

## Validation of an early warning system for enteric disorders in broiler farming

Emanuela Tullo<sup>a</sup>, Federica Borgonovo<sup>b\*</sup>, Ilaria Fontana<sup>a</sup>, Guido Grilli<sup>c</sup>, Marcella Guarino<sup>a</sup>, Valentina Ferrante<sup>a</sup>

<sup>a</sup>*Department of Environmental Science and Policy, Università degli Studi di Milano, Via Celoria, 10, 20133 Milan, Italy*

<sup>b</sup>*Department of Health, Animal Science and Food Safety, Università degli Studi di Milano, Via Celoria 10, 20133, Milan, Italy*

<sup>c</sup>*Department of Veterinary Medicine, Università degli Studi di Milano, Via Celoria, 10, 20133 Milan, Italy*

federica.borgonovo@unimi.it

### Abstract

Enteric disorders represent a major health issue in intensive farming; these pathologies could be caused by bacteria, viruses and parasites and are a major cause of performances reduction. Monitoring poultry health status takes a key role for farming management in the reduction of chemicals/drugs and their costs.

Nowadays, antibiotics are commonly and preventively used in intensive farming system; therefore, this practice could result in the antibiotic resistance phenomena. For this reasons, there is the global interest in reducing the use of antimicrobials (AM). This could be possible through Precision Livestock Farming (PLF), that can provide valuable information for the early detection of health problem in intensive farming, in virtue of the combination of cheap technologies and specific algorithms.

The experimental trial was carried out in the facilities of the Università degli Studi di Milano located in Lodi.

One hundred and twenty Ross 308 one-day-old chicks were split into two separate boxes (A and B) with standardised management conditions. Air samples from both groups were analysed through a chemical sensors array and were processed with a multivariate statistical software.

This study aims to develop a PLF diagnostic tool, sensible to the variation of volatile organic compounds, to promptly recognise enteric problems in intensive farming, supporting veterinarians and enabling specific treatments in case of disease. The innovative approach of this methodology is the capability to provide reliable real-time information and repeatable responses. The advantage of early warning is the possibility to use non-conventional therapies instead of AM to treat animals.

**Keywords:** early warning system; poultry farming; health problem; intensive farming; PLF; volatile organic compounds

### Introduction

Enteric disorders represent a major health issue in intensive farming; bacteria, viruses and parasites could cause these pathologies.

One of the most common enteric disorder in poultry farming is Coccidiosis that is caused by protozoa of the family Eimeriidae and Coccidia. These parasites are present

in almost every poultry farms. Most species belong to the genus *Eimeria* and infect various poultry intestine tracts.

Clinical disease occurs only after ingestion of sporulated oocysts by susceptible birds. Infections may last up to 4-7 days with parasite replication in host cells causing extensive damage to the intestinal mucosa (Chapman, 2014). The infestation affects especially the digestive tract of young animals; symptoms include lack of appetite and diarrhoea with the consequent drop in the productive performances.

Environmental conditions and high animal density, features of intensive poultry farming, might promote coccidiosis development (McDougald & Fitz-Coy, 2013).

Subclinical infestations have consequences on poultry performance too, with serious economic losses, poor product quality and increase in carcass condemnation at slaughter (Williams, 1999). Nowadays, the available diagnosis technique consist in the counts of the oocysts in the faeces or in the evaluation of lesions provoked by coccidia in different intestinal tracts of dead/culled animals (Johnson & Reid, 1970). However, these methods are time consuming, not ethical and just few laboratories perform this practice.

Due to the ease of pathologies transmission in high density-confined environment of intensive farming, diagnostic techniques must be rapid and sufficiently low-cost to avoid an improper use of medication (McDougald & Fitz-Coy, 2013). Nowadays, antibiotics are commonly and preventively used in intensive farming system; therefore, this practice could result in the antibiotic resistance phenomena. For this reasons, there is the global interest in reducing the use of antimicrobials (AM). Indeed, reducing the use of medication might contribute to reduce antimicrobials resistance that is considered a serious threat for public health (Alali *et al.*, 2009; Berge *et al.*, 2005).

This could be possible through Precision Livestock Farming (PLF) that can provide valuable information for the early detection of health problem in intensive farming, in virtue of the combination of cheap technologies and specific algorithms (Tullo *et al.*, 2013).

The PLF approach can be easily applied at farm level (Wathes, 2010) supporting farmers with early warning systems for the management of complex biological production processes. For instance, PLF tool based on the collection of sounds (Fontana *et al.*, 2015; Fontana *et al.*, 2016; Guarino *et al.*, 2008; Hemeryck *et al.*, 2015; Meen *et al.*, 2015; Moura *et al.*, 2008; Silva *et al.*, 2009; Vandermeulen *et al.*, 2016; Vandermeulen *et al.*, 2015), images (Aydin *et al.*, 2010; Berckmans *et al.*, 2008; Dawkins *et al.*, 2012; Demmers *et al.*, 2012; Guzhva *et al.*, 2016; Ismayilova *et al.*, 2013; Matthews *et al.*, 2016; Porto *et al.*, 2015; Romanini *et al.*, 2012; Shao & Xin, 2008; Tullo *et al.*, 2016) and data from sensors (Caja *et al.*, 2016; Neethirajan, 2017) have been widely used to monitor animal health welfare and production performance.

Image analysis has been widely used in many species to investigate thermal comfort.

The prompt reaction to any change in health, welfare and productive status is the key for the reduction in drugs usage and for the improvement of animal wellbeing.

For these reasons it is necessary to develop an alternative control system to promptly detect the onset of the infestation. Odour and air quality from livestock can give indication on animals health, since the odours from litter strongly depend on the features of animal faeces. Therefore, enteric problems are characterised by different chemical odour properties (Sohn *et al.*, 2008).

In this scenario, a system based on sensors was used as an early and non-invasive detection of any health problem in intensive farming. This system uses non-specific gas sensors that, are sensitive to a wide range of volatile compounds (organic and

inorganic), and is able to classify and identify the odours analysed using a pattern recognition system.

The pattern recognition procedure consists in a comparative and qualitative analysis among different odour samples and it is responsible for the discrimination and classification of sensor data into different clusters. Thus, any new odour sample is assigned to a specific class, based on the sensor output, identifying the presence of a particular chemical pattern (identification process) (Green *et al.*, 2006).

This study aims to develop a PLF diagnostic tool, sensible to the variation of volatile organic compounds (VOCs), to promptly recognise enteric problems in intensive farming, supporting veterinarians and enabling specific treatments in case of disease.

## **Material and Methods**

The experimental trial was carried out in the facilities of the Università degli Studi di Milano located in Lodi and lasted for 45 days.

One hundred and twenty Ross 308 one-day-old chicks were placed at day 0 and split in two separate boxes (A and B) with standardised ventilation, rearing conditions and diet. The feed in group A was added with coccidiostatic.

Both boxes measured 2x3m, the floor was covered with wood shavings and the stocking density was 30 kg/m<sup>2</sup>.

Litter sampling was performed in six different locations within the box and the level of infestation was weekly evaluated according to the method of Mc Master (Conway & McKenzie, 2007). The gold standard for coccidiosis is the oocyst count per gram (opg).

Meanwhile, air sampling was carried out following recommendations described in the European Standard EN 13725 (CEN, 2003). Air samples were drawn into Nalophan<sup>®</sup> bags using a special sampler working with the lung principle. The sampler draws the air directly into the bag by evacuating the tightly closed atmospheric pressure vessel in which it was placed (Dincer *et al.*, 2006).

Air samples were analysed through a chemical sensors array and were processed with a multivariate statistical software.

In order to discriminate between air samples collected in the two groups, firstly data were analysed using the principal component analysis (PCA), then were processed using the discriminant analysis (LDA) and 'Odour-prints' were successively compared.

PCA is a statistical procedure useful for data analysis and classification and it is the most widely used pattern recognition method for the elaboration of the sensor responses.

LDA is a classification procedure, which maximises the variance between groups categories (defined with the PCA) and minimises the variance between categories, taking into account the distance between and within different classes.

## **Results and Discussion**

During the production cycle group B developed an infection of coccidiosis. At day 21 the oocyst count was 250 opg, while at day 35 the infection reached the highest value of 37.000 opg.

The score plot in Figure 1 reports the results of PCA performed on samples collected at day 21 and 35 in both groups (group A – no coccidiosis, group B – coccidiosis).

The analysis showed that, the system was able to find differences between air samples collected in group A and group B, even when the oocysts in group B were in the order of 250 opg.

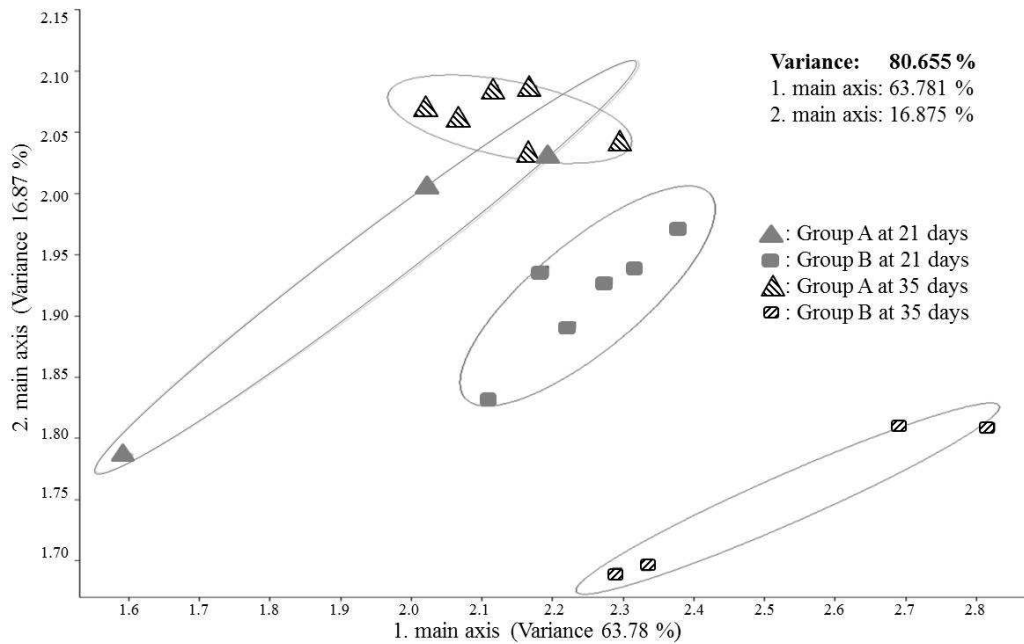


Figure 1. Score plot performed group A and group B at day 21 and 35 of the cycle production.

The score plot, reported in Figure 1, shows that the sum of variances is higher than 80% and the majority of the data was explained by the first axis (63.7% of the total variance). This score plot shows also that samples can be clustered into two groups; the first one (group A at day 21 and 35) represents the broilers that did not develop the coccidiosis while the other one (group B at day 21 and 35) identifies those animals that developed the pathology. Anyway, Figure 1 clearly shows how samples collected on group B at day 35 are highly separated from samples collected both on group A (at day 21 and 35) and on group B (at day 21). This separation of samples might be related to the high number of oocyst found in the litter of group B at day 35 (37,000 opg); indeed, at that moment, broilers were highly infested by coccidia. Furthermore, as shown in Table 1 the discrimination power reached high values (0.70 – 0.80), indicating the good severability of classes (group A and B).

Table 1. Discrimination power among group A and B at day 21 and 35

	Group A day 21 (0 opg)	Group B day 21 (250 opg)	Group A day 35 (0 opg)	Group B day 35 (37k opg)
Group A day 21 (0 opg)		0.76	0.69	0.65
Group B day 21 (250 opg)	0.76		0.83	0.76
Group A day 35 (0 opg)	0.69	0.83		0.71
Group B day 35 (37k opg)	0.65	0.76	0.71	

This system based on chemical sensors is able to recognize at an early stage the difference in features of air sampled in the two groups. This ability related to litter sampling may represent a diagnostic tool for an early detection of health problems due to enteric disorders.

## Conclusions

The results of this preliminary study might be useful for the development of an alternative diagnostic tool to promptly detect the onset of infestations through the monitoring of air quality in poultry houses.

New samples should be collected at farm level, to get a sufficiently large database to define more reliable ‘odour-prints’ of litter samples collected in broiler houses.

Moreover, this system might be a very useful tool to detect enteropathy in farmed animals kept under controlled environmental conditions. Further studies will be necessary to develop and implement an automated tool able to automatically and promptly recognise enteric disorders in intensive farming.

The innovative approach of this methodology is the capability to provide reliable real-time information and repeatable responses. The advantage of early warning is the possibility to use non-conventional therapies instead of AM to treat animals.

The results showed in this study have been patented under the Italian patent application number 102016000059153 on 9<sup>th</sup> June 2016.

## References

Alali, W., Scott, H., Christian, K., Fajt, V., Harvey, R., Lawhorn, D. 2009. Relationship between level of antibiotic use and resistance among *Escherichia coli* isolates from integrated multi-site cohorts of humans and swine. *Preventive Veterinary Medicine* **90**, 160-167.

Aydin, A., Cangar, O., Ozcan, S.E., Bahr, C., Berckmans, D. 2010. Application of a fully automatic analysis tool to assess the activity of broiler chickens with different gait scores. *Computers and Electronics in Agriculture* **73**, 194-199.

- Berckmans, D., Leroy, T., Borgonovo, F., Costa, A., Aerts, J.M., Guarino, M. 2008. Real-time measurement of pig activity in practical conditions. In: *Proc. 8th International Livestock Environment Symposium, ILES 2008* Iguassu Falls, Brazil, 957-962.
- Berge, A., Atwill, E.R., Sisco, W. 2005. Animal and farm influences on the dynamics of antibiotic resistance in faecal *Escherichia coli* in young dairy calves. *Preventive Veterinary Medicine* **69**, 25-38.
- Caja, G., Castro-Costa, A., Knight, C.H. 2016. Engineering to support wellbeing of dairy animals. *Journal of Dairy Research* **83**, 136-147.
- Chapman, H. 2014. Milestones in avian coccidiosis research: a review. *Poultry Science* **93**, 501-511.
- Conway, D., McKenzie, M. 2007. *Poultry Coccidiosis. Diagnostic and Testing Procedures*. 168. Wiley-Blackwell, Iowa, USA.
- Dawkins, M.S., Cain, R., Roberts, S.J. 2012. Optical flow, flock behaviour and chicken welfare. *Animal Behaviour* **84**, 219-223.
- Demmers, T., Cao, Y., Parsons, D.J., Gauss, S., Wathes, C.M. 2012. Simultaneous Monitoring and Control of Pig Growth and Ammonia Emissions. In: *Proc. 9th International Livestock Environment Symposium 2012, ILES 2012* Valencia, Spain, 452-458.
- Dincer, F., Odabasi, M., Muezzinoglu, A. 2006. Chemical characterization of odorous gases at a landfill site by gas chromatography–mass spectrometry. *Journal of chromatography A* **1122**, 222-229.
- Fontana, I., Tullo, E., Butterworth, A., Guarino, M. 2015. An innovative approach to predict the growth in intensive poultry farming. *Computers and Electronics in Agriculture* **119**, 178-183.
- Fontana, I., Tullo, E., Scrase, A., Butterworth, A. 2016. Vocalisation sound pattern identification in young broiler chickens. *Animal* **10**, 1567-1574.
- Green, G.C., Chan, A.D., Goubran, R.A. 2006. An investigation into the suitability of using three electronic nose instruments for the detection and discrimination of bacteria types. In: *Proc. Engineering in Medicine and Biology Society, 2006. EMBS'06. 28th Annual International Conference of the IEEE* 1850-1853.
- Guarino, M., Jans, P., Costa, A., Aerts, J.-M., Berckmans, D. 2008. Field test of algorithm for automatic cough detection in pig houses. *Computers and Electronics in Agriculture* **62**, 22-28.
- Guzhva, O., Ardö, H., Herlin, A., Nilsson, M., Åström, K., Bergsten, C. 2016. Feasibility study for the implementation of an automatic system for the detection of social interactions in the waiting area of automatic milking stations by using a video surveillance system. *Computers and Electronics in Agriculture* **127**, 506-509.
- Hemeryck, M., Berckmans, D., Vranken, E., Tullo, E., Fontana, I., Guarino, M., Van Waterschoot, T. 2015. The Pig Cough Monitor in the EU-PLF project: Results and multimodal data analysis in two case studies. In: *Proc. 7th European Conference on Precision Livestock Farming, ECPLF 2015* 147-155.

- Ismayilova, G., Costa, A., Fontana, I., Berckmans, D., Guarino, M. 2013. Labelling the Behaviour of Piglets and Activity Monitoring from Video as a Tool of Assessing Interest in Different Environmental Enrichments. *Annals of Animal Science* **13**, 611-621.
- Johnson, J., Reid, W.M. 1970. Anticoccidial drugs: lesion scoring techniques in battery and floor-pen experiments with chickens. *Experimental parasitology* **28**, 30-36.
- Matthews, S.G., Miller, A.L., Clapp, J., Plötz, T., Kyriazakis, I. 2016. Early detection of health and welfare compromises through automated detection of behavioural changes in pigs. *The Veterinary Journal* **217**, 43-51.
- McDougald, L., Fitz-Coy, S. 2013. Coccidiosis, Disease of Poultry, 13th ed. Iowa State Press Ames, 1148-1163.
- Meen, G.H., Schellekens, M.A., Slegers, M.H.M., Leenders, N.L.G., van Erp-van der Kooij, E., Noldus, L.P.J.J. 2015. Sound analysis in dairy cattle vocalisation as a potential welfare monitor. *Computers and Electronics in Agriculture* **118**, 111-115.
- Moura, D.J., Silva, W.T., Naas, I.A., Tolón, Y.A., Lima, K.A.O., Vale, M.M. 2008. Real time computer stress monitoring of piglets using vocalization analysis. *Computers and Electronics in Agriculture* **64**, 11-18.
- Neethirajan, S. 2017. Recent advances in wearable sensors for animal health management. *Sensing and Bio-Sensing Research* **12**, 15-29.
- Porto, S.M., Arcidiacono, C., Anguzza, U., Cascone, G. 2015. The automatic detection of dairy cow feeding and standing behaviours in free-stall barns by a computer vision-based system. *Biosystems Engineering* **133**, 46-55.
- Romanini, C.E.B., Viazzi, S., Borgonovo, F., Costa, A., Guarino, M., Leroy, T., Berckmans, D. 2012. Farm animals monitoring tool based on image processing technique. In: *Proc. 1st International Workshop on Veterinary Biosignals and Biodevices, VBB 2012, in Conjunction with BIOSTEC 2012* Vilamoura, Algarve, Portugal, 60-68.
- Shao, B., Xin, H. 2008. A real-time computer vision assessment and control of thermal comfort for group-housed pigs. *Computers and Electronics in Agriculture* **62**, 15-21.
- Silva, M., Exadaktylos, V., Ferrari, S., Guarino, M., Aerts, J.M., Berckmans, D. 2009. The influence of respiratory disease on the energy envelope dynamics of pig cough sounds. *Computers and Electronics in Agriculture* **69**, 80-85.
- Sohn, J.H., Hudson, N., Gallagher, E., Dunlop, M., Zeller, L., Atzeni, M. 2008. Implementation of an electronic nose for continuous odour monitoring in a poultry shed. *Sensors and Actuators B: Chemical* **133**, 60-69.
- Tullo, E., Fontana, I., Gottardo, D., Sloth, K., Guarino, M. 2016. Technical note: Validation of a commercial system for the continuous and automated monitoring of dairy cow activity. *Journal of Dairy Science* **99**, 7489-7494.
- Tullo, E., Fontana, I., Guarino, M. 2013. Precision livestock farming: An overview of image and sound labelling. In: *Proc. 6th European Conference on Precision Livestock Farming, ECPLF 2013* Leuven, Belgium, 30-38.
- Vandermeulen, J., Bahr, C., Johnston, D., Earley, B., Tullo, E., Fontana, I., Guarino, M., Exadaktylos, V., Berckmans, D. 2016. Early recognition of bovine respiratory disease in calves using automated continuous monitoring of cough sounds. *Computers and Electronics in Agriculture* **129**, 15-26.

Vandermeulen, J., Bahr, C., Tullo, E., Fontana, I., Ott, S., Kashiha, M., Guarino, M., Moons, C.P.H., Tuytens, F.A.M., Niewold, T.A., Berckmans, D. 2015. Discerning pig screams in production environments. *PloS one* **10**.

Wathes, C.M. 2010. The prospects for precision livestock farming. *Journal of the Royal Agricultural Society of England* **171**, 26-32.

Williams, R. 1999. A compartmentalised model for the estimation of the cost of coccidiosis to the world's chicken production industry. *International journal for parasitology* **29**, 1209-1229.