicken droppings and serum protein (Spearmans rho 0.65, p<0.001).

Table 4: Allergic sensitization profiles among poultry farm workers

Allergen	**Overall Prevalence (%) (n=230)**	**Atopic Prevalence (%) (n=78)**	**Non-atopic Prevalence (%) (n=152)**
IgE to house dust mites			
- *D. pteronyssinus*	57 (25)	53 (68)***	4 (3)
- *D. farinae*	54 (23)	54 (69)***	0 (0)
IgE to poultry associated allergens			
- Storage mite (*L. destructor*)	29 (13)	26 (33)***	3 (2)
- Sunflower seed (*Helianthus annuus*)	29 (13)	27 (35)***	2 (1)
- Mould mix (*Penicillium chrysogenum, C. herbarum, A. fumigatus, C. albicans, A. alternata, S. rostrata*)	18 (8)	16 (21)***	2 (1)
IgE to chicken-specific allergens			
- Droppings	14 (6)	12 (15)***	2 (1)
- Feathers	5 (2)	5 (6)**	0 (0)
- Meat	2 (0.87)	2 (3)*	0 (0)
- Serum protein	1 (0.43)	1 (1)	0 (0)
Sensitization to at least one chicken specific allergen	19 (8)	17 (22)***	2 (1)
Sensitization to at least one poultry work related allergen	56 (24)	50 (64)***	6 (4)

Chi-square * p<0.05, ** p<0.01, *** p<0.001

Pulmonary function tests

The results of pulmonary function indices are presented in Table 5. Although 19% of the workers had FEV_1 less than 80% of predicted, only 7% of the workers showed evidence of probable chronic obstructive lung disease (FEV_1 /FVC % predicted less than the 5th centile). Significantly more males than females showed evidence of airway obstruction (FEV_1/FVC < 70%). Between 7% and 8% of workers showed evidence of asthma based on having airway inflammation (FeNO>50ppb) and reversible airway obstruction on spirometry respectively. Overall, the prevalence of non-atopic asthma (NAA=10%) was higher than atopic (AA=6%) and probable occupational asthma (OA=5%).

Table 5: Pulmonary function indices among poultry farm workers

Pulmonary function indices	Overall (n=230)	Males	Females
FEV_1 (litres)	3.13± 0.70	3.36±0.66	2.55±0.44**
FVC (litres)	3.77±0.80	4.07±0.69	3.03±0.48**
FEV_1 % predicted	89.11±12.90	88.44±13.77	90.74±10.75
FEV_1/FVC	0.83±0.06	0.83±0.07	0.84±0.04
No. with FEV_1/FVC <70%	9 (5)	9 (7)	0 (0)*
No. with FEV_1<80% predicted	36 (19)	30 (23)	6 (11)
FEV_1 increase ≥12% and ≥200 ml post-bronchodilator	14 (8)	11 (8)	3 (6)
FeNO >50 ppb	17 (7)	11 (7)	6 (8)
FEV_1/FVC ratio post-bronchodilator increase(<5th percentile)	12 (7)	11 (8)	1 (2)

* p<0.05, ** p<0.001; Reference values are from the European Community for Coal and Steel (ECCS)

Host risk factors associated with clinical endpoints of interest

Simple logistic regression analysis revealed that increasing age was significantly associated with features suggestive of probable obstructive lung disease (OR=1.10, 95% CI: 1.03-1.16) (Table 6). Analysis of the association between atopic status and health outcomes of interest revealed that, atopy was associated with presence of airway inflammation (FeNO>50 ppb; OR=3.04, 95% CI: 1.11-8.34), sensitization to chicken specific allergens (OR=20.90, 95% CI: 4.69-93.21), sensitization to at least one poultry work related allergen (OR=43.45, 95% CI: 17.00-111.05) and having probable occupational asthma (OR=6.47, 95% CI: 1.70-24.68). Workers sensitized to chicken specific allergens were more likely to report adverse reactions following ingestion of poultry products (OR=5.46, 95% CI: 1.28-23.18). Sensitization to at least one poultry work related allergen was more likely to occur in males (OR=2.03, 95% CI: 1.00-4.13). Subjects with work related ocular nasal symptoms were more likely to be obese (OR=2.17, 95% CI: 1.07-4.40) and report work related skin symptoms (OR=3.05, 95% CI: 1.13-8.25).

Table 6: Host risk factors associated with allergic respiratory disease and asthma in poultry workers

	Age	**Gender (male)**	**Atopy**	**Work related skin symptoms**	**Obesity (BMI >30 kg/m^2 vs BMI <30 kg/m^2)**	**Smoking (current versus never)**	**Poultry product ingestion related reactions†**
Outcome	**OR (95% CI)**						
Work-related ocular-nasal symptoms	1.01 (0.98-1.04)	0.61 (0.35-1.06)	1.03 (0.60-1.78)	**3.05 (1.13-8.25)**	**2.17 (1.07-4.40)**	0.93 (0.55-1.57)	2.90 (0.73-11.53)
Work related chest symptoms	0.98 (0.96-1.02)	0.68 (0.36-1.28)	0.97 (0.51-1.84)	1.45 (0.53-3.97)	1.58 (0.74-3.38)	0.29 (0.49-1.71)	2.26 (0.62 to 8.35)
Sensitization to chicken specific allergens	0.95 (0.90-1.01)	2.70(0.76-9.59)	**20.90 (4.69-93.21)**	1.26 (0.27-5.90)	0.55 (0.12-2.50)	1.52 (0.59-3.89)	**5.46 (1.28-23.18)**
Sensitization to at least one poultry work related allergen	0.98 (0.95-1.02)	**2.03 (1.00-4.13)**	**43.45 (17.00-111.05)**	1.37 (0.50-3.76)	0.92 (0.41-2.08)	1.31 (0.72-2.40)	2.15 (0.59-7.93)
FEV_1 increase ≥12% and ≥200 ml post-bronchodilator	1.00 (0.94-1.06)	1.57 (0.42-5.87)	0.31 (0.67-1.41)	0.79 (0.10-6.50)	0.39 (0.05-3.10)	2.21 (0.71-6.87)	1.55 (0.18 to 13.43)
FeNO >50 ppb	0.96 (0.90-1.01)	0.86 (0.31-2.42)	**3.04 (1.11-8.34)**	*	1.05 (0.29-3.86)	0.41 (0.13-1.33)	*
Atopic asthma	0.98 (0.92-1.04)	0.75 (0.24-0.36)	*	0.87 (0.11-7.05)	0.88 (0.19-4.16)	0.42 (0.11-1.60)	*
Non-atopic asthma	1.00 (0.96-1.05)	1.39 (0.52-3.67)	*	1.00 (0.22-4.61)	0.44 (0.10-1.95)	1.82 (0.76-4.34)	2.37 (0.47-11.89)
Probable OA	1.03 (0.97-1.09)	0.95 (0.27-3.25)	**6.47 (1.70-24.68)**	0.95 (0.12-7.78)	1.69 (0.43-6.53)	0.94 (0.29-3.05)	2.11 (0.25-18.18)

*OR not calculable; OR: each OR is a separate unadjusted regression model, †Self-reported adverse reactions to poultry products following ingestion

Environmental risk factors associated with allergic respiratory disease and asthma

In unadjusted logistic regression models workers with work related ocular nasal (OR=2.89, 95%CI: 1.69-4.94) and chest symptoms (OR=4.89, 95%CI: 2.44-9.78) were more likely to report an episode of exposure to high gas, dust and fumes (GDF) causing asthma like symptoms (Table 7).

Workers with work-related chest symptoms were more likely to work in the rearing department (OR=2.51, 95% CI: 1.01-6.24). Presence of reversible airway obstruction was positively associated with the casual work status (OR=6.35, 95% CI: 1.44-28.00) as well as employment in small broiler farms (OR=10.07, 95% CI: 1.01-104.61). Sensitization to chicken specific allergens was associated with casual work status (OR=4.21, 95% CI: 1.04-17.10) and workers sensitised to at least one poultry work related allergen were more likely to have <2 years compared to >6 years of employment (OR=2.91, 95% CI: 1.15-7.34). Moreover, non-atopic asthma was more likely to be found in casual workers (OR=6.31, 95% CI: 1.71-23.24) as well as those working in automated laying farms (OR=8.00, 95% CI: 1.01-68.26). These associations remained essentially similar in the multivariate logistic regression after adjusting for gender and atopy (Table 8).

In further analysis of sensitisations trends, the proportion of workers sensitised to at least one poultry work related allergen declined with increasing years of employment (chi-square trend p=0.023) (Table 9).

Table 7: Environmental risk factors associated with allergic respiratory disease and asthma in poultry workers

	Casual versus permanent work	**Years of employment <2years versus >6years)**	**Rearing (versus other areas of work)**	**Automated versus manual egg laying activities (n=83)**	**Employment in small versus large broilers † (n=68)**	**Episode of high GDF exposure causing asthma symptoms**
Outcome	**OR (95% CI)**					
Work-related ocular-nasal symptoms	0.38 (0.10-1.44)	0.87 (0.42-1.82)	1.82 (0.75-4.45)	0.47 (0.20-1.16)	0.69 (0.22-2.14)	**2.89 (1.69-4.94)**
Work related chest symptoms	*	0.96 (0.37-2.51)	**2.51 (1.01-6.24)**	1.78 (0.67-4.73)	0.50 (0.10-2.54)	**4.89 (2.44-9.78)**
Sensitization to chicken specific allergens	**4.21 (1.04-17.10)**	2.54 (0.47-13.69)	1.12 (0.24-5.22)	0.97 (0.23-4.18)	*	0.61 (0.23-1.61)
Sensitization to at least one poultry work related allergen	1.03 (0.27-3.97)	**2.91 (1.15-7.34)**	1.19 (0.44-3.19)	0.85 (0.30-2.36)	0.43 (0.11-1.68)	0.93 (0.51-1.70)
FEV_1 increase ≥12% and ≥200 ml post bronchodilator	**6.35 (1.44-28.00)**	2.49 (0.48-13.04)	1.50 (0.31-7.27)	*	**10.07 (1.01-104.61)**	0.88 (0.29-2.66)
FeNO >50 ppb	2.71 (0.54-13.49)	0.56 (0.13-2.45)	1.29 (0.27-6.04)	0.98 (0.13-7.27)	0.92 (0.09-9.47)	0.57 (0.20-1.60)
Atopic asthma	1.56 (0.19-13.11)	0.56 (0.13-2.45)	1.79 (0.37-8.65)	0.63 (0.10-4.00)	2.88 (0.17-48.66)	0.93 (0.30-2.86)
Non-atopic asthma	**6.31 (1.71- 23.24)**	1.14 (0.36-3.64)	0.89 (0.19-4.08)	**8.00 (1.01-68.26)**	3.13 (0.57-17.20)	1.00 (0.07-17.67)
Probable OA	*	0.36 (0.07-1.96)	1.98 (0.41-9.67)	3.08 (0.31-30.87)	*	0.78 (0.24-2.50)

Each OR represents a separate unadjusted regression model; * OR not calculable

†Broiler houses with ≤ 20,000 chickens =small broilers, > 20,000 chickens = large broilers; FeNO: fractional exhaled nitric oxide; OA: occupational asthma; GDF: Gas, Dusts and Fumes,

Table 8: Multivariate logistic regression models of environmental risk factors associated with allergic respiratory disease and asthma of in poultry workers

	Casual versus permanent work	**Years of employment <2years versus >6years**	**Rearing (versus other areas of work)**	**Automated versus manual egg laying activities (n=83)**	**Employment in small versus large broilers † (n=68)**	**Episode of high GDF exposures causing asthma symptoms**
Outcome		**OR (95% CI)**				
Work-related ocular-nasal symptoms	0.44 (0.12-1.71)	1.04 (0.49-2.23)	2.29 (0.91-5.74)	0.46 (0.18-1.14)	0.66 (0.21-2.06)	**2.76 (1.61-4.75)**
Work related chest symptoms	*	1.06 (0.39-2.88)	**3.17 (1.22-8.29)**	1.68 (0.61-4.66)	0.48 (0.09-2.54)	**4.79 (2.38-9.64)**
Sensitization to chicken specific allergens	**6.04 (1.02-35.86)**	2.03 (0.35-11.84)	0.91 (0.17-4.83)	1.36 (0.23-7.85)	*	0.53 (0.19-1.50)
Sensitization to at least one poultry work related allergen	1.03 (0.17-6.44)	**4.03 (1.18-13.80)**	1.08 (0.28-4.23)	1.56 (0.30-8.11)	0.31 (0.06-1.53)	0.75 (0.33-1.69)
FEV_1 increase ≥12% and ≥200 ml post-bronchodilator	**5.67 (1.18-27.11**)	2.26 (0.40-12.64)	1.2 (0.24-6.20)	*	**11.57 (1.04-128.97)**	1.01 (0.33-3.13)
FeNO >50 ppb	3.38 (0.61-18.69)	0.53 (0.12-2.48)	1.46 (0.29-7.41)	1.28 (0.14-11.86)	0.91 (0.09-9.38)	0.52 (0.18-1.48)
Atopic asthma**	1.78 (0.20-15.79)	0.61 (0.13-2.87)		0.64 (0.10-4.07)	2.82 (0.17-47.68)	
Non-atopic asthma**	**5.04 (1.33-19.02)**	1.05 (0.32-3.46)	0.80 (0.17-3.74)	**8.03 (1.01-68.57)**	3.07 (0.56-16.84)	1.19 (0.48-2.95)
Probable OA	*	0.31 (0.05-1.81)	2.36 (0.42-13.29)	3.81 (0.35-41.54)	*	0.69 (0.21-2.31)

Each OR represents a separate model adjusted for gender and atopy; * OR not calculable; ** Atopy not adjusted for in this model

†Broiler houses with ≤ 20,000 chickens=small broilers, > 20,000 chickens = large broilers; FeNO: fractional exhaled nitric oxide; OA: occupational asthma; GDF: Gas, Dusts and Fumes (GDF)

Table 9: Association between employment duration and sensitization to poultry work-related allergens

	Prevalence of sensitization to at least one poultry work related allergen (%)		
Years of employment	Yes	No	Total
<2 years	19 (33)	38 (67)	57 (100)
2-6 years	29 (25)	87 (75)	116 (100)
>6 years	8 (14)	49 (86)	57 (100)
Total	56 (24)	174 (76)	230 (100)

Chi-squared test for trend p=0.023

DISCUSSION

The findings of this study confirms that poultry workers are at increased risk of allergic sensitization and asthma. Moreover, it demonstrates that sensitization to poultry work related allergens develops early during employment within the first two years. The study also suggests that tasks characterised by high particulate mass and endotoxin exposures are strongly associated with non-atopic asthma, which is the most prevalent asthma phenotype in this group of workers.

Exposure assessment data revealed that the levels of particulate mass, endotoxin and (1-3) β-D-glucans were highly variable between the five poultry farming processes conducted in departments. Workers in hatchery, rearing farms and automated laying farms were exposed to particulate mass concentrations above the Occupational Exposure Limit (OEL) of 10mg/m^3 (ACGIH) [13]. Furthermore, workers in all five poultry farming processes were exposed to endotoxin levels above the 90 EU/m^3 OEL set by DECOS [14]. While no specific OEL exists for (1-3) β-D-glucan, the levels found in this study are within the range previously demonstrated in polish poultry houses (0.8-6886 ng/m^3) [15].

The mean inhalable particulate mass concentration of poultry dust reported in this study is higher than those reported in several previous studies done in France and Canada [16–18] but similar to levels found in earlier studies in North Carolina poultry farms reported by Lenhart et al [19]. Most previous studies reporting lower levels of particulate mass measured area samples rather than personal environmental samples that are regarded as a closer estimate of the exposures inhaled by workers, hence their possible underestimation of true exposures. Moreover, while this current study reported total inhalable particulate mass concentrations,

most previous studies have reported only on respirable dust, which is generally lower than the inhalable dust concentrations.

The mean airborne endotoxin levels found in this study are similar to levels reported previously [18,20]. However, earlier studies have shown endotoxin concentration to vary extensively, with some studies reporting higher levels [7,18,21] and others having lower levels [22–25] compared to the current study. This points out the highly variable nature of endotoxin found in different geographical settings despite similar work processes. Furthermore, this variation can also be explained by factors such as use of area versus personal sampling methods. .

In the current study, the highest particulate mass concentrations were measured in the rearing sections of the farms with poultry catchers having the highest endotoxin and (1-3) β-D-glucan levels. The high particulate mass concentrations found in rearing farms could be due to labour intensive tasks performed such as weighing, vaccination as well as beak-trimming. Rearing farms involved in this study house up to 60 000 chickens at any one time and every chicken has to be weighed and vaccinated at regular intervals. These tasks increase animal activity in a confined area which disperses dust particulate from surface dust in addition to feathers, dander and serum protein. In a similar manner, increased animal activity associated with bird catching tasks could explain the high endotoxin and (1-3) β-D-glucan levels observed among catchers. Specific poultry farming processes influencing animal activity have been shown to increase the airborne concentration of microorganisms [26]. A Swiss study reported high levels of endotoxin among poultry catchers similar to what was found in this current study [25].

In this study, large broiler farms (housing > 20 000 chickens) had much higher particulate mass concentrations and endotoxin levels than small broiler farms (housing≤ 20 000 chickens). This finding is consistent with previous studies in which particulate mass and endotoxin concentrations have been shown to increase with increasing number of birds [27]. This may be due to the increase in biomass (calculated as the product of the number of birds and their individual weight) as well as higher levels of skin debris and feathers found as a result of the large number of birds.

In this study, lower personal dust exposure levels were expected in automated laying farms since the egg collection task was engineered to reduce the amount of time spent by workers in poultry houses. Interestingly, much higher particulate mass, (1-3) β-D-glucan and endotoxin levels were measured in the automated laying farms compared to manual farms. These higher levels may be attributable to the regular feather dusting tasks in the automated laying farms to prevent accumulation of dust and feathers in the conveyor belts affecting their function. The dusting process thereby contributes to excessive poultry dust exposure even though these workers spend less time in these automated laying poultry houses.

The findings of this study demonstrated that one third of workers were atopic with sunflower seed and storage mites being the most common sensitizers (13%), on the other hand chicken droppings were the most common chicken specific allergen (6%). The prevalence of atopy reported in this study is similar to previous studies conducted among poultry workers [4,17]. The specific sensitization to sunflower seeds is probably due to inhalation of sunflower seed husk used as bedding in poultry houses. Occupational exposure to sunflower seed with subsequent sensitization has been reported in animal food processing and in bakery workers [28,29]. Chicken droppings have also previously been reported as the most common sensitisers in other poultry farms in South Africa [4]. Chicken droppings are known to

contain excreted serum protein antigens, which may explain a strong correlation (Spearmans rho 0.65, $p<0.001$) between chicken droppings and serum protein sensitization in this study.

In the current study the prevalence of non-atopic asthma (10%) was higher than atopic (6%) and probable occupational asthma (5%) phenotypes. A high prevalence of non-atopic asthma in this population may suggest that asthma symptoms associated with poultry dust exposure are mediated largely by non-allergenic mechanisms. This study has demonstrated that the prevalence of work related ocular nasal and chest symptoms is similar to previous studies [17,30]. The prevalence of asthma reported in this study (7%) is comparable to Portuguese poultry farmers (6.4%) [30] but lower than Spanish farmers (14%) [21]. A higher prevalence of asthma reported in the Spanish study may have been due to different epidemiological definitions of asthma used. In the current study 7% of workers had probable allergic airway inflammation (FeNO>50ppb) with overall mean FeNO value of 19ppb. This is slightly higher compared to findings of a recent study done in North Carolina by Kearny et al, reporting mean FeNO levels of 16.8ppb [31] with few workers having levels >50ppb (Kearney GD, personal communication, April 2016). These differences may be due to higher poultry dust exposures found in our study. Although no environmental measurements were reported in the North Carolina study, only 19% poultry workers reported heavy dusty work compared to 72% of the workers in our study.

Host factors such as atopy, male gender, obesity, work-related skin symptoms, and poultry product ingestion related reactions were positively associated with allergic sensitization and respiratory outcomes. Atopy was associated with airway inflammation, sensitization to at least one chicken or poultry work related allergen as well as occupational asthma. Atopy has been consistently reported as a strong risk modifier for sensitization to work-related high molecular weight agents [32]. The association between male gender and sensitization to

poultry work related allergens may be attributable to the gendered distribution of work, as has been reported in other work settings [33]. In this study individuals with work related ocular nasal symptoms were more likely to have work related skin symptoms (OR=3.05, 95% CI: 1.13-8.25) or be obese (OR=2.17, 95% CI: 1.07-4.40). Recent studies have suggested a causal link between atopic dermatitis and subsequent development of rhinitis, asthma and other atopic disorders [34]. The dysfunctional skin barrier associated with dermatitis may act as a site of allergic sensitization inducing Th2 immunity that predisposes individuals to allergic nasal reactions and airway hyperactivity [35]. On the other hand, overweight or obesity has been shown to increase the risk of non-allergic rhinitis in adults, especially in males [36]. These results suggest that the risk of respiratory outcomes may be reduced by interventions targeting work related skin symptoms and obesity. Another interesting finding of this study was that workers sensitized to chicken specific allergen were more likely to have poultry ingestion related allergic reaction (OR=5.46, 95% CI: 1.28-23.18). This is consistent with other studies demonstrating an association between sensitization to chicken antigens (feathers, droppings, serum) and food allergy with de-novo sensitization due to ingestion or inhalation [37].

The findings of this study showed that workers with ocular-nasal or chest symptoms were more likely to report an episode of high GDF exposure. This is similar to other studies reported in previous literature [38,39]. Notably GDF exposure was described as an independent risk factor for job change due to respiratory symptoms in a Swedish study [40]. This may explain the finding that all workers who reported job change due to respiratory symptoms reported an episode of high exposure to GDF causing respiratory symptoms in the current study. This study also showed that workers with work related chest symptoms were more likely to be from the rearing department (OR=2.51, 95% CI: 1.01-6.24). This may be

due to high particulate mass concentration levels found in this department. Moreover, non-atopic asthma was more likely to be found in workers from the automated laying farms (OR=8.00, 95% CI: 1.01-68.26) compared to those working in the manual laying farms. This may be attributable to the high particulate mass, (1-3) β-D-glucan and endotoxin concentrations found in the automated laying farms.

Despite large broiler farms having higher airborne particulate mass and endotoxin concentrations compared to small broilers farms, data from this study demonstrated that reversible obstructive airway pattern was more likely in workers from small broiler farms (OR=10.07, 95% CI: 1.01-104.61). This is contrary to findings from a previous study in which a significant dose-response relationship was demonstrated between wheeze and large farms housing >500 birds (OR=1.39,95% CI:1.10- 1.75) [2]. It is probable that this discrepancy could be attributed to the healthy workers effect present in the current study, and also previously documented [30]. The healthy worker effect may also explain the declining pattern of sensitization observed with increasing years of service. Moreover, an increased risk of sensitization in the first 2 years of employment as shown in this study (OR=2.91, 95% CI: 1.15-7.34), confirms that sensitization to occupational allergens occurs mainly during the first 2 years of exposure to an agent [41]. This finding has important implications for prevention since it provides a window of opportunity for appropriate timing of medical surveillance which should be done more frequently in the first 2 years of employment [41].

This study reports an increased risk of sensitization, reversible airway obstruction and non-atopic asthma in casual workers. This finding is consistent with previous studies that have demonstrated casual workers to have an increased likelihood of exposure to occupational hazards and poorer occupational health and safety outcomes compared to permanent workers [42,43]. This is due to a number of factors including casual workers being unfamiliar with

their workplace processes and the related hazards; unaware of safe working practices; using poor quality personal protective equipment; subjected to constant changes in their tasks; pressurized to take on manual and more hazardous work with limited supervision or training; and are usually not confident to raise safety issues for fear of having their jobs terminated [43].

Although this study demonstrated statistically significant positive associations between several risk factors and some health outcomes of interest, it was unable to do so for other outcomes. This could be due to the lack of statistical power as a result of a small sample size. Moreover, the study may have been affected by selection bias as a result of the healthy worker effect. Finally, due to the cross-sectional nature of this study, it was difficult to demonstrate temporal relationships between exposure and health outcome variables of interest. Future studies should explore in more detail the dose-response relationships using the various exposure metrics that are identifiable for poultry dust.

In conclusion, this study confirmed that poultry workers are exposed to high concentrations of particulate dust, endotoxins and (1-3) β-D-glucans which are significantly above the recommended levels to protect respiratory health. Additionally, the non-atopic asthma phenotype was the most common asthma phenotype and work in the rearing department demonstrating a higher risk to respiratory health. Preventive measures, including appropriate training, are recommended to reduce respiratory health risks, particularly in novice workers.

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SECTION D: APPENDICES

APPENDIX 1: LETTER OF APPROVAL FROM THE UNIVERSITY OF WITWATERSRAND HUMAN RESEARCH ETHICS COMMITTEE

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Ms Tanusha Singh

CLEARANCE CERTIFICATE	**M10646**
PROJECT	Allergic Sensitisation and Work-Related Asthma among Poultry Workers in South Africa
INVESTIGATORS	Ms Tanusha Singh.
DEPARTMENT	Diepartment of Immunology
DATE CONSIDERED	25/06/2010
DECISION OF THE COMMITTEE*	Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 16/08/2010 **CHAIRPERSON** Signed
(Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable
cc: Supervisor :

DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and **ONE COPY** returned to the Secretary at Room 10004, 10th Floor, Senate House, University.
I/We fully understand the conditions under which I am/we are authorized to carry out the abovementi research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. **I agree to a completion of a yearly progress report.**
PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

APPENDIX 2: LETTER OF APPROVAL FROM THE UNIVERSITY OF CAPE TOWN HUMAN RESEARCH ETHICS COMMITIEE

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: lamees.emjedi@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

02 September 2015

HREC REF: 601/2015

Prof M Jeebhay
Public Health & Family Medicine
Falmouth Building
Medical School

Dear Prof Jeebhay

PROJECT TITLE: ALLERGIC SENSITIZATION AND WORK RELATED ASTHMA AMONG POULTRY WORKERS IN SOUTH AFRICA (MMed-candidate-Dr D Ngajilo)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th September 2016.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

We acknowledge that the MMed student, Dorothy Ngajilo will also be involved in this study.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Yours sincerely

Signed

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938
This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

APPENDIX 3: INFORMATION SHEET AND CONSENT FORM

Listed in Schedule 3 Part A: National Public Entities – Practice No.5200296

NATIONAL HEALTH LABORATORY SERVICE

NATIONAL INSTITUTE

FOR

OCCUPATIONAL HEALTH

A Division of the National Health Laboratory Service

Immunology & Microbiology Section

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Information sheet and consent form

Good day,

We, Onnicah Matuka and Tanusha Singh, medical scientists from the National Institute for Occupational Health (NIOH) would like to invite you to consider participating in a research study entitled *"*Allergic sensitisation and work-related asthma among poultry workers in South Africa". These investigations will allow the NIOH to gain an understanding of non-allergic asthma in poultry workers.

Why this study?

Occupational health problems are not uncommon in the workplace. Therefore monitoring or assessing exposure to hazardous biological substances is a fundamental step to improving the quality of life for workers. Previous research studies have shown that poultry farm workers are exposed to a wide range of agents from dust derived from the litter, feathers, feed, excreta and microorganisms which could cause respiratory disease. In South Africa no study has reported associations between exposure to airborne biological agents and respiratory health effects in poultry workers, therefore the (NIOH) would like to investigate these agents. The study will include ~ 346 South African poultry workers (excluding pregnant women) and we anticipate that the collection of data will take 2-3 weeks per farm. The measurements will include personal air sampling, questionnaire, blood tests and lung function tests.

What do we expect from the participants in this study?

If you agree to participate in this study you will be asked to complete a questionnaire, which will provide us with important information on respiratory symptoms and other medical conditions as well as employment history. During the course of the study a doctor or nurse will use sterile (free of germs) equipment to take 10ml (~2 teaspoons) of blood to be tested if you have allergy (specific IgE and phadiotops) and inflammation by testing allergic mediators ((IL-7, IL-3, IL-4, IL-5, Eotaxin and GM-CSF) and non-allergic mediators (IL-8, IL-1β, IL-6, IL-10, TNF-α) in each blood sample . A

registered clinical technologist will test the strength of your lungs by doing some breathing tests (Spirometry). You will be asked to blow several times into a machine which measures how well your lungs are working. You will be asked to repeat the breathing test after you first breathe in a small amount of a medication (salbutamol). This test helps us find out if you may have a breathing problem like asthma. You will also be asked to blow 2-3 times into a NIOXMINO machine, which measures nitric oxide produced by the airways. This machine is used to detect if a person has allergic airway inflammation which is present in asthma or rhinitis.

You will wear a personal sampling pump connected to a filter for a minimum duration of 240 minutes while performing your daily duties so that we can collect the air on your breathing zone to test if you are exposed to hazardous biological agents. The dust collected in the air during your work activity will also be used to determine the effectiveness of the controls in place Analysis will include total dust, moulds and their by-products (glucan and MVOCs), mites concentration in bulk samples.

Are there any risks involved for participating in the study?

Wearing the dust measuring equipment is not a risk to you and it will not interfere with your work. You may feel a little dizzy for a short while and have slight bruising during the blood tests. You may also have mild shortness of breath, cough, chest tightness, wheezing, chest soreness or headache during asthma tests, which last only a few minutes. A doctor or nurse will always be available in case of any emergency.

The potential benefits for the participants:

The indirect benefit of the project is creating a safer and healthy working environment for you and your fellow colleagues. All participants will be given a written copy of their laboratory test results and what they mean and at the end of the study (after 4 years) we will present our findings at the farms and answer to all questions. Participants may also benefit from early diagnosis of asthma and treatment thus preventing the increasingly high incidence of work-related asthma. Those with identified work-related asthma problems will also be given the names of specialists for referral. Employed personnel with work-related asthma would be entitled to compensation and would be given assistance in applying for it.

Participants will not be paid for participating in the study.

May I withdraw from the study?

Yes, your participation in this study is entirely voluntary; you can decline to participate or withdraw at any time during the course of the study with no penalty.

Is confidentiality assured?

Yes, all information and research data obtained during the course of this study will be strictly confidential. Data forms will be coded and will not have your name but a coding system that will be used.

If you are happy to take part in the study, please read and sign the attached consent form.

Address study queries to Onnicah or Tanusha at National Institute for Occupational Health,

PO Box 4788, Johannesburg 2000. Email onnicah.matuka@nioh.nhls.ac.za or tanusha.singh@nioh.nhls.ac.za , Tel 011 712 6475 or 6487 Fax 011 712 6426

For ethics queries or complaints please contact Anisa Keshav at keshava@research.wits.ac.za Tel 011 717 1234 of the Human Research Ethics Committee.

✂

I …… (*Print name*) hereby confirm that I have been informed of the nature (including risks and benefits) of the study "Allergic sensitisation and work-related asthma among poultry workers in South Africa" outlined in the information sheet and agree to be part of this study.

PARTICIPANT Survey no.……

.....	……	……
Print Name	Signature	Date and Time

WITNESS

.....	……	……
Print Name	Signature	Date and Time

APPENDIX 4: PRETEST INSTRUCTIONS

NIOH OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG POULTRY WORKERS OF SOUTH AFRICA – 2011

PRETEST INSTRUCTIONS

Surname: _____

First Name: _____

Work number of participant: _____

YOUR VISIT/APPOINTMENT is at _____ (time) on the _____ (date)

You will have the following tests on this visit:

1. A member of the research team will explain the project, obtain your permission to participate, and ask you a list of questions.
2. Members of the research team will conduct the lung function and NIOX tests.
3. A nurse will take a specimen of blood for the allergy test from you on this visit.
4. Please bring this form with you when you come for testing.

Reason for doing these tests:

These tests will enable us to find out whether you are allergic to some of the things you are exposed to at work, such as feathers, chicken droppings and mites in the bedding. In this way we will be able to find ways of protecting the health of workers working on broiler farms.

BEFORE YOU COME FOR TESTING IT IS VERY IMPORTANT THAT YOU FOLLOW THE INSTRUCTIONS BELOW TO ENSURE ACCURATE RESULTS.

1. **DO NOT** smoke any tobacco or cigarettes during the **2 hours** before your appointment.

2. Please ask your staff **NOT TO EAT/DRINK 1 HOUR** before their assessment.

3. We will start with the NIGHT SHIFT AROUND **9AM**, please ask them to adhere to the above.

4. If you are taking asthma medicines:
    - Do not use any asthma tablets/syrup (for example Nuelin, Theodur, Euphyllin Retard) during the **24 hours (1 full day)** before your appointment.
    - Do not use any bronchodilator asthma inhaler pumps (for example Ventolin, Venteze, Asthavent, Atrovent), during the **12 hours** before your appointment.

5. If you are taking any medicine or using any skin cream for allergies (itchy nose/eyes/skin rash) or for flu (for example, Sinutab, Allergex, Actifed, Zyrtec):
    - Do not use any of these medicines for the **3 days** before your appointment since this may keep the allergy test from identifying which things you are allergic to.

6. Please bring with you on the visit any medicine/s from a doctor or clinic that you may be taking at the time to show it to the interviewer. Please bring the bottles or packets with you so that the interviewer can record the name of the medicine.

IF YOU FORGET OR FEEL YOU MUST USE YOUR MEDICINES, COME ANYWAY, WE WILL TALK WITH YOU AND DECIDE WHICH TESTS CAN BE DONE ON THAT DAY.

APPENDIX 5: ENGLISH QUESTIONNAIRE

NIOH OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG POULTRY WORKERS OF SOUTH AFRICA - 2011

ENGLISH QUESTIONNAIRE
Note: Answer ALL questions. Insert a cross (X) where appropriate

Survey Number	

A. IDENTIFICATION DATA

1	Surname						
2	First name/s						
3	Address						
4	Work number						
5	Date of birth:	Day		Month		Year	
6	Gender:					Male	
						Female	
7	Home Language:					English	
						Afrikaans	
						Setswana	
						Other	
8	Interviewer's **name**						
9	Date of interview:	Day		Month		Year	
10	Farm name:						
	Site or location						
	Province						

11	Are you a casual or permanent worker?	Casual Permanent	☐ ☐
12,1	Date of last work shift? Day _____ Month _____	Year	_____
12,2	Which shift did you work today?	6.00-16.30 (7.5h) 6.00-16.30 (9h) 16.30 - 6.00am Other	☐ ☐ ☐ ☐
	Specify:		

B. HEALTH PROBLEMS

Wheeze and tightness in the chest

1	Have you ever had wheezing or whistling in your chest in the past? ***If YES, go on to Question 1.1*** ***If NO, skip to Question 2***	Yes No	☐ ☐
1,1	If yes, when was the first time you had these symptoms. Month _____	Year	_____
1,2	Have you had wheezing or whistling in your chest at any time in the **last 12 months?** ***If YES, go on to Question 1.2.1*** ***If NO, skip to Question 3***	Yes No	☐ ☐
1.2.1	Have you been short of breath when the wheezing noise was present?	Yes No	☐ ☐
1.2.2	Have you had this wheezing or whistling when you did not have a cold or flu?	Yes No	☐ ☐
2	Have you been woken up with a feeling of tightness in your chest at any time in the **last 12 months?**	Yes No	☐ ☐

	Shortness of breath		
3	Have you had an attack of shortness of breath that came on during the daytime when you were at rest at any time in the **last 12 months?**	Yes No	☐ ☐
4	Have you had an attack of shortness of breath that came on following running or exercise at any time in the **last 12 months?**	Yes No	☐ ☐
5	Have you been woken by an attack of shortness of breath at any time in the **last 12 months?**	Yes No	☐ ☐

	Cough and phlegm from the chest		
6	Have you been woken by an attack of coughing at any time in the **last 12 months?**	Yes No	☐ ☐
7	Do you usually cough first thing in the morning?	Yes No	☐ ☐
8	Do you usually cough during the rest of the day, or at night? ***If YES, go on to Question 8.1*** ***If NO, skip to Question 9***	Yes No	☐ ☐
8,1	Do you cough like this on most days/nights for as much as three or more months in each of the last two years?	Yes No	☐ ☐
9	Do you usually bring up any phlegm from your chest first thing in the morning?	Yes No	☐ ☐
10	Do you usually bring up any phlegm from your chest during the day, or at night? ***If YES, go on to Question 10.1*** ***If NO, skip to Question 11***	Yes No	☐ ☐

10,1	Do you bring up phlegm like this on most days/ nights for as much as three or more months in each of the last two years?	Yes No	☐ ☐

Breathing

11	Do you ever have trouble with your breathing? ***If YES, go on to Question 11.1*** ***If NO, skip to Question 12***	Yes No	☐ ☐
11,1	Do you have this trouble: ***Give all options at once*** ***Insert a cross (X) next to one answer only***		
	a) continuously so that your breathing is never quite right?		☐
	b) repeatedly, but it goes away completely between the times when it troubles you?		☐
	c) only rarely?		☐
12	Are you disabled from walking by a condition other than heart or lung disease? ***If YES, state the condition*** _____ ***and go on to Question 13*** ***If NO, go to Question 12.1***	Yes No	☐ ☐
12,1	Are you troubled by shortness of breath when hurrying on level ground or walking up a slight hill? ***If YES, go on to Question 12.1.1*** ***If NO, skip to Question 13***	Yes No	☐ ☐
12.1.1	Do you get short of breath walking with other people of your own age on level ground?	Yes No	☐ ☐
12.1.2	Do you have to stop for breath when walking at your own pace on level ground?	Yes No	☐ ☐

Asthma

13	Have you ever had asthma?	Yes	
	If YES, go on to Question 13.1 ***If NO, skip to Question 13.8***	No	
13,1	If yes, was this confirmed by a doctor?	Yes	
		No	
13,2	How old were you when you were told you have asthma?		
	Give all options at once ***Insert a cross (X) next to one answer only***		
	a) Only before you were 17 years old		
	b) Only at the age of 17 years or older		
	c) Both		
	The following references to "attack" of asthma refers to episodes of wheezing, shortness of breath, chest tightness or cough attributed to asthma		
13.3.1	How old were you when you had your first attack of asthma?	years old	
13.3.2	How old were you when you had your most recent attack of asthma?	years old	
13,4	Which months of the year do you usually have attacks of asthma?		
	Answer ALL questions, insert a cross (X)		
13.4.1	January/February	Yes	
		No	
13.4.2	March/April	Yes	
		No	

13.4.3	May/June	Yes No	☐ ☐
13.4.4	July/August	Yes No	☐ ☐
13.4.5	September/October	Yes No	☐ ☐
13.4.6	November/December	Yes No	☐ ☐
13,5	Have you had an attack of asthma in the last **12 months?** ***If YES, go on to Question 13.5.1*** ***If NO, skip to Question 13.6***	Yes No	☐ ☐
13.5.1	How often have you had an attack of asthma in the **last 12 months?** ***<u>Give all options at once</u>*** ***Insert a cross (X) next to one answer only***		
	a) Every day		☐
	b) More than 2 times a week		☐
	c) More than 1 time per month		☐
	d) 3 to 12 times in the whole year		☐
	e) 1 to 2 times in the whole year		☐
13,6	Are your chest symptoms caused by, or made worse by any of the following: ***Answer all questions, insert a cross (X)***		
13.6.1	Contact with animals/pets	Yes No	☐ ☐
13.6.2	Grass or flowers	Yes No	☐ ☐

13.6.3	Heavy exercise	Yes No	☐ ☐
13.6.4	Breathing cold air	Yes No	☐ ☐
13.6.5	Dusts or sprays at work	Yes No	☐ ☐
13.6.6	Tobacco smoke	Yes No	☐ ☐
13.6.7	Change in the weather	Yes No	☐ ☐

13,7 Do your chest symptoms seem better or worse when you are away from work (for example, on weekends, off-shift and annual leave)?

Give all options at once

Insert a cross (X) next to ONE answer only

a) Stay the same ☐
b) Get better ☐
c) Get worse ☐

13,8 Does being at work ever make your chest tight or wheezy? Yes ☐ No ☐

If YES, go on to Question 13.8.1
If NO, skip to Question 13.9

13.8.1 When did you first notice having problems with chest tightness or wheeze at work? Month _____ Year _____

13.8.2 Is there anything that you work with **(specify feather, bedding, feed, litter, manure &/or premix)** or any other substance that causes you to have these chest symptoms? Yes ☐ No ☐

If YES, go on to Question 13.8.3
If NO, skip to Question 13.9

13.8.3 What do you think is causing these symptoms?

13,9 Have you ever had to change or leave your work area, either temporarily or permanently, in this ~~broiler~~ farm or any other ~~broiler~~ farm because of any chest symptoms? Yes ☐ No ☐

If YES, go on to Question 13.9.1
If NO, skip to Question 13.10

13.9.1 What type of job were you doing when this happened?

13.9.2 Was this a job in this ~~broiler~~ farm? Yes ☐ No ☐

If YES, go on to Question 13.9.2.1
If NO, skip to Question 13.10

13.9.2.1 What area/section did you move to?

13.9.2.2 What job did you do there?

13.9.2.3 Did your symptoms improve when you changed jobs? Yes ☐ No ☐

13,10 Have you ever worked in a job or jobs that exposed you to vapours, gas, dust **(e.g. feed, litter, bedding, feathers)** or fumes? Yes ☐ No ☐

If YES, go on to Question 13.10.1.
List the jobs beginning with the most recent
If NO, skip to Question 13.11

13.10.1 What was or is this job?

(if current job write 'current job' and specify)

13.10.2 Before that?

13.10.3	Before that?		
13,11	Has there ever been an instance when you inhaled a large amount of vapour, gas, dust or fumes in any of these jobs that resulted in you developing a tight chest, wheeze or cough? ***If YES, go on to Question 13.11.1. If NO, skip to Question 13.12***	Yes No	
13.11.1	What was or is this job?	(if current job write 'current job' and specify)	
13,12	Are you using any medicines, including inhalers/ pumps, nebulizers, syrups or tablets, for asthma or breathing problems? ***If YES, go on to Question 13.12.1, showing examples of each. If NO, skip to question 13.13***	Yes No	
13.12.1	Which medicines?		
13.12.2	Do you take these medicines every day even when you do not have any trouble breathing?	Yes No	
13,13	Have you ever been treated for any of the following: ***Answer all questions***		
13.13.1	Repeated chest infections as a child	Yes No Unknown	
13.13.2	Tuberculosis (TB)	Yes No Unknown	

13.13.3	Chronic bronchitis	Yes No Unknown	☐ ☐ ☐

Nose and eye symptoms

14	Have you ever had any nose or eye problems or allergies such as hay fever? ***If YES, go on to Question 14.1 Answer all questions*** ***If NO, skip to Question 14.4***	Yes No	☐ ☐
14,1	How old were you when you first noticed these symptoms?	Years old	☐
14,2	During the past 12 months have you had **two or more** episodes of:		
14.2.1	Sneezy, itchy or runny nose when you did not have a cold or flu?	Yes No	☐ ☐
14.2.2	Red, itchy or watery eyes	Yes No	☐ ☐
14.2.3	Do you usually have the nose or eye symptoms at any particular time of the year?	Yes No	☐ ☐
14.2.3.1	If YES, which is the **worst** season? ***<u>Give all options at once</u>*** ***Insert a cross (X) next to ONE answer only*** a) Winter b) Spring c) Summer d) Autumn ***If YES to any of question 14.2, go on to Question 14.3*** ***If NO, skip to Question 14.4***		☐ ☐ ☐ ☐

14,3 Do your nose or eye symptoms seem better or worse when you are away from work (for example, on weekends, off-shift and annual leave?)

Give all options at once

Insert a cross (X) next to ONE answer only

a) Stay the same
b) Get better
c) Get worse

14,4 Does being at work ever cause you to have sneezy/ itchy/runny nose or red/itchy/watery eyes? Yes / No

If YES to any one of the above, go on to Question 14.4.1
If NO, skip to Question 14.6

14.4.1 Since when have you been having these symptoms at work? Month _____ Year _____

14.4.2 Is there anything that you work with **(e.g. feed, bedding, litter, manure &/or premix)** or any other substance that causes you to have these symptoms? Yes / No

If YES, go on to Question 14.4.3
If NO, skip to Question 14.5

14.4.3 What do you think is causing these symptoms?

_____

14,5 Are you using any medicines, including nose sprays, drops, tablets or injections, for your nose or eye symptoms at present? Yes / No

If YES, go on to Question 14.5.1
If NO, go on to Question 14.6

Present a chart with different allergy medicines
(N.B. a worker might show you his/her medicines).

14.5.1 Which medicines?

_____

_____

14,6	Did you have hay fever (itchy or watery eyes/nose) as a child?	Yes No	

Skin symptoms

15	Have you ever had any kind of skin problem either at home or at work? ***If YES, go on to Question 15.1*** ***If NO, skip to Question 15.4.4***	Yes No	
15,1	How old were you when you **first** noticed this skin problem?	Years old	
15,2	During the past **12 months** have you had any skin problems that occurred **2 or more times**? ***If Yes, which of the following problems did you have?*** ***Go through each option in the table below and cross (X) the appropriate response.***	Yes No	

			Forearms/hands		**Whole body**	
15.2.1	Itchy or scratchy skin	Yes No			Yes No	
15.2.2	Hives (bommels)	Yes No			Yes No	
15.2.3	Dry,scaly skin	Yes No			Yes No	
15.2.4	Redness of the skin	Yes No			Yes No	
15.2.5	Blisters or weeping skin	Yes No			Yes No	
15.2.6	Burning skin	Yes No			Yes No	

15.2.7	Start with an hour of contact with a substance (feed, litter, bedding, etc) or food item (e.g. chicken)	Yes No		Yes No	
15.2.8	Warts	Yes No		Yes No	
15.2.9	Other	Yes No		Yes No	
	Specify:				

If YES, to any of the above go on to Question 15.3
If NO, skip to Question 15.4

15,3 Do your skin problems seem better or worse when you are away from work (for example, on weekends, off-shift and vacations)?

<u>Give all options at once</u>
Insert a cross (X) next to one answer only

a) Stay the same	
b) Get better	
c) Get worse	

15,4	Does being at work ever cause you to have skin problems?			Yes No	

If YES, go on to Question 15.4.1
If NO, skip to Question 15.4.4

15.4.1	Since when have you been having these skin problems at work?	Month		Year	
15.4.2	Is there anything that you work with **(e.g. feed, bedding, litter, manure &/or premix)** or any other substance that makes these skin problems worse?			Yes No	

If YES, go on to Question 15.4.3
If NO, skip to Question 15.4.4

15.4.3 What do you think is causing these skin problems?

15.4.4	Have you ever bruised or injured your fingers or hands while working in the ~~broiler~~ farm?	Yes No	☐ ☐
15.4.5	Do you wear gloves while working? ***If YES, go on to Question 15.4.5.1*** ***If NO, skip to Question 15.5***	Yes No	☐ ☐
15.4.5.1	How often do you wear these gloves while working? ***Give all options at once*** ***Insert a cross (X) next to one answer only***		
	a) most of the time		☐
	b) less than half the time		☐
	c) occasionally		☐
15,5	Do the tasks of your current job involve wet work? ***Give all options at once*** ***Insert a cross (X) next to one answer only***		
	a) most of the time		☐
	b) less than half the time		☐
	c) occasionally		☐
	d) no wet work		☐
15,6	How many times do you wash your hands in the course of a day? ***Give all options at once*** ***Insert a cross (X) next to one answer only***		
	0 times		☐
	1 time		☐
	2-3 times		☐
	4-5 times		☐
	6 or more		☐

15,7	Are you using any medicines, including any creams or ointments, for your skin problems at present? ***If YES, go on to Question 15.7.1*** ***If NO, skip to next question 15.8***	Yes No	
15.7.1	Which medicines?		
15,8	Did you have eczema as a child?	Yes No	

Other allergic conditions

16	Are you allergic to insect stings or bites? ***If YES, go on to Question 16.1*** ***If NO, skip to Question 17***	Yes No	
16,1	What kind of reactions do you have?		
16.1.1	Breathing difficulty, feeling faint, fever?	Yes No	
16.1.2	Redness, itching or swelling at the sting site	Yes No	
16.1.3	Other Specify:	Yes No	
17	Have you ever had any difficulty with your breathing after taking medications or injections that you did not have before? ***If YES, go on to Question 17.1*** ***If NO, skip to 18***	Yes No	
17,1	Which medicines?		

18	When you are near animals (such as cats, dogs or horses), near feathers (including pillows, quilts or duvets), near grass and flowers, or in a dusty part of the house, do you ***ever***		
18,1	Start to cough?	Yes No	
18,2	Start to wheeze?	Yes No	
18,3	Get a tight chest?	Yes No	
18,4	Start to feel short of breath?	Yes No	
18,5	Get a runny/stuffy nose or sneeze?	Yes No	
18,6	Get itchy or watery eyes?	Yes No	
18,7	Get itchy skin/rash?	Yes No	
19	Have you ever had an illness or trouble caused by eating a particular type of food/fruit? ***If YES, go on to Question 19.1*** ***If NO, skip to Section C: Family history***	Yes No	
19,1	What type of food/fruit was this?		
	Did this illness or trouble include:		
19.1.1	Itchy skin or rash	Yes No	

19.1.2	Diarhoea or vomiting	Yes No	☐ ☐
19.1.3	Runny or stuffy nose	Yes No	☐ ☐
19.1.4	Severe headaches	Yes No	☐ ☐
19.1.5	Breathlessness/tight chest/wheeze	Yes No	☐ ☐
19.1.6	Other Specify:	Yes No	☐ ☐
19,2	Was the food canned or preserved?	Yes No	☐ ☐
19,3	Do you experience these problems when you drink fizzy drinks also?	Yes No	☐ ☐

C. FAMILY HISTORY

1	Do/did any members of your family (blood relatives) ever have any kind of allergies? ***<u>Do not include relatives by marriage</u>*** ***If family history is completely unknown (subject is adopted, etc.), mark UNK and do not complete table. Move to next section***	Yes No Unknown	☐ ☐ ☐

If YES, complete table below. Insert a cross (X) in the appropriate block for each option

		No one in family	Yes, present in family			Do not know
			Parent	Brother/sister	Child	
1,1	Hay fever					
1,2	Eczema					

1,3	Asthma					
1,4	Chicken related allergy (including egg allergy)					
1,5	Other Specify:					

D. SMOKING HISTORY

1	Have you ever smoked tobacco (cigarettes or pipe) for as long as a year? ***'YES' means at least 20 packs of cigarettes or 360 grams of tobacco in a lifetime or at least one cigarette/day for one year*** ***If YES, go on to Question 1.1*** ***If NO, skip to Question 2***	Yes No	
1,1	How old were you when you started smoking?	Years old	
1,2	Do you now smoke? ***'YES' means smoking tobacco in the last month or more*** ***If YES, go on to Question 1.2.1-2*** ***If NO, skip to Question 1.3***	Yes No	
1.2.1-2	How much do you now smoke on average?		
1.2.1	Number of cigarettes per day		
1.2.2	Pipe tobacco in grams/week		
1,3	Have you stopped smoking completely? ***If YES, go on to Question 1.3.1*** ***If NO, skip to Question 1.4***	Yes No	

1.3.1	How old were you when you stopped smoking completely?	Years old	
1.3.1.1	How many years in total did you smoke cigarettes? (Do not include the years you stopped before you started again)	Years	
1.3.2	On average of the entire time you smoked, how much did you smoke?		
1.3.2.1	Number of cigarettes per day		
1.3.2.2	Pipe tobacco in grams/week		
1,4	Do you or did you inhale the smoke?	Yes No	
2	Have you been regularly exposed to tobacco smoke from other people smoking cigarettes or pipe in the last 12 months? ***'Regularly' means on most days or nights***	Yes No	

E. DIETARY HISTORY/DOMESTIC ACTIVITIES

1 How often have you eaten the following chicken products in the last 12 months?

Go through each chicken product option and insert a cross (X) in the block for each option

		Daily	1/week	2-3/week	1/month	2-3/month	Never
1,1	Chicken polony						
1,2	Chicken viennas						
1,3	Chicken steaklets						
1,4	Chicken burger						

1,5	Chicken nuggets						
1,6	Chicken bites						
1,7	Chicken canned food						
1,8	Chicken liver						
1,9	Chicken soup						
1,10	Chicken stock cubes						
1,11	Eggs						
1,12	Other						
	Specify:						

2	Have you changed your diet or avoided certain chicken (eg. chicken/eggs) products because they do not agree with you when you eat them?	Yes No	
	If YES, go on to Question 2.1 ***If NO, skip to next Section F on HEALTH & SAFETY***		
2,1	What chicken products have you avoided?		
3	Do you prepare chicken at home?	Yes No	
	If YES, go on to Question 3.2 ***If NO, go to Question 3.1***		
3,1	If no, does any one else prepare chicken food at home?	Yes No	
3,2	How often do you prepare chicken food at home?		
	a) once a month		
	b) 2-3 times a month		
	c) 2-3 times per week		
	d) once a week		
	e) everyday		

3,3	What do you prepare? a)meat b)chicken soup c)chicken lasagne d) Other Specify:	
3,4	How often do you use the chicken products? a) once a month b) 2-3 times a month c) 2-3 times per week d) once a week e) everyday	

F. HEALTH AND SAFETY EDUCATION AND TRAINING

1	What are the hazards associated with chicken farming?		
2	Have you had any health and safety training on how to protect yourself when working in the chicken farm?	Yes No	

G. WORK HISTORY IN CHICKEN INDUSTRY

1	How long have you been working at this ~~broiler~~ farm?	Years		Months	
	Present job				
2	How long have you been working in your current job?	Years		Months	
3	In which division are you currently working? ***Refer to exposure area list to identify division***				

a) Broiler
b) Breeding
c) Factory
d) Outsourced services
e) Administration
f) Other

Specify:

3,1 In which area are you currently working?

a) Rearing
b) Laying
c) Hatchery
d) Grading **(specify below type of grading e.g. egg or chicks)**
e) Cleaning
f) Transport
g) Broiler
h) Free range
i) Catching
j) Hanging
k) Defeathering
l) Eviceration
m) Packing/dispatch
n) Rendering
o) Bedding
p) Administration
q) Washing team
r) Other

Specify:

3,2 What is your job/task in this area?

Job Title

Get a short description of the job

3,3 What materials do you work with?

Answer one or more questions by inserting a cross (X)

	a) Chicks	Yes	
		No	
	b) Chickens	Yes	
		No	
	c) Eggs	Yes	
		No	
	d) Feed	Yes	
		No	
	e) Bedding	Yes	
		No	
	f) Litter	Yes	
		No	
	g) Other	Yes	
		No	
	Specify:		
3,4	Do you ever do other jobs during your shift on a regular basis (almost every day)?	Yes	
		No	
	If **Yes**, which jobs?		
3,5	How much dust would you say your current job produces: ***Give all options at once*** ***Insert a cross (X) next to ONE answer only***		
	a) None		
	b) A little		
	c) An average amount		
	d) A lot		
3.5.1	Does your job involve cleaning activities? ***Give all options at once*** ***If YES, continue with 3.5.1 (one or more), If NO go to 3.5.2***	Yes	
		No	

3.5.1.1	General cleaning	Yes	
		No	
3.5.1.2	Clean water/feed system	Yes	
		No	
3.5.1.3	Clean equipment	Yes	
		No	
3.5.1.4	Clean PPE	Yes	
		No	
3.5.1.5	Clean nest boxes	Yes	
		No	
3.5.1.6	Clean egg trays	Yes	
		No	
3.5.1.7	Clean crates	Yes	
		No	
3.5.1.8	Other	Yes	
		No	
	Specify:		

3.5.2	How far do you work from the source of the dust? ***Give all options at once*** ***Insert a cross (X) next to ONE answer only***		
	a) Right next to the source		
	b) About 1-2 metres away		
	c) More than 3 metres away		
	d) Does not apply		
3,6	Do you use any personal protective equipment on a regular basis (almost every day) while doing your job? ***If NO, skip to Question 4*** ***If YES, continue with Question 3.6.1***	Yes No	
3.6.1	Which of the following personal protective		

	equipment do you use on a **regular** basis (almost every day)?		
3.6.1.1	Goggles:	Yes No	☐ ☐
3.6.1.2	Gloves:	Yes No	☐ ☐
3.6.1.3	Mask:	Yes No	☐ ☐
3.6.1.4	Aprons:	Yes No	☐ ☐
3.6.1.5	Other: Specify:	Yes No	☐ ☐
	If NO to all of the previous questions, skip to Question 4 If YES to any one of the above questions, continue with Question 3.6.2		
3.6.2	How long have you been wearing the personal protective equipment on a regular basis (almost every day) while working?		
3.6.2.1	Goggles	Years	☐
3.6.2.2	Gloves:	Years	☐
3.6.2.3	Mask:	Years	☐
3.6.2.4	Aprons:	Years	☐
3.6.2.5	Other:	Years	☐
	<u>Previous jobs in present farm</u>		
4	Before doing this job at this farm, did you do a different job here?	Yes No	☐ ☐
	If NO, skip to question 5		

If YES, continue with question 4.1

4,1 What other jobs did you do here?

Start with the first job and work forward, getting a one-line description of each job. If casual worker, denote each period of employment as a separate job. For continuous years of seasonal work consider as one job (provided no broken years service)

Job 1

4.1.1 Area/section

4.1.2 Job Title

Get a short description of the job

4.1.3 Permanent/casual:

4.1.4 How long did you work in this job?

Years

Months

4.1.5 In which area were you working?

a) Rearing
b) Laying
c) Hatchery
d) Grading **(specify below type of grading e.g. egg or chicks)**
e) Cleaning
f) Transport
g) Broiler
h) Free range
i) Catching
j) Hanging
k) Defeathering
l) Eviceration

	m) Packing/dispatch		
	n) Rendering		
	o) Bedding		
	p) Administration		
	q) Washing team		
	r) Other		
	Specify:		
4.1.6	What materials did you work with?		
	Answer one or more questions by inserting a cross (X)		
	a) Chicks	Yes	
		No	
	b) Chickens	Yes	
		No	
	c) Eggs	Yes	
		No	
	d) Feed	Yes	
		No	
	e) Bedding	Yes	
		No	
	f) Litter	Yes	
		No	
	g) Other	Yes	
		No	
	Specify:		
4.1.7	How much dust would you say that this job produced:		
	<u>Give all options at once</u>		
	Insert a cross (X) next to ONE answer only		
	a) None		
	b) A little		
	c) An average amount		
	d) A lot		

4.1.8	Does your job involve cleaning activities? ***Give all options at once*** ***If YES, continue with 4.1.8 (one or more), If NO go to 4.1.9***	Yes No	
4.1.8.1	General cleaning	Yes No	
4.1.8.2	Clean water/feed system	Yes No	
4.1.8.3	Clean equipment	Yes No	
4.1.8.4	Clean PPE	Yes No	
4.1.8.5	Clean nest boxes	Yes No	
4.1.8.6	Clean egg trays	Yes No	
4.1.8.7	Clean crates	Yes No	
4.1.8.8	Other Specify:	Yes No	
4.1.9	How far did you work from the source of the dust? ***Give all options at once*** ***Insert a cross (X) next to ONE answer only*** a) Right next to the source b) About 1-2 metres away c) More than 3 metres away d) Does not apply		
4.1.10	Did you use any personal protective equipment on a regular basis (almost every day) while doing your	Yes No	

	job? ***If NO, skip to Question 4.2.1*** ***If YES, continue with Question 4.1.10***		
4.1.10.1-5	Which of the following personal protective equipment did you use on a regular basis (almost every day)?		
4.1.10.1	Goggles:	Yes No	
4.1.10.2	Gloves:	Yes No	
4.1.10.3	Mask:	Yes No	
4.1.10.4	Aprons:	Yes No	
4.1.10.5	Other: ***If NO to all of the previous questions, skip to Question 4.2.1*** ***If YES to any one of the above questions, continue with Question 4.1.11***	Yes No	
4.1.11	How long have you been wearing the personal protective equipment on a regular basis (almost every day) while working?		
4.1.11.1	Goggles	Years	
4.1.11.2	Gloves:	Years	
4.1.11.3	Mask:	Years	
4.1.11.4	Aprons:	Years	
4.1.11.5	Other:	Years	
	Job 2		
4.2.1	Area/section		

4.2.2	Job Title		
	Get a short description of the job		
4.2.3	Permanent/casual:		
4.2.4	How long did you work in this job?	Years Months	
4.2.5	In which area were you working?		
	a) Rearing		
	b) Laying		
	c) Hatchery		
	d) Grading **(specify below type of grading e.g. egg or chicks)**		
	e) Cleaning		
	f) Transport		
	g) Broiler		
	h) Free range		
	i) Catching		
	j) Hanging		
	k) Defeathering		
	l) Eviceration		
	m) Packing/dispatch		
	n) Rendering		
	o) Bedding		
	p) Administration		
	q) Washing team		
	r) Other		
	Specify:		
4.2.6	What materials did you work with?		
	Answer one or more questions by inserting a cross (X)		
	a) Chicks	Yes No	
	b) Chickens	Yes	

		No	
	c) Eggs	Yes	
		No	
	d) Feed	Yes	
		No	
	e) Bedding	Yes	
		No	
	f) Litter	Yes	
		No	
	g) Other	Yes	
		No	
	Specify:		
4.2.7	How much dust would you say that this job produced: ***Give all options at once*** ***Insert a cross (X) next to ONE answer only***		
	a) None		
	b) A little		
	c) An average amount		
	d) A lot		
4.2.8	Does your job involve cleaning activities? ***Give all options at once*** ***If YES, continue with 4.2.8 (one or more), If NO go to 4.2.9***	Yes No	
4.2.8.1	General cleaning	Yes	
		No	
4.2.8.2	Clean water/feed system	Yes	
		No	
4.2.8.3	Clean equipment	Yes	
		No	

4.2.8.4	Clean PPE	Yes No	
4.2.8.5	Clean nest boxes	Yes No	
4.2.8.6	Clean egg trays	Yes No	
4.2.8.7	Clean crates	Yes No	
4.2.8.8	Other	Yes No	
	Specify:		

4.2.9 How far did you work from the source of the dust?

Give all options at once

Insert a cross (X) next to ONE answer only

a) Right next to the source	
b) About 1-2 metres away	
c) More than 3 metres away	
d) Does not apply	

4.2.10 Did you use any personal protective equipment on a regular basis (almost every day) while doing your job? Yes / No

If NO, skip to Question 4.3.1
If YES, continue with Question 4.2.10

4.2.10.1-5 Which of the following personal protective equipment did you use on a regular basis (almost every day)?

4.2.10.1	Goggles:	Yes No	
4.2.10.2	Gloves:	Yes No	

4.2.10.3	Mask:	Yes No	
4.2.10.4	Aprons:	Yes No	
4.2.10.5	Other:	Yes No	
	If NO to all of the previous questions, skip to Question 4.3.1 ***If YES to any one of the above questions, continue with Question 4.2.11***		
4.2.11	How long have you been wearing the personal protective equipment on a regular basis (almost every day) while working?		
4.2.11.1	Goggles	Years	
4.2.11.2	Gloves:	Years	
4.2.11.3	Mask:	Years	
4.2.11.4	Aprons:	Years	
4.2.11.5	Other:	Years	

Job 3

4.3.1	Area/section	
4.3.2	Job Title	
	Get a short description of the job	
4.3.3	Permanent/casual:	
4.3.4	How long did you work in this job?	Years Months

4.3.5	In which area were you working?		
	a) Rearing		
	b) Laying		
	c) Hatchery		
	d) Grading **(specify below type of grading e.g. egg or chicks)**		
	e) Cleaning		
	f) Transport		
	g) Broiler		
	h) Free range		
	i) Catching		
	j) Hanging		
	k) Defeathering		
	l) Eviceration		
	m) Packing/dispatch		
	n) Rendering		
	o) Bedding		
	p) Administration		
	q) Washing team		
	r) Other		
	Specify:		
4.3.6	What materials did you work with?		
	Answer one or more questions by inserting a cross (X)		
	a) Chicks	Yes	
		No	
	b) Chickens	Yes	
		No	
	c) Eggs	Yes	
		No	
	d) Feed	Yes	
		No	
	e) Bedding	Yes	
		No	
	f) Litter	Yes	
		No	

	g) Other	Yes	
		No	
	Specify:		
4.3.7	How much dust would you say that this job produced: ***Give all options at once*** ***Insert a cross (X) next to ONE answer only***		
	a) None		
	b) A little		
	c) An average amount		
	d) A lot		
4.3.8	Does your job involve cleaning activities? ***Give all options at once*** ***If YES, continue with 4.3.8 (one or more), If NO go to 4.3.9***	Yes No	
4.3.8.1	General cleaning	Yes	
		No	
4.3.8.2	Clean water/feed system	Yes	
		No	
4.3.8.3	Clean equipment	Yes	
		No	
4.3.8.4	Clean PPE	Yes	
		No	
4.3.8.5	Clean nest boxes	Yes	
		No	
4.3.8.6	Clean egg trays	Yes	
		No	
4.3.8.7	Clean crates	Yes	
		No	
4.3.8.8	Other	Yes	

		No	
	Specify:		
4.3.9	How far did you work from the source of the dust? ***Give all options at once*** ***Insert a cross (X) next to ONE answer only***		
	a) Right next to the source		
	b) About 1-2 metres away		
	c) More than 3 metres away		
	d) Does not apply		
4.3.10	Did you use any personal protective equipment on a regular basis (almost every day) while doing your job? ***If NO, skip to Question 4.4.1*** ***If YES, continue with Question 4.3.10***	Yes No	
4.3.10.1-5	Which of the following personal protective equipment did you use on a regular basis (almost every day)?		
4.3.10.1	Goggles:	Yes No	
4.3.10.2	Gloves:	Yes No	
4.3.10.3	Mask:	Yes No	
4.3.10.4	Aprons:	Yes No	
4.3.10.5	Other: ***If NO to all of the previous questions, skip to Question 4.4.1*** ***If YES to any one of the above questions, continue with Question 4.3.11***	Yes No	
4.3.11	How long have you been wearing the personal protective		

	equipment on a regular basis (almost every day) while working?		
4.3.11.1	Goggles	Years	
4.3.11.2	Gloves:	Years	
4.3.11.3	Mask:	Years	
4.3.11.4	Aprons:	Years	
4.3.11.5	Other:	Years	

Job 4

4.4.1	Area/section		
4.4.2	Job Title		
	Get a short description of the job		
4.4.3	Permanent/casual:		
4.4.4	How long did you work in this job?	Years Months	
4.4.5	In which area were you working?		
	a) Rearing		
	b) Laying		
	c) Hatchery		
	d) Grading **(specify below type of grading e.g. egg or chicks)**		
	e) Cleaning		
	f) Transport		
	g) Broiler		
	h) Free range		
	i) Catching		
	j) Hanging		
	k) Defeathering		

	l) Eviceration		
	m) Packing/dispatch		
	n) Rendering		
	o) Bedding		
	p) Administration		
	q) Washing team		
	r) Other		
	Specify:		
4.4.6	What materials did you work with?		
	Answer one or more questions by inserting a cross (X)		
	a) Chicks	Yes	
		No	
	b) Chickens	Yes	
		No	
	c) Eggs	Yes	
		No	
	d) Feed	Yes	
		No	
	e) Bedding	Yes	
		No	
	f) Litter	Yes	
		No	
	g) Other	Yes	
		No	
	Specify:		
4.4.7	How much dust would you say that this job produced:		
	<u>Give all options at once</u>		
	Insert a cross (X) next to ONE answer only		
	a) None		
	b) A little		
	c) An average amount		
	d) A lot		

4.4.8	Does your job involve cleaning activities? ***Give all options at once*** ***If YES, continue with 4.3.8 (one or more), If NO go to 4.3.9***	Yes No	
4.4.8.1	General cleaning	Yes No	
4.4.8.2	Clean water/feed system	Yes No	
4.4.8.3	Clean equipment	Yes No	
4.4.8.4	Clean PPE	Yes No	
4.4.8.5	Clean nest boxes	Yes No	
4.4.8.6	Clean egg trays	Yes No	
4.4.8.7	Clean crates	Yes No	
4.4.8.8	Other Specify:	Yes No	
4.4.9	How far did you work from the source of the dust? ***Give all options at once*** ***Insert a cross (X) next to ONE answer only***		
	a) Right next to the source b) About 1-2 metres away c) More than 3 metres away d) Does not apply		
4.4.10	Did you use any personal protective equipment	Yes	

	on a regular basis (almost every day) while doing your job?	No	
	If NO, skip to Question 5 ***If YES, continue with Question 4.4.10***		
4.4.10.1-5	Which of the following personal protective equipment did you use on a regular basis (almost every day)?		
4.4.10.1	Goggles:	Yes No	
4.4.10.2	Gloves:	Yes No	
4.4.10.3	Mask:	Yes No	
4.4.10.4	Aprons:	Yes No	
4.4.10.5	Other:	Yes No	
	If NO to all of the previous questions, skip to Question 5 ***If YES to any one of the above questions, continue with Question 4.4.11***		
4.4.11	How long have you been wearing the personal protective equipment on a regular basis (almost every day) while working?		
4.4.11.1	Goggles	Years	
4.4.11.2	Gloves:	Years	
4.4.11.3	Mask:	Years	
4.4.11.4	Aprons:	Years	
4.4.11.5	Other:	Years	
	<u>Previous work in other chicken farms</u>		

5	Have you worked in any other chicken farms in the past two years?	Yes No	

If NO, skip to question 6
If YES, continue with question 5.1

5,1	Why did you change jobs?	

5,2	What is the total amount of time you have worked in the chicken industry before you started working in this ~~broiler~~ farm?	Years Months	

Previous work experience

6 Name all the previous workplaces that you have worked in, when not working in this farm or before coming to work in this farm:

Start with the most recent job and work backwards (including all other farms and jobs done)

Name of Company	What did the company make?	Job title (what did you do?)	Date started (year)	Date ended (year)	Total years

THANK YOU FOR ANSWERING THE QUESTIONNAIRE

APPENDIX 6: EXHALED NITRIC OXIDE /LFT PRE-TEST DATA COLLECTION SHEET

NIOH OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG POULTRY WORKERS OF SOUTH AFRICA - 2011

EXHALED NITRIC OXIDE /LFT PRE-TEST DATA COLLECTION SHEET

Card 1

Survey Number _____ 1-3

A. IDENTIFICATION DATA

1.Surname _____

2. First name/s _____

3. Work number _____ 4-9

4. Date of birth: Day_____Month_____Year____ 10-15

5. Interviewer's initials _____ 16

6. Date of interview:

Day____Month_____Year_____ 17-22

7. Farm name: _____ 23-24

B.HEALTH PROBLEMS

Recent chest infections

1. Have you had the flu, sinusitis or a chest infection in the past 3 weeks?

Yes (1)
No (2) 25

2. Are you being treated for Tuberculosis (TB)?

Yes (1)
No (2) 26

2.1 If yes, for how long? _____months _____weeks 27-30

If YES, to question no 2, indicate to person that the tests will not be done today. Schedule another appointment in three months time since the start of TB medication. If the person has already had three months of treatment,proceed with the rest of the screening questions and the post-bronchodilator test.

3. Have you had a heart attack or stroke in the last month?

Yes (1)
No (2) 31

4. Do you have epilepsy?

Yes (1) ☐ 32
No (2)

5. Have you had an operation on your brain, eyes, chest or stomach (in the last 12 months)?

☐ 33
☐☐ 34-35

If **YES** to any of the above **Q3-5**, indicate to the person that the lung function tests will not be done. If NO, proceed with the rest of the screening questions

6.For women:

6.1 Are you Pregnant? Yes (1) ☐ 36
No (2)

6.2 Are you Breastfeeding? Yes (1) ☐ 37
No (2)

If **Pregnant**, indicate to the person that the **Lung Function Test will not be done** today, but proceed with NIOX

If **Breastfeeding**, proceed with Lung Function Test with Post-Bronchodilator. Proceed with the rest of the screening questions.

C. ALCOHOL CONSUMPTION

1. Do you drink alcohol?

Yes (1) ☐ 38
No (2)

1.1 If yes, when have you last consumed alcohol?

1-2 hours ago (1) ☐ 39
1 day ago (2)
1 week ago (3)

1.2 How much alcohol did you consume?

_____ ☐☐ 40-41

D. MEDICATION USAGE (show booklet)

1. Are you taking any medicine/s from a doctor or clinic at the moment for asthma, and or hayfever?

Yes (1) ☐ 42
No (2)

1.1 If yes, what are you taking and when last did you take them?

Names		No. of hours since last dose	
_____		_____	43-44
_____	_____	_____	45-46
_____	_____	_____	47-48

2. Are you taking any medicine/s from a doctor or clinic at the moment for any heart condition, or your eyes?

Yes (1)
No (2) 49

If short-acting beta-2-agonist or anti-cholinergic inhalers used in the **last 4** hours or long-acting MDI or theophylline used in **last 8** hours, reschedule spirometry and counsel accordingly.

E. GREEN VEGETABLE CONSUMPTION

1. How often do you eat the following vegetable products?

Type of product	Daily	1 to 3 times a week	1 to 3 times per month	Never	
1.1 Green salad	1	2	3	4	50
1.2 Spinach & other green leafy vegetables	1	2	3	4	51

2. When did you last consume green salad and/or spinach/other green leafy vegetables?

1-2 hours ago (1)
1 day ago (2)
1 a week ago (3) 52

F. PHYSICAL ACTIVITY

1. Do you exercise?

Yes (1)
No (2) 53

2. When was the last time you exercised?

1-2 hours ago (1)
1 day ago (2)
1 week ago (3) 54

G. SPIROMETRY/LUNG FUNCTION TEST

1. Have you ever had a spirometry/lung function test?

Yes (1)
No (2) 55

2. If yes, when last did you blow into a lung function machine?

1-2 hours ago	(1)	☐ 56
1 day ago	(2)	
1 week ago	(3)	
> a week ago	(4)	

H. RECENT FOOD INTAKE

1. Did you drink coffee, tea or coca-cola in the last 6 hours?

Yes	(1)	☐ 57
No	(2)	

2. Did you have anything to eat or drink in the last hour?

Yes	(1)	☐ 58
No	(2)	

3. Have you smoked in the last hour?

Yes	(1)	☐ 59
No	(2)	

If YES to Question 2 or 3, reschedule test for at least 1 hour later the same day or another date.

APPENDIX 7: LUNG FUNCTION TEST DATA COLLECTION SHEET

NIOH OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG POULTRY WORKERS OF SOUTH AFRICA – 2011
LUNG FUNCTION TESTS DATA COLLECTION SHEET

CARD 1

Record Number [ ][ ][ ] 1-3

Work number [ ][ ][ ][ ][ ][ ] 4-9

Date [ ][ ][ ][ ][ ][ ] 10-15
DAY MONTH YEAR

1. Subject's blood pressure [ ][ ][ ] systolic

[ ][ ][ ] diastolic

2. Subject's age YEARS [ ][ ] 16-17

3. Subject's gender MALE [ ] FEMALE [ ] 18

4.1 Subject's height CENTIMETRES [ ][ ][ ] 19-21

4.2 Subject's weight KILOGRAMS [ ][ ][ ] 22-24

5. When did you last work in the poultry farm? Date [ ][ ] [ ][ ] [ ][ ] 25-30

BASELINE SPIROMETRY

6. **PREDICTED FEV_1** [ ][ ][ ] 31-33

7. **INITIAL FEV_1 and FVC**
(up to 8 attempts)

	FEV_1	FVC	
1	[ ]	[ ][ ] [ ][ ]	34-39
2	[ ]	[ ][ ] [ ][ ]	40-45
3	[ ]	[ ][ ] [ ][ ]	46-51
4	[ ]	[ ][ ] [ ][ ]	52-57
5	[ ]	[ ][ ] [ ][ ]	58-63

7.1 Number of rejected attempts [ ] 64

8. **Best INITIAL FEV_1 as % of predicted FEV_1** [ ][ ][ ] 65-67
(divide best results from No. 7 by results from No. 6)

BRONCHODILATOR CHALLENGE ONLY

9. **FEV_1 and FVC**

	FEV_1		FVC			
9.1 Record Best two technically satisfactory	[ ]	[ ][ ]	[ ]	[ ][ ]	68-73
Manoeuvres (up to 8 attempts)	[ ]	[ ][ ]	[ ]	[ ][ ]	74-79

9.2 Number of rejected attempts [ ] 80

CARD 2

10. **Best POST-BRONCHODILATOR FEV_1 as % of initial FEV_1** [ ][ ][ ] 1-3
(divide best results from No. 9 by best results from No. 7)

11. General comments:

_____

_____

12. Technologist initial's _____ [ ] 4

13. Room temperature: _____ [ ][ ] 5-6
(degrees celcius)

NO YES
14 Lung function record appended [ ] [ ] 7

APPENDIX 8: EXHALED NITRIC OXIDE DATA COLLECTION SHEET

NIOH OCCUPATIONAL ALLERGY AND ASTHMA STUDY AMONG POULTRY WORKERS OF SOUTH AFRICA - 2011
EXHALED NITRIC OXIDE DATA COLLECTION SHEET

Card 1

Survey Number _____ [ ][ ][ ] 1-3

Date: _____

Time _____

Ambient NO concentration (ppb) _____ [ ][ ][ ] 4-6

Ambient temperature (degrees celcius) _____ [ ][ ] 7-8

1. Subject's blood pressure systolic _____ [ ][ ][ ] 9-11
diastolic _____ [ ][ ][ ] 12-14

2. Subject's age (in years) _____ [ ][ ] 15-16

3.1 Subject's height (in centimetres) _____ [ ][ ][ ] 17-19

3.2 Subject's weight (in kilograms) _____ [ ][ ][ ] 20-22

4. Gender: Male (1) [ ] 23
Female (2)

5. Effort number (start) _____ [ ][ ][ ] 24-26

6.1 FENo measurement (ppb) 1st effort _____ [ ][ ][ ] 26-28
NIOX machine number of efforts completed _____ [ ][ ][ ][ ] 29-32

6.2 FENo measurement (ppb) 2nd effort _____ [ ][ ][ ] 33-35
NIOX machine number of efforts completed _____ [ ][ ][ ][ ] 36-39

7. FENO printout appended
Yes (1) [ ] 40
No (2)

APPENDIX 9: GUIDELINES FOR AUTHORS FOR CHOSEN JOURNAL (OCCUPATIONAL AND ENVIRONMENTAL MEDICINE)

INSTRUCTIONS FOR AUTHORS

EDITORIAL POLICY

Occupational and Environmental Medicine is an international, peer-reviewed scientific journal covering the broad, multidisciplinary fields of occupational medicine and environmental health. Our goal is to help clinicians, researchers and others keep up to date with the latest developments in occupational and environmental health throughout the world.

We publish high-quality original research and peer-assessed reviews in the areas of occupational and environmental epidemiology, medicine, exposure assessment, hygiene, ergonomics, and psychology; and the evaluation of interventions in controlling risks to the health of workers.

Although the journal's main focus is primary research, we also seek to further the professional education of occupational physicians and other practitioners and to provide a

lively forum for discussion of current concerns related to occupational and environmental health.

Because the scope of occupational and environmental health is extraordinarily broad, *Occupational and Environmental Medicine* welcomes original research and systematic reviews on a wide range of health outcomes and their potential determinants. Industry- and population-based epidemiologic studies are considered relevant, as are assessments of workplace or ambient exposures. Clinical trials and other human experimental research into questions relevant to occupational and environmental health are also welcome. Methodological investigations and applications of mathematical modeling are considered, as well. In general, *Occupational and Environmental Medicine* does not publish reports of single clinical cases or results of laboratory-based research that does not include human subjects, unless of extraordinary interest.

Submission to *Occupational and Environmental Medicine* implies that the work described has not been accepted for publication elsewhere, that it is not under consideration for publication elsewhere and does not duplicate material already published.

OPEN ACCESS

Authors can choose to have their article published Open Access for a fee of £1,950 (plus applicable VAT).

COLOUR FIGURE CHARGES

During submission you will be asked whether or not you agree to pay for the colour print publication of your colour images. This service is available to any author publishing within this journal for a fee of £250 per article. Authors can elect to publish online in colour and

black and white in print, in which case the appropriate selection should be made upon submission.

LANGUAGE POLISHING SERVICE

If you are not a native English speaker, we recommend that you have your manuscript edited by a native speaker prior to submission. Professional editing will improve the grammar, spelling and punctuation of your manuscript, providing clear language which will mean that reviewers and editors are better able to concentrate on the scientific content of the paper. Click here for more information.

ARTICLE TYPES AND WORD COUNTS

The word count excludes the title page, abstract, tables, acknowledgements and contributions and the references. For guidance on how to improve your graphs and tables please view these BMJ demonstration videos.

Supplementary material (e.g. additional tables, figures and text files) can be published online only and is not included in the word count. We advise that supplementary files are kept to a minimum and your cover letter must justify why you think its appropriate to be included with your submission.

Information on our publication turnaround times and acceptance rates can be found here.

ORIGINAL ARTICLE

Original articles should follow the following guidelines:

Word count: up to 4500 words

Structured abstract: up to 250 words (use the headings Objectives, Methods, Results, and Conclusions)

Tables/Illustrations: up to 5

References: up to 40 (more may be allowable for systematic reviews)

Keywords: up to 3 to assist with indexing

Please consult the journal's guidelines regarding the presentation of statistical data

ADDITIONAL INFORMATION ON FORMATTING YOUR PAPER

Papers are considered on the understanding that they are submitted solely to this Journal and do not duplicate material already published elsewhere. In cases of doubt, where part of the material has been published elsewhere, the published material should be included with the submitted manuscript to allow the editor to assess the degree of duplication.

Papers should follow the requirements of the International Committee of Medical Journal Editors (BMJ 1991;302:338-41). Papers and references must be typed in double spacing. SI units should be used.

Please include a box offering a thumbnail sketch of what your article adds to the literature, for readers who would like an overview without reading the whole article. This box should be titled 'What this paper adds' and should consist of 3-5 single-sentence bullet points, as follows: 1-2 sentences summarising the state of scientific knowledge on the subject before the study was done and why the study was needed; 1-2 sentences summarising what we know as a result of this study that wasn't known before, and, where appropriate, 1 sentence noting any important policy or practice implications of the research.

PRESENTATION OF STATISTICAL DATA IN OEM

The Journal does not have fixed policies regarding the presentation of statistical data, but we strongly encourage authors to observe some simple guidelines to ensure that numerical information is presented in a clear and informative manner, as follows:.

In general, measures of the estimated magnitude of effect or association (for example, rate ratios or differences in means) should be used to present the results of analyses that contrast groups or samples. The presentation of statistical test results without an estimate of effect size is less informative and is therefore discouraged.

Epidemiologic measures of association (the rate ratio, odds ratio, risk difference, and so on) are preferred for contrasts of disease frequency.

The presentation of regression coefficients is discouraged except in certain circumstances involving data (for example, lung function) measured on a continuous scale. Units for regression coefficients should always be given.

Confidence intervals should be presented for measures of association whenever possible. P-values for tests of no association are generally not necessary when confidence intervals are provided.

When presented, p-values should be given as quantitative values rather than relative to a cut point for statistical significance (for example, p=0.032, rather than p

Other types of statistical tests, including goodness of fit tests, tests of homogeneity and tests for trend, may be informative in some situations. Where such tests are considered appropriate, reporting their results and the associated p-values is necessary.

The number of significant digits that should be reported in numerical data is rarely the default number provided by statistical software. The number of decimal places or significant digits that is appropriate for a given analysis is a matter of judgment, but it should be consistent with the size of the sample, the analytical precision of the measurements and the nature of the data. For example, a response rate of 64.7% could be misleading if reported from a study of 20 people, but a rate ratio of 1.017 might be entirely appropriate in a study on the effects of air pollution in a large city with several million exposed residents.

PLAGIARISM DETECTION

BMJ is a member of CrossCheck by CrossRef and iThenticate. iThenticate is a plagiarism screening service that verifies the originality of content submitted before publication. iThenticate checks submissions against millions of published research papers, and billions of web content. Authors, researchers and freelancers can also use iThenticate to screen their work before submission by visiting www.ithenticate.com.

COVER LETTER

Your cover letter should inform the Editor of any special considerations regarding your submission, including but not limited to:

Details of related papers by the same author(s) already published or under consideration for publication.

Details of previous reviews of the submitted article.

Copies of related papers, previous Editors’ and reviewers' comments, and responses to those comments can be submitted using the File Designation "Supplementary file for Editors only".

Editors encourage authors to submit previous communications as doing so is likely to expedite the review process.

MANUSCRIPT FORMAT

The manuscript must be submitted as a Word document. PDF is not accepted. The manuscript should be presented in the following order:

Title page.

Abstract, or a summary for case reports (Note: references should not be included in abstracts or summaries).

Main text separated under appropriate headings and subheadings using the following hierarchy: BOLD CAPS, bold lower case, Plain text, Italics.

Tables should be in Word format and placed in the main text where the table is first cited.

Tables must be cited in the main text in numerical order.

Acknowledgments, Competing Interests, Funding and all other required statements.
Reference list.

Images must be uploaded as separate files (view further details under the Figures/illustrations section). All images must be cited within the main text in numerical order and legends should be provided at the end of the manuscript.

Appendices should be uploaded using the File Designation "Supplementary File" and cited in the main text.

Please remove any hidden text headers or footers from your file before submission.

STYLE

Abbreviations and symbols must be standard. SI units should be used throughout, except for blood pressure values which should be reported in mm Hg.

Whenever possible, drugs should be given their approved generic name. Where a proprietary (brand) name is used, it should begin with a capital letter.

Acronyms should be used sparingly and fully explained when first used.

FIGURES/ILLUSTRATIONS

Images must be uploaded as separate files. All images must be cited within the main text in numerical order and legends should be provided at the end of the manuscript.

COLOUR IMAGES AND CHARGES

For certain journals, authors of unsolicited manuscripts that wish to publish colour figures in print will be charged a fee to cover the cost of printing. Refer to the specific journal's instructions for authors for more information.

Alternatively, authors are encouraged to supply colour illustrations for online publication and black and white versions for print publication. Colour publication online is offered at no charge, but the figure legend must not refer to the use of colours.

FILE TYPES

Figures should be submitted in TIFF or EPS format. JPEG files are acceptable in some cases. A minimum resolution of 300 dpi is required, except for line art which should be 1200 dpi.

Histograms should be presented in a simple, two-dimensional format, with no background grid.

During submission, ensure that the figure files are labelled with the correct File Designation of “Mono Image” for black and white figures and “Colour Image” for colour figures.

Figures are checked using automated quality control and if they are below the minimum standard you will be alerted and asked to resupply them.

Please ensure that any specific patient/hospital details are removed or blacked out (e.g. X-rays, MRI scans, etc). Figures that use a black bar to obscure a patient’s identity are NOT accepted.

TABLES

Tables should be in Word format and placed in the main text where the table is first cited. Tables must be cited in the main text in numerical order. Please note that tables embedded as Excel files within the manuscript are NOT accepted. Tables in Excel should be copied and pasted into the manuscript Word file.

Tables should be self-explanatory and the data they contain must not be duplicated in the text or figures. Any tables submitted that are longer/larger than 2 pages will be published as online only supplementary material.

MULTIMEDIA FILES

You may submit multimedia files to enhance your article. Video files are preferred in .WMF or .AVI formats, but can also be supplied as .FLV, .Mov, and .MP4. When submitting, please ensure you upload them using the File Designation "Supplementary File - Video".

REFERENCES

Authors are responsible for the accuracy of cited references and these should be checked before the manuscript is submitted.

CITING IN THE TEXT

References must be numbered sequentially as they appear in the text. References cited in figures or tables (or in their legends and footnotes) should be numbered according to the place in the text where that table or figure is first cited. Reference numbers in the text should be inserted immediately after punctuation (with no word spacing)—for example, [6] not [6].

Where more than one reference is cited, these should be separated by a comma, for example, [1, 4, 39]. For sequences of consecutive numbers, give the first and last number of the sequence separated by a hyphen, for example, [22-25]. References provided in this format are translated during the production process to superscript type, and act as hyperlinks from the text to the quoted references in electronic forms of the article.

Please note that if references are not cited in order the manuscript may be returned for amendment before it is passed on to the Editor for review.

PREPARING THE REFERENCE LIST

References must be numbered consecutively in the order in which they are mentioned in the text.

Only papers published or in press should be included in the reference list. Personal communications or unpublished data must be cited in parentheses in the text with the name(s)

of the source(s) and the year. Authors should request permission from the source to cite unpublished data.

Journals from BMJ use a slightly modified version of Vancouver referencing style (see example below). The style template is available via Endnote. Note that The BMJ uses a different style.

BMJ REFERENCE STYLE

List the names and initials of all authors if there are 3 or fewer; otherwise list the first 3 and add 'et al.' (The exception is the Journal of Medical Genetics, which lists all authors). Use one space only between words up to the year and then no spaces. The journal title should be in italic and abbreviated according to the style of Medline. If the journal is not listed in Medline then it should be written out in full.

Example references

Journal article

13 Koziol-Mclain J, Brand D, Morgan D, et al. Measuring injury risk factors: question reliability in a statewide sample. *Inj Prev* 2000;6:148–50.

Chapter in book

14 Nagin D. General deterrence: a review of the empirical evidence. In: Blumstein A, Cohen J, Nagin D, eds. Deterrence and Incapacitation: Estimating the Effects of Criminal Sanctions on Crime Rates. Washington, DC: National Academy of Sciences 1978:95–139.

Book

15 Howland J. Preventing Automobile Injury: New Findings From Evaluative Research. Dover, MA: Auburn House Publishing Company 1988:163–96.

Abstract/supplement

16 Roxburgh J, Cooke RA, Deverall P, et al. Haemodynamic function of the carbomedics bileaflet prosthesis [abstract]. *Br Heart J* 1995;73(Suppl 2):P37.

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Websites are referenced with their URL and access date, and as much other information as is available. Access date is important as websites can be updated and URLs change. The "date accessed" can be later than the acceptance date of the paper, and it can be just the month accessed.

Electronic journal articles

Morse SS. Factors in the emergency of infectious diseases. *Emerg Infect Dis* 1995 Jan-Mar;1(1). www.cdc.gov/nciod/EID/vol1no1/morse.htm (accessed 5 Jun 1998).

Electronic letters

Bloggs J. Title of letter. *Journal name* Online [eLetter] Date of publication. url eg: Krishnamoorthy KM, Dash PK. Novel approach to transseptal puncture. *Heart* Online [eLetter] 18 September 2001. http://heart.bmj.com/cgi/eletters/86/5/e11#EL1

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1. Alwick K, Vronken M, de Mos T, et al. Cardiac risk factors: prospective cohort study. *Ann Rheum Dis* Published Online First: 5 February 2004. doi:10.1136/ard.2003.001234

How to cite articles with a DOI once they have appeared in print

1. Vole P, Smith H, Brown N, et al. Treatments for malaria: randomised controlled trial. *Ann Rheum Dis* 2003;327:765–8 doi:10.1136/ard.2003.001234 [published Online First: 5 February 2002].

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Additional figures and tables, methodology, raw data, etc may be published online only as supplementary material. If your paper exceeds the word count you should consider if any parts of the article could be published online only. Please note that these files will not be copyedited or typeset and will be published as supplied, therefore PDF files are preferred.

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Some journals also encourage authors to submit translated versions of their abstracts in their local language, which are published online only alongside the English version. These should be uploaded using the File Designation “Abstract in local language”.

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Statistical analyses must explain the methods used.

RESEARCH REPORTING GUIDELINES

Authors are encouraged to use the relevant research reporting guidelines for the study type provided by the EQUATOR Network. This will ensure that you provide enough information for editors, peer reviewers and readers to understand how the research was performed and to judge whether the findings are likely to be reliable.

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