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Quality control of pollen identification and quantification exercise for the AusPollen Aerobiology Collaboration Network: a pilot study

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Abstract

Pollen monitoring in Australia has rapidly expanded, particularly in response to needs identified following the world's most devastating thunderstorm asthma epidemic in Melbourne, 2016. Pollen identification and quantification are integral processes of establishing a standardised pollen monitoring network. A pilot study was designed to assess proficiency in these processes of counters who contribute to daily pollen information at established pollen monitoring sites of the AusPollen Aerobiology Collaboration Network. Counters were instructed to count grass and other pollen along four longitudinal transects of two reference slides at lens magnification of 400×. Participants were asked about their experience, training and usual practice in pollen counting via an online questionnaire. Of the 44 counters invited, 15 consented to participate. Reported pollen concentration values were compared to an approximation of the assigned true concentration values. Overall, 86% of reported values were within acceptable ranges of variation from assigned values. Apparent courting proficiency could have been affected by study limitations including slide quality and high/low pollen concentrations. Although counting performance did not appear to be related to experience and amount of training received, the majority of participants were not highly experienced or trained and the number of participants was small. It was not possible in this pilot study to make conclusions regarding relationships between training or experience and counter proficiency. Evaluation of counter proficiency is an important step in providing more accurate pollen concentrations, which are integral to local daily pollen forecasts for optimum day-to-day management of pollen-related conditions.

Keywords

Quality control
Pollen, grass pollen
Standardisation
Aerobiology

1. Background

Since the granting of the NHMRC AusPollen Partnership in 2016, the pollen monitoring network in Australia has rapidly expanded.

This expansion further escalated following the devastating asthma epidemic in Melbourne, 2016 (Thien et al. 2018; Inspector-General for Emergency Management 2017). Currently, the AusPollen Aerobiology Collaboration Network consists of 22 active pollen monitoring sites (http://www.neii.gov.au/nemsr) including the founding sites of the AusPollen Partnership (https://www.pollenforecast.com.au/), the AirRater Program (https://airrater.org/) and the Victorian Thunderstorm Asthma Pollen Surveillance Project (https://www2.health.vic.gov.au/public-health/environmental-health/climate-weather-and-public-health/thunderstorm-asthma/forecasting-faqs).

The AusPollen Partnership embarked on processes for standardising pollen monitoring in Australia (Medek et al. 2016; Beggs et al. 2015; Davies et al. 2015; Haberle et al. 2014). The first national Australian Airborne Pollen and Spore Monitoring Network Interim Standard and Protocols document (Interim Standard and Protocols) that outlines minimum requirements and recommended best practice for all pollen monitoring processes was published as a health professional paper (Beggs et al. 2018) and adopted and applied by the existing Australian pollen monitoring sites since 2017. Additionally, newly established sites have been audited against a checklist of ten aspects including pollen counting specified in the Interim Standard and Protocols.

A key aim in the progress towards national standardised pollen monitoring was to evaluate the counting performance of pollen counters (counters) who contribute daily pollen data. This will ensure the reliability of pollen forecasts that are used directly by members of the community, clinicians and government agencies to improve the health and quality of life of individuals with allergic respiratory diseases.

2. Methods

The pilot quality control (QC) of pollen identification and quantification exercise (pilot study) was conducted from May to July 2018. All 44 counters from the AusPollen Aerobiology Collaboration Network were invited to participate. The approach was modelled on the QC of counting processes of the well-established European and Spanish Aerobiology Networks (Galán et al. 2014; Oteros et al. 2013).

Two reference slides containing different levels of grass pollen (GP) and other pollen (OP) commonly present in the Australian environment were circulated to participating sites. Counters were provided with detailed instructions based on the minimum requirements and recommended best practice in pollen counting articulated in the Interim Standard and Protocols. Participants were requested to count four longitudinal transects by light microscopy with lens magnification of 400× and to complete an online questionnaire about their experience, training and usual counting practice.

3. Data analysis

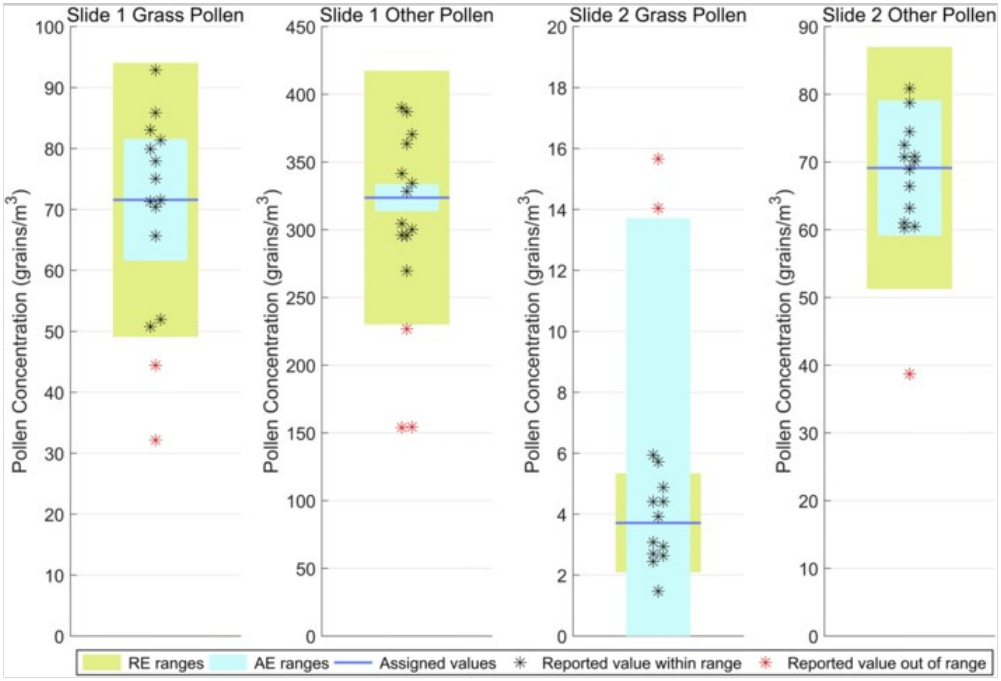
The data analysis method and approach of Galán et al. (2014) was applied. ~~Participants' reported values~~ Values reported by participants were compared with assigned values, each of which is an approximation to a true value. Assigned values were calculated as the mean values of all participant-reported concentrations of the sample population, excluding significant extreme values, so-called statistical outliers (identified using interquartile range and *z*-scores methods). It was also examined whether using the average of three experienced counters' concentrations determined by counting the entire exposed slide would be superior to using an assigned value based on counting by recommended best practice encompassing four longitudinal transects. Absolute (AE) and relative errors (RE) and acceptable ranges of AE and RE were determined for each participant using equations of Galán et al. (2014). Only when both errors occurred were reported values considered to fall outside of the acceptable range of the assigned value. Outcomes of the questionnaire regarding the experience, training and usual counting practice of counters were analysed. All information given by the counters was non-identifiable during the analysis.

4. Results

Of the 44 counters invited, 15 consented to participate. Participants were from New South Wales, Queensland, Tasmania and Victoria. All 15 participants provided GP and OP concentrations for slide 1, while 14 participants provided GP and OP concentrations for slide 2. Most of the pollen concentration values reported (50/58) were within the acceptable range of variation from the assigned values based on the RE and AE (Fig. 1). Overall eight values were outside of acceptable ranges of variation including two of 15 values on slide 1 and two of 14 values for slide 2 for GP. Four counters reported one value (out of four) and two counters reported two values (out of four) that were determined to be unacceptable. Recommendations for additional training were made to these counters.

Fig. 1

Pollen counting proficiency results of participating pollen counters in Australia. *RE* relative error, *AE* absolute error



The majority of the participants (80%) started pollen monitoring after 2015, mostly having one season of experience (Fig. 2). Most (80%) had attended face-to-face training with 60% indicating they received small group training, while just 33% had taken part in a workshop. Most participants (87%) received training one or two times. One person normally uses latitudinal counting method, while all other participants (93%) regularly count longitudinal transects. More than a half of the examined group (60%) have a usual practice of counting two longitudinal transects. Most participants (87%) routinely count GP and pollen from other taxa, as well as ruptured pollen.

Fig. 2

Questionnaire outcomes regarding the experience and usual practice of participating counters in Australia. *LO* longitudinal, *LA* latitudinal

Experience and training	Start of pollen monitoring	
	Training	Face to face training
		Small group training
		Workshop
	Number of training sessions	
Usual practice	Counting method	
	Number of transects	
	Count grass as well as other types of pollen	
	Count ruptured pollen	

5. Discussion and conclusions

In this pilot study, nearly all reported values (50/58) were within acceptable ranges of variation from the assigned pollen concentration values. Considering the newness of the Australian pollen monitoring network, 86% counting accuracy is a satisfactory initial outcome. For reported GP values, 13% for slide 1 and 14% for slide 2 were outside acceptable values, which is within the error range observed by Galán et al. (2014) who reported significant errors for GP values of between none and 18% of 10–34 participants. The eight of 58 values observed outside of acceptable concentration ranges should be considered in the light of limitations of this study. Almost half of

the counters commented on poor visibility of reference slides which may have hampered identification and quantification of pollen. However, just two out of six participants who reported unsatisfactorily values were amongst those who questioned the slide quality. By chance, slide 1 had a high OP (324 grains/m^3) and counting took longer than anticipated, while slide 2 had a low GP (4 grains/m^3), which may also have influenced the counting accuracy.

It appeared that using the average of three experienced counters' pollen concentrations determined by counting the entire exposed slide was not markedly superior to using the assigned value.~~, as some counts varied between experienced counters by more than 10%~~. However, coefficients of variation for reference values based on the whole slide counting method were less than the coefficients of variation for the assigned values, expect for grass pollen on slide two which had a very low concentration. If ~~experienced~~ reference counters follow the same method for counting as required from participants (four longitudinal transects) rather than the whole slide, the resultant value should be closer approximations to an assigned true values than reference values based on counting the whole slide surface. In addition, it is suggested here that experienced counters could perform three counting cycles over three different days in order to yield better reference values.

Most participants were not highly experienced or trained, having only started since 2015. It appeared in this first small cohort of relatively inexperienced participants that duration of experience in pollen counting and amount of training were not associated with the counter accuracy. However, conclusions regarding the influence of experience and training on accuracy cannot be made at this stage due to the small participant's number. There is a possibility for improvement by applying the recommended best practice of counting a minimum of three, or the highly recommended four transects instead of two, and by providing additional training to counters.

Quality systems are essential for consistency when multiple individuals generate aerobiology data as inputs to daily pollen forecasts. End-user and stakeholder confidence in the accuracy of pollen data is critical to continued inter-sector engagement with agencies that provide weather, air quality and public health services. Standardisation of pollen counting processes, including monitoring of counter proficiency, is also necessary for research purposes, such as research on pollen forecasting and health impacts. Into the future, high-quality pollen records will continue to remain necessary reference datasets ~~with the~~ in the context of development and adoption of new automated pollen monitoring approaches (Oteros et al. 2015; Crouzy et al. 2016). We suggest that regular evaluation of counter proficiency should become an integral part of internal quality assurance and improvement processes for all pollen monitoring networks, but to date there is little published on this topic. This study contributes one of the few reports internationally on QC of counter proficiency and is the first from the newly established AusPollen Aerobiology Collaboration Network.

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Compliance with ethical standards

Conflict of interest JMD declares that QUT owns patents and patent applications (AU2008/316301; US PTO 14/311944; PCT/AU2015/050348; PCT/AU2014/000630/WO2014_201499) for which she is a named inventor. JMD leads the NHMRC AusPollen Partnership Project (GNT 1116107) with matching cash and in-kind co-sponsorship from The Australasian Society for Clinical Immunology and Allergy, Asthma Australia, Bureau of Meteorology (BOM), Commonwealth Scientific and Industrial Research Organisation, Stallergenes Australia and ~~Meteorology~~ Federal Office of Meteorology and Climatology MeteoSwiss, Switzerland. ~~She~~ JMD is an investigator of the BOM's Victorian Thunderstorm Asthma Pollen Surveillance Project and has received grants from the NHMRC, Australian Research Council (DP170101630; DP190100376), National Foundation for Medical Research Innovation, the Allergy and Immunology Foundation of Australasia, Asthma Australia, Queensland University of Technology and contracted research grant from Stallergenes (France), in-kind provision of materials from ThermoFisher (Sweden) and services from Sullivan Nicolaides Pathology (QLD, Australia). JMD's institute has received Honorarium payments and travel expenses for education sessions and conference presentations from Stallergenes Australia, GlaxoSmithKlien, Wymedical, and Meda Pharmaceuticals. ~~Associate Professor Paul J. Beggs (~~PJB~~)~~ received research grants from Australian Research Council Project (DP170101630) and NHMRC Project (APP1116107) and has been consulted/contacted by BOM (2017-2020) and Office of Environment and Heritage, NSW (2018-2019). Other authors declare that they have no conflict of interest.

Ethical approval All procedures performed in this study involving human participants were in accordance with the ethical standards of QUT and the Australian Guidelines for Responsible Conduct of Research including the 1964 Helsinki declaration. The study was approved by the QUT Human Research Ethics Committee (1800000161).

Informed consent Informed consent was obtained from all study participants.

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