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The use of systematic reviews for decision making in animal production medicine and policy

by

Christa Kalberer Irwin

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Veterinary Preventive Medicine

Program of Study Committee:
Annette M O'Connor, Major Professor
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Iowa State University

Ames, Iowa

2010

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Dedication

This thesis is dedicated first to my children, who have continually "rolled with the flow" without complaint, readily taking on arduous responsibilities and developing life skills to be proud of, supported me with their endearing comfort and empathy, livened me with their tireless energy and childish antics, and for whom I endeavor to challenge to hone their talents and skills and seek fulfilling opportunities. I hope I have provided them the best example of dedication, perseverance, life-long learning and pursuit of success.

In addition, my family and close friends deserve my gratitude for their tireless support during these years. Their interest and enthusiasm for my undertakings and achievements have made us all better educators, as well, better educated.

Finally, I wish to thank those who have mentored me through this thesis work – I have learned far more than books can provide. With this more thorough and skillful toolset, I feel confident to approach challenges more holistically and with structure, forethought and design ideas, to incorporate the epidemiologic methods I have learned to the problems I encounter.

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Table of Contents

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INTRODUCTION: THESIS ORGANIZATION

This thesis consists of four chapters. The first chapter briefly familiarizes the reader with the systematic review methodology and describes possible approaches to incorporating the key systematic review concepts into reviews of laboratory-based microbial research. In this regard, Chapter 1 is an introduction to the conduct of reviews, their application in other disciplines and the possible application of the systematic review methodology to microbial sciences. Chapter 2 is an empirical application of the systematic review methodology to microbial sciences using persistence of influenza virus as an example. The review in Chapter 2 identified significant gaps in research design and poor reporting, therefore Chapter 3 is an example of one of the sequelae to the systematic review methodology. Chapter 3 is a publication about the approaches used to study the topic areas and potential recommendations for changes that may improve applicability. Finally, Chapter 4 concludes the thesis, and discusses the important differences, and unique challenges, between the current well-developed systematic review methodology for interventions and the reality of research synthesis in microbial sciences.

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CHAPTER 1. THE USE OF SYSTEMATIC REVIEW METHODOLOGY IN AGRI-FOOD SCIENCE: DISCUSSION ON HOW ASPECTS OF SYSTEMATIC REVIEWS APPLY TO MICROBIOLOGY AS WELL AS POLICY

Introduction

Systematic reviews are a method of synthesizing research from multiple information sources. Systematic reviews are currently employed extensively in human health intervention research. The purpose of systematic reviews is to distill the findings of a large body of research into a useful, more manageable form for application using a systematic and transparent approach (36). In this respect, systematic reviews are invaluable in policy and decision-making arenas, including clinicians, researchers and government agencies, particularly in human medicine (21,36). Systematic reviews also have a critical role in documenting areas where scientific findings are consistent or areas where a great deal of uncertainty remains. In this role systematic reviews can guide funding decisions for future research and reduce unnecessary duplication of research. Although originally developed for intervention studies, systematic reviews have been applied to other research types such as diagnostic test evaluation and causation, however the methodologies are not as well developed. The disciplines that have incorporated the systematic review methodology also extend beyond human health management to criminology, education and ecology (9,18-20,44,68).

Despite over 20 years of use in human health intervention decision making, the systematic review methodology is relatively new in veterinary science. In the past decade systematic reviews have become more visible in food safety and animal health (2,12,39,39,48,52,53). Recently, the European Union Food Safety authority has incorporated the systematic review methodology into risk assessments (15). Still, in the United States it is rare for policy or decision-making bodies to request or incorporate systematic reviews when regarding animal health, zoonoses, or food safety.

Perhaps not surprisingly the application of systematic review methodology is absent from the

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laboratory-based microbial sciences. However, considering the importance of laboratory-based microbial sciences to policy-making in animal health, veterinary related public health and food safety it seems likely that the application of the systematic review methodology will also increase. Compared to the major human health issues which are usually chronic and non infectious in nature, e.g. diabetes, obesity and cancer, microbial organisms are dominant in animal health, veterinary related public health and food safety. Appreciating this fact, coupled with the unique and routine capability to directly infect targets (animals or food) with the organism of interest, it is reasonable to assume microbiologic research will always play an integral and directly relevant role in policy-making in and among these fields. Examples of the contribution of microbial research to these areas include work on understanding pathogenic mechanisms and the ecology of nationally and internationally important organisms such as avian influenza, swine influenza, Salmonella, *E. coli*, and PRRSV. Primary research about these organisms frequently informs microbial risk assessments and subsequent trade implications. Based on the importance of this area of microbial information to microbial risk assessments, it is easy to anticipate that key features associated with systematic reviews, in particular transparency and evidentiary value, will eventually be requested for microbial data that informs risk assessments. In this regard, the systematic review methodology, potential gaps in knowledge identified by reviews, and increased transparency in the translation of primary research would benefit microbiological scientists and policy makers utilizing their research findings.

What is a systematic review?

As mentioned, systematic reviews are one of several methods employed to synthesize research findings from multiple studies. The most commonly used approach to research synthesis in the bench sciences appears to be narrative reviews. Systematic and narrative reviews differ in their design and outcome expectations and are therefore fundamentally distinct. A systematic review is a scientific study itself. There are many discussions regarding these difference (8,8,34-36,52), but the main areas in which a systematic review qualifies as a scientific study are regarding the presence of a “methods” section, a focused question, an explicit systematic approach to evaluate the published material under review (a

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design protocol), a detailed description of quality criteria used to review the literature, and a synthesis of the data extracted during the review, in the form of a meta-analysis or a summary of the gaps in information identified during the analysis.

Although descriptions of systematic reviews are reported in numerous other publications (3,21,26,30,32,36,43,47,52), a brief synopsis follows for completeness. A systematic review must clearly state a specific, well-defined question to be answered, which includes key elements. For intervention or risk factor reviews the key components are the population, intervention, and outcome of interest. Reviews of different question types may have different key components. Next, an exhaustive literature search, inclusive of electronic and hand searching, is performed. Content experts are used to ensure completeness of the search terms. The purpose of the comprehensive search is to minimize selection bias of publications included in the review. The bias that occurs in a systematic review due to a narrow literature search is referred to as retrieval bias i.e., papers easily retrieved are incorporated, and publications with positive results often find their way into easily retrieved journals (28). Comprehensive searches are also performed to minimize citation bias, caused by preferential selection of citations due to familiarity or significant results (56). Another step to reduce bias at the outset of the review occurs at de-duplication, when citations from different databases are combined. It is common for a systematic review to have criteria to restrict inclusions of apparently redundant studies (identical author(s), trial(s), outcome(s)) to a single, most current or complete manuscript, when multiple, seemingly duplicated citations are found. This is in effort to remove multiple publication bias due to redundancies in reporting of highly publicized and presented studies (56).

Following identification of the literature, the citations are formally screened for relevance to the review question. This screening is a rapid process, based on the title and abstract of the citation. Citations excluded at the relevance screening stage are not assessed further. After relevance screening, primary research that is relevant is formally assessed using quality criteria. Content experts are also used to develop or outline the quality criteria detailed in the review, against which the identified relevant literature is judged. The criteria are directly related to the purpose of the review. From the quality studies, relevant data is then extracted

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using a systematic, not selective, method and a meta-analysis may be performed to synthesize the body of information into a reportable outcome. At any point in the assessment, studies are removed or excluded if established criteria are not met. Finally, the review seeks publication, using a scientifically based, transparent format, inclusive of a discussion not only on the results of the analysis, but of what is lacking in the available evidence. Systematic reviews must always address potential sources of bias that may have been involved in the review or review process, so readers can evaluate not only the value of the information to their field of interest, but also the validity of the systematic review itself.

Illustration of differences between narrative and systematic reviews

The difference between narrative and systematic reviews is easily illustrated by comparing the highly publicized review by the PEW Trust commissioned report regarding industrial farm animal production (46), and the systematic methodology inherent in the systematic review of the association of community health and proximity to animal feeding operations (38). Due to the structured, transparent and criterion based methodology of the systematic review, the interpretation of the literature and outcomes of these reviews are vastly different. In the forward of the PEW report, it was noted that input from stakeholders and citizens were incorporated, the Commission used their expertise and experience to create objective conclusions, and the Commission had access to the most current information and expertise in the fields of concern. Deans or professors from various respected School of Public Health comprised a third of the commission (5/15), and the commission also contained two independent cattle ranchers. Other types of farming enterprises and livestock production veterinary representation were notably absent.

The Commissioned report sought to be an evidence based review, of material submitted by a wide range of stakeholders, interested parties, hearings etc. However, it was difficult to assess the extent to which bias was introduced into the conclusion of the review as no criteria were described stating the screening and qualifying standards by which the literature provided to the Commission would be reviewed. The lack of this information made it easier for groups who disliked the review conclusions to dismiss the process as biased and lacking

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transparency.

For example, it was easy to infer that information bias was likely present due to the design of the report, since the information used was not limited to primary research, and selection bias was present as well, as all literature offered and identified as relevant to the topic was included if deemed useful by the commission, however the commission did not specify what was useful. Because no quality assessment was performed, and because all literature, not just scientific literature, was used to create this narration, it would be imprudent to construct a synthesis, inclusive of all information, of the overall benefits or detriments of industrial farm animal production and offer recommendations for federal actions, which was the prescribed intent of the document. The PEW commission report is a narrative review. It included a history of animal production, and cited numerous references equally (including HSUS, the Des Moines Register and numerous conference proceedings), without restriction, quality assessment, or admission of biases. In no case, did a systematic assessment of the quality of study design, reporting or validity of the data of citations enter the equation for weighting the information used to inform the conclusion of the review.

This is juxtaposed by O'Connor et al. (38), where the material and methods described the relevance and qualifying criteria for inclusion into the CAFO review, the validity assessment imposed on the qualifying manuscripts, and the assessment for bias or confounding within those qualifying studies. Such an approach enables readers to readily assess the potential for bias in the review conclusions. The systematic review collected 4908 citations, found only 28 potentially relevant studies, and of those only 9 qualified as relevant. Of the 9, two studies did not account for potential confounding, several studies assessed multiple comparisons, which is a concern for small studies or where adjustments are not included, and the potential for selection bias among qualified studies, due to the lack of random selection of subjects, was identified. Ultimately, data from 5 relevant, qualifying and valid studies were extracted and the synthesis concluded not only that there was inconsistency in the evidence of association, but there was also inconsistency in the dose-response relationship between exposure to a CAFO and respiratory disease.

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The PEW Commissioned report clearly showed a lack of the critical review process which should be a requirement for documents which assist decision makers or are used within policy discussion settings. Systematically reviewed literature, because it is knowingly performed in a transparent, reproducible and methodologic manner, is more applicable to and arguable for use in policy decision making. It is disappointing that, due to the notoriety of the PEW Trust, this report was well received and is currently used to instruct policy, but it demonstrates the obvious and pressing need for more systematic reviews in the public sector as well as a request for more systematic reviews by governing officials.

How have systematic reviews been applied for decision making?

Use in the human and veterinary medical fields

Systematic reviews have most frequently focused on how suitable and efficacious interventions are in specific circumstances. Interventions reviewed by systematic reviews in human medicine are often related to drug or other medical therapy (6). Originally these reviews have assessed randomized controlled trials and provide stronger evidence for decision making (as opposed to opinion or anecdote), for clinicians. In addition, Evidence Based Medicine has gained ground in the medical field, and this model of medical practice utilizes much of the critical appraisal tools systematic reviews provide, particularly adopting the systematic methodology and comprehensive approach to understanding the current status of science. Because of this, systematic reviews are being increasingly valued and utilized in the medical profession (1,3,23,25,30).

Food animal medicine systematic reviews have included lameness in cattle (22), meta-analyses of beef cattle production (66), and public health and food safety topics (2,11,16,24,67,69). In the food safety or antibiotic use in livestock arena, the value of a systematic review is quite clear, as there is a significant amount of information, of varying qualities, which must be acknowledged but critiqued, to attain as high a quality and thorough a review as possible, concisely and transparent of biases, because of the public nature of the topic.

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Detailed example of the use of systematic reviews to policy-making in human medicine

Recently, the US Preventive Services Task Force, after performing a systematic review on 5 screening modalities for reducing mortality due to breast cancer, overturned current dogma on the utility and true efficacy of breast cancer screening methods for women under 50 years of age (61). The comprehensive and systematically reviewed literature focused on the benefits versus the harms of each modality of breast cancer screening. The Task Force concluded there was insufficient evidence to recommend (and therefore recommended against) routine screening mammography for breast cancer in women under 50 yrs of age. In addition, it stated there was insufficient evidence to assess the benefit of mammography screening in women over 75 years of age. The Task Force also acknowledged there was insufficient evidence to recommend alternatives to film mammography screening using other diagnostic tools such as digital mammography or MRI. The Task Force did recommend that women between 50-74 years of age should have biennial mammography screening. The directive for the Task Force was to review the evidence and determine the overall benefits and harm of screening for breast cancer, in order to summarize the reproducible science regarding breast cancer screening techniques for policy and health care decision makers. Certainly the Task Force knew the information would not be well received, as it challenged public perception and currently accepted practices, but the group was charged with putting data behind a previous recommendation, and the previous policy was found to lack defensible evidence. This is one of the benefits provided by a systematic review. In the medical field and regarding public policies on human health, systematic reviews provide officials better information about the scientific information available, using a transparent method of literature assessment, to enable more informed decision-making.

Detailed example of the use of systematic reviews to policy-making in veterinary medicine

More focused examples of the use of systematic reviews and their application to food animal veterinary medicine or policies that impact veterinary medicine include the report of the Joint Expert Advisory Committee on Antibiotic Resistance (JETACAR) in Australia, and the assessment of a recombinant bovine somatotropin (rBST) license application for Canada. The JETACAR report used systematic reviews to evaluate the evidence of antibiotic resistance in

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specific food-borne pathogens originating from food animals. Subsequent to the reviews, the committee performed a qualitative risk assessment, assessing the likelihood of the passage of resistance through animal food to humans (identifying and classifying the hazard, assessing the exposure and characterizing the risk). Finally the committee summarized their findings with a proposal for a resistance management program and recommendations regarding regulations, surveillance, reporting, education and research (24). This report was provided to governing officials and resultant surveillance programs were developed and implemented (65). Because of the importance of the topic to stakeholders and the potential for criticism, the comprehensive and transparent nature of the systematic review component of the JETACAR process was seen as advantageous by policy makers.

In a similar fashion, Health Canada approached the Canadian Veterinary Medical Association (CVMA) and asked that an expert panel be formed to assess the primary literature on rBST, for product efficacy and adverse animal health affects before licensing in Canada. The panel convened and performed a systematic review on all available literature, finding 1777 citations on the topic and screening for studies investigating only lactating dairy cows, published in peer reviewed journals and written in English (14). Of the eighty-six relevant studies, 53 qualified to be used in a meta-analysis. The resultant meta-analysis was not favorable to longevity of dairy cows, although milk output was increased by 11-15% in multiparous cows: body condition was adversely affected as well as lameness (55% increased risk); there was increased risk for mastitis (25%) and failure to conceive (40%) and an apparent increased risk for culling in treated multiparous cows as well (13,14). Because of these meta-analyses on the treatment effects on dairy cows, Health Canada did not grant a license for the use of rBST in Canadian dairy production. Health Canada also approached the Royal College of Physicians and Surgeons requesting a similar review of the human safety aspects of rBST. The Royal College of Physicians and Surgeons found the product safe for human exposure.

The Value of "failed" systematic reviews: Identification of gaps

As discussed the primary purpose of systematic reviews is to combine data and inform

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policy. However it does occur that a systematic review finds insufficient data to inform policy. When a systematic review is unable to reach a summary effect estimate on the original question, it is said to have "failed". In these circumstances, systematic reviews play the important (though often unpopular) role of identifying gaps in reporting, research, or study execution (37). Through this identification of insufficient or inadequate information, the inability to reach a conclusion or the degree of uncertainty identified by the review is also useful for policy makers as well as clinicians (45).

When systematic reviews were incipient to human medicine, it was readily apparent that some systematic reviews "failed" because of lack of research, and this lead to increased funding in those areas. However, also importantly, it was clear that some systematic reviews "failed" because the information from primary studies were executed and/or presented in a manner that made interpretation difficult or impossible (4). Similar issues have been found in veterinary science. The evidence shows a considerable amount of heterogeneity in parameters investigated between studies, particularly in the livestock and food-safety realm, as well, significant room for improvement in reporting and execution of study design (10,17,39,42,48-51,64,67,69). These findings have led to an entirely new endeavor that is separate but strongly associated with systematic reviews i.e., the development of reporting guidelines.

Reporting guidelines list expected parameters that should be included in the report to enable readers to assess the internal and external validity of a study. Reporting guidelines are available for many study designs (59). These guidelines aim to ensure that the information needed to accurately assess studies has been detailed. The REFLECT statement is the first reporting guidelines for animal studies (40). The rationale for quality reporting is the generally accepted concept that if the details of study design are not transparent or reproducible, the quality of the resultant data cannot be properly assessed for sources of bias. What started as a collaboration to establish a set of guidelines to improve and provide a systematic approach to reporting randomized clinical control trials in human medicine (CONSORT statement) (33), has evolved into a systematic approach for reporting in many human medical fields. The efficacy and relevance of the Cochrane Collaboration

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methodology has been quickly understood and adopted for observational studies (62,63), diagnostic test evaluation (7), outbreak reporting (57), qualitative research (60) and meta-analyses (31,58). The process of writing a systematic review has a similar "code of conduct", and is well described in the PRISMA statement (29,32).

"Failed" systematic reviews have also served the function of providing empirical evidence for sources of heterogeneity in study results, including the identification of design features as a source of this bias in primary research. For example, systematic reviews of interventions have found an association to be present between treatment effect and whether a study was randomized or blinded, identifying the observation of a stronger effect when treatment groups were not randomized or blinded (10,27,54,55).

How might systematic reviews be applied to laboratory microbial sciences?

In the microbial sciences, the types of questions that might be answered using the systematic review methodology are broad. Systematic reviews in small animal medicine or surgical interventions are common (5,41), and similarly there are numerous microbial or antimicrobial intervention studies available in the literature which could be reviewed systematically, e.g. "what is the effect of compound "x" on expression of gene(s) "y"?" It is also common to question microbial population characteristics such as survival, mutation and transmission. A systematic review methodology could be employed to combine data from multiple studies to provide a summation of the effect (23). Similarly, studies on the prevalence, presence or level of a factor could be combined over multiple studies, to obtain a better estimate than that obtained from one study alone or a narrative review. For example, multiple studies conducted on the effect of refrigeration of survival of bacteria in food matrixes could be synthesized in meta-analyses. The pooled estimate would be a quantitative value and would have a reduced confidence interval or more realistic estimates of variation than can be obtained from a single study. Such information could then be incorporated into a risk assessment. Further because the estimate was obtained using a process that can itself be assessed for bias, the rationale for the estimate is more defensible for public policy makers.

The following chapters of this thesis manuscript provide an example of the application of

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these concepts to microbial primary research. As with many forms of critical evaluation, there are critiques of systematic reviews and the use of the systematic review process, and a few key concerns will be discussed in Chapter 4. However, a common misguided opinion should be clarified, in that the systematic methodology does not stifle independent or creative thought in study design, rather, it recommends whatever design and/or analysis is used should be reported transparently. In addition, systematic reviews are arduous, time-consuming and can be costly. They are efficacious when used in topics of contention, but should otherwise be limited to purposes where there is a substantive need for a critical assessment and statistical summary of the literature on a given topic. Narrative reviews are useful as historical perspectives and for reporting progression of knowledge in given fields of study, and they are definitely valuable in these regards. However, as this thesis intends to show, when decisions requiring evidence need to be made, particularly regarding policy, it is paramount that the evidence be valid, repeatable and defensible. If, because of poor reporting or gaps of knowledge the information is unattainable, as in the case described here, the microbiologic community should convene and review how to provide evidence which will sustain a systematic review in the near future. Microbiologists should understand that direct application of laboratory science to the field has become routine. In this way, better, more rigorous scientific reporting and assessment is necessary, to ensure beneficial contributions to both animal and public health.

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CHAPTER 2. USING THE SYSTEMATIC REVIEW METHODOLOGY TO EVALUATE FACTORS THAT INFLUENCE THE PERSISTENCE OF INFLUENZA IN ENVRIONMENTAL MATRICES

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Abstract

Understanding factors that influence the persistence of influenza in an environment without host animals is critical to decision making for down times, set back distances and eradication programs in livestock production systems. This systematic review identified literature describing persistence of influenza in environmental samples i.e. air, water, soil, feces and fomites. An electronic search of PUBMED, CAB, AGRICOLA, Biosis and Compendex was performed and citation relevance was determined according to the aim of the review. Quality assessment of relevant studies was performed using criteria from experts in virology, disease ecology and environmental science. 9760 abstracts were evaluated, 40 appeared to report the persistence of influenza in environmental samples. Evaluation of full texts revealed 19 of the 40 studies were suitable for review as they described virus concentration measured at multiple sampling times with virus detectable at least twice. Within the 19 studies, there was significant heterogeneity in study design, quantification methods and outcome reporting. Seven studies reported survival in air (six published before 1970), seven in water (five published after 1990), two in feces and three on surfaces. All three fomite and five air studies addressed human influenza, and all water and feces studies pertained to avian influenza. Outcome measurements were transformed to half-lives, and resultant multivariate mixed linear regression models identified influenza surviving longest in water, compared with air, feces and fomites. Temperature was a statistically significant predictor of persistence over all matrices as well as air matrix specifically. Salinity and pH were significant predictors in water conditions.

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Introduction

The aim of this review was to summarize the findings from experiments that report persistence of influenza in the environment. The motivation for the review was to provide better science-based information to inform policies that will impact livestock producers and surrounding communities. The period of time that influenza viruses persist in environmental matrices (e.g., air, soil, feces, water, fomites) and factors that affect that period should inform many decisions in regulatory livestock disease control. Avian and equine influenza are World Organisation for Animal Health (OIE) notifiable diseases and OIE strongly advises all its members to notify the disease linked with the now called "pandemic H1N1 2009" virus to the OIE when detected in animals. For avian influenza control measures include quarantine and depopulation, while for the pandemic H1N1 2009 and quarantine may be imposed by the member nation. During outbreaks of highly pathogenic avian influenza (HPAI) in the US, infected premises are depopulated and a period of quarantine imposed before new animals can be introduced (74). Further, legislative initiatives have requested consideration of the distance pathogens associated with animal health , including avian and swine influenza virus, may travel between infected facilities when establishing guidelines for the granting permits for new livestock production facilities, otherwise referred to as set-back distances (26). The period of time influenza virus can be reasonably expected to persist in environmental matrices without amplifying hosts should form the basis for these depopulation times and set back distances. Given the growing importance of influenza viruses and the need for science informed public policy, the purpose of this review was to summarize the literature reporting the persistence of influenza virus in environmental matrixes to better inform these regulatory decisions. The objective of this review was therefore to use the systematic review methodology to answer the question, "What is the evidence for an association between humidity, temperature, UV intensity and media composition and the persistence of influenza virus in air, soil, feces, water and on fomites?"

Materials and Methods

The approach to reporting the systematic review follows the guidelines for reporting systematic reviews called the PRISMA statement (40) with modifications where needed as

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the PRISMA statement refers mainly to intervention studies rather than bench science applications.

Definitions

Study: A manuscript reporting primary research.

Experiment: A research trial described within a single study.

Observation: A single persistence measurement derived from a complete set of persistence data over time, within an experiment. This individual persistence data per time interval (and parameter) or summary outcome for an experiment was the extracted information for this meta-analysis. The raw data was in varying formats including virus concentration per time interval, $\log_{10}$ transformed virus concentration per time interval, the slope of the persistence line, percent recovery from starting concentration post-equilibration, and actual half-live calculations.

Systematic review methodology: The systematic review methodology is a formalized approach to conducting a critical review of the literature and has been applied to the policy making process in clinical sciences, social sciences, food safety regulation and environmental sciences (7,24,52,61,68,81). The methodology has several key principles designed to limit the incorporation of biased scientific results or the selective use of particular scientific results into review conclusions: transparency, comprehensiveness, and quality assessment. Transparency refers to the reporting of all aspects of the review to enable the reader to assess the validity of the review process and potential biases. Comprehensiveness refers to a broad approach to identifying the literature to be considered for the review. Quality assessment refers to the evaluation of the primary research for the presence of study design features necessary for valid primary research. Studies failing to report key features are not included in the summation of findings. A consequence of this approach is that well executed but poorly reported studies cannot be differentiated from poorly executed but accurately reported studies. Systematic reviews have four formalized steps: 1) literature search, 2) relevance screening, 3) quality assessment and data extraction, and 4) data analysis and summation. In clinical sciences, some systematic reviews are registered, and have published protocols; this

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review did use a working protocol but it was not registered as there is no mechanism for registering reviews outside the clinical sciences.

Literature search

An electronic literature search in PUBMED [1948 to present], CAB [1910 to present], AGRICOLA [1970 to present], Biosis [1926 to present] and Compendex [1884 to present] was conducted. Terms that described influenza virus and persistence in environmental matrices were identified in the National Agricultural Thesaurus and the PUBMED MESH database after consulting review papers (2,6,62,78). The searches were designed to capture the population of interest i.e., influenza virus, the outcome of interest, i.e., persistence, and the environmental matrices. Boolean terms were used to combine terms within a string (OR) and between strings (AND) (Appendix A1, Appendix A2, Appendix A3, Appendix A4).

The search used in PUBMED for the water matrix was as follows (influenza OR influenzavirus OR Orthomyxoviridae OR Influenzavirus C OR influenza C OR Influenza A OR Influenzavirus A OR H1N1 OR H2n2 OR H3N2 OR H3N8 OR H2N3 OR H5N2 OR H7N7 OR H9N2 OR (Influenza in Birds) OR Influenza B OR influenzavirus B OR (Hemagglutinin Glycoproteins) OR Human influenza) AND ((virus or viral or microbial or microbe) and (pathogenicity OR survivability OR survival OR stability OR infectivity OR infection OR infective OR "infective dose" OR infect OR viability OR "environmental stability" OR inactivation OR transmission)) AND (water OR wetland* OR waterway OR watershed OR pH OR manure OR feces OR faeces or faecal shedding OR fecal shedding OR wastewater OR effluent OR irrigation OR drying OR desiccation OR desiccating OR lyophilization OR lyophilized OR water microbiology). Retrieved citations were stored in reference management software (Reference Manager v 11, Berkeley, CA). Duplicate citations were removed by electronic and hand scanning the electronic database. When multiple instances of the same citation were identified, the most complete citation was retained. After de-duplication, citations were uploaded to a web-based systematic review software for coordination of the review (SRS v4, Trial Stat, Ottawa, Ontario, Canada).

Hand searching of the reference lists of relevant papers and previously published narrative

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reviews was conducted as the review progressed i.e., after a paper or review was identified as relevant to the review. Two reviewers evaluated the reference list and identified potentially relevant citations. If the electronic search had not captured the citation, it was added to the web-based systematic review software.

Relevance screening

The purpose of relevance screening in the systematic review methodology is to rapidly remove citations not relevant to the review, as the literature search process should be highly sensitive with low specificity. Eligible studies were primary research papers that reported persistence of influenza virus in the environmental matrices.

Two levels of relevance screening were used. For level one relevance screening, each citation was reviewed independently by a primary and secondary reviewer. The primary reviewers were: a BVSc with doctoral degree in epidemiology, a BVSc with Master's degree in epidemiology, a scientist with a bachelor of science and a DVM completing a masters training in epidemiology. The secondary reviewers were DVM's, three with MS degrees and a PhD candidate. The secondary reviewers participated in a 60-minute training session about the review process and the reviews aims.

The level one relevance screening questions were:

Question 1) Is the full publication written in English? Possible responses were yes, no, and can't tell.

Question 2) What type of publication does the abstract or title describe? Possible responses were: primary research, simulation model, review, report, survey, testimonial, editorial, opinion and can't tell.

Question 3) Given the article is primary research is influenza virus the focus microbe of the abstract or title? Possible responses were yes, no, can't tell, and not applicable.

Question 4) Given the article is primary research does the abstract or title describe a project involving environmental samples, such as, but not limited to air, feces, fecal slurry,

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soil and, water? Possible responses were yes, no, can't tell, and not applicable.

Citations advanced to the 2nd relevance screening if the responses of both reviewers were: Question 1) yes or can't tell, Question 2) primary research or can't tell, Question 3) yes or can't tell, and Question 4) yes or can't tell.

The second relevance screening was conducted using the full manuscript with two independent primary reviewers (CI, AOC). The questions for the second level of relevance screening were:

Question 1) Does this manuscript pass level 1 screening questions (English, primary research, about influenza and includes environmental sampling)? Possible responses were yes or no.

Question 2) Does the manuscript provide at least 2 observations of the same virus? Possible responses were yes, no or not applicable i.e. doesn't pass level 1 screening.

Citations advanced to the next level of the review if the responses to both questions were yes from at least 1 reviewer.

Quality assessment and data extraction

The purpose of the quality assessment was to identify primary research that described the key features required in an experiment assessing virus persistence in environmental matrices. To identify these key features, content experts in virology, environmental science, and disease ecology were consulted and the purpose of the review described. The key feature identified was measurement of the virus using a quantifiable concentration assay. The rationale behind this feature was to enable determination of virus decay. Appropriate concentration assays identified were $TCID_{50}$, EID_{50}, LD_{50}, MP_{50}, PFU, and ELD_{50}. Experiments using haemagglutination assays were considered inadequate as this assay measures chicken erythrocyte haemagglutination rather than virus activity. Experiments that reported the percentage of dead animals, embryos, or the presence or absence of the virus were excluded as these assays quantitate an infection rather than the persistence of virus. Further, the content experts concluded that each experiment should describe the influenza strain, the virus

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passages prior to the experiment, the environmental matrix, the method of spiking the environmental matrix with the virus, the study duration and sampling intervals, the environmental parameters (i.e. temperature, relative humidity, salinity, pH) under which the experiment was conducted, and at least 2 sample periods where virus continued to be detected. For the manuscripts that passed the second level of relevance screening the presence of these features was evaluated by two reviewers independently (CI, AOC). Manuscripts that did not describe these features were not included in the data extraction and summation.

One reviewer (CI) was responsible for extracting data from the studies that passed quality assessment. When unclear a second reviewer was consulted. For each experiment, extracted information included the matrix (i.e. air, feces, water, and fomites) and conditions relevant to each matrix: i.e. temperature (°C), pH, salinity (ppm of NaCl). Experiments that described the temperature as room temperature were inferred to have been conducted at 22 °C. When relative humidity was reported as room air humidity, this was inferred to be <30% relative humidity. Fresh and tap water were inferred to be 0 ppm NaCl.

Virus concentration was extracted for all time points for all experiments with the exception of aerosol experiments. Based on the recommendation of a content expert, measurements of virus concentration made during the equilibration time were not included in the calculation of virus half-life for aerosolization experiments. For example, if an experiment documented a change in decay rate from sampling at or before 15 minutes, to a gradual and uniform viral concentration reduction thereafter, the results from the first 15 minutes were omitted from the calculation of virus half-life as losses due to the differences in droplet sizes and virus settling within the aerosolization chamber. If not reported in the text or tables, data were extracted from graphs when possible.

Data analysis and summation

The aim of data analysis and summation was to describe the persistence of influenza virus reported in the experiments and the association of environmental matrixes with persistence. To compare across experiments, the extracted results were converted to viral half-life, as this

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measure was independent of starting viral concentration or unit of measure.

For each experiment the predicted half-life of the virus was calculated based on the extracted data (CI). First, a least squares regression model was used to estimate the decay slope of the persistence of the virus (the decay slope, $\beta_{persistence}$) in the set conditions of the experiment as previously described (8,66,67) (Equation 1).

$$y = \alpha + \beta_{persistence}x + \varepsilon$$

(Equation 1)

where y was the concentration of virus in $\log_{10}$ of units used in the study, x was the time (days), α was the intercept, $\beta_{persistence}$ was the slope of the regression line and ε was the residual error. If the experiment had already calculated the coefficient β (the decay slope), this was used unchanged in further analyses. Using $\beta_{persistence}$ from equation 1, the half-life of the virus ($t_{1/2}$) was calculated using Equation 2. (10)

$$t_{1/2} = -log_{10}2/\beta_{persistence}$$

(Equation 2)

To describe the association between the explanatory variables and the outcome, log-transformed virus half life ($\log_{10}$ $t_{1/2}$), multivariate models were used to obtain adjusted associations for all fixed effects (Equations 3, 4, 5). The multivariate model was a linear mixed regression model (PROC MIXED, SAS v. 9.2, SAS Institute Inc. Cary, NC, USA). Additionally, a quad contrast was tested for significance to determine whether there was evidence for non-linearity in the categories of temperature, salinity and relative humidity (because pH was a binomial factor, it was not assessed in this fashion). The method of estimation for the variance components was restricted maximum likelihood with a Kenward-Rodger correction for standard errors and degrees of freedom. In all models environmental variables were included as fixed effects. To account for the nested random effect of study within matrix, as well as the between study variations of parameters, study and fixed effect interactions with study were included in each model as random effects i.e.

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study*temperature, study*relative humidity, study*water source, study*salinity, study*pH.

For all models, biologically sensible interactions between fixed effects were assessed and removed if the likelihood ratio test indicated these were not significant with $p<0.10$, or if there was insufficient data representation within levels of the main effects to make valid comparisons between the effect levels. Model assumptions were assessed by evaluating the form of residual values versus fitted values plot, a quantile-quantile plot and a histogram of the distribution of residuals. The model was determined appropriate if the mean of the residual values versus fitted values plot was centered around 0, the Q-Q plot was essentially a positive linear line, and the histogram showed normal distribution around 0.

For all fixed main effects, the null hypothesis was that the main effect was not associated with virus log $t_{1/2}$. The main effect was evaluated using the Type III sum of squares test in PROC MIXED (SAS) and if the p-value was less than 0.05 the effect was considered significant. If the main effect was significant, the Tukey-Kramer test for multiple comparisons was used to make pairwise comparisons within that fixed main effect for polychotomous variables. The group mean differences (Δ) were estimated by point estimates, and 95 percent confidence intervals and p-values adjusted by Tukey-Kramer method were reported.

Point estimates near zero indicate relative equivalence to the log $t_{1/2}$ of the referent. For all models the interpretation of the point estimate within each effect was related to the half-life ratio, where 10^{Δ} estimated the multiplicative affect of each parameter or category of an effect, compared to the referent. Values of 10^{Δ} greater than the null value one suggest the response is associated with increased $t_{1/2}$ compared to the referent, and values of 10^{Δ} less than the null value one suggest the response is associated with decreased $t_{1/2}$ compared to the referent. Inclusion of null value one in the 95% confidence interval of 10^{Δ} signified the p-value of the Tukey-Kramer test was > 0.05.

Three models were constructed. The first model evaluated virus log $t_{1/2}$ across matrices, therefore the explanatory fixed effects were matrix (4 level categorical variable = water, air, feces, fomites) and temperature ($^{\circ}$C) categorized into three levels (2 to 12°C, 17 to <27°C,

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and ≥27°C) which followed a natural grouping from the studies themselves. Temperatures were rounded to the nearest whole number for categorization. Two random effects were included in the overall model: study nested within the matrix, and an interaction term between study and temperature (Equation 3). The code for the models is included in Appendix A7.

$$y_{ijkl} = \mu + matrix_i + temperature_j + study_k(matrix_i) + study_k(matrix_i)*temperature_j + \varepsilon_{ijkl}$$

(Equation 3)

where y_{ijkl} denotes the log of virus half-life ($\log_{10} t_{1/2}$) for the lth observation of the kth study of the matrix i and temperature j, and the coefficients on the right hand side of equation denote the groups means, e.g. $matrix_i$ denotes the mean response in matrix group i.

The subsequent models were matrix specific. For the analysis evaluating virus log $t_{1/2}$ in aerosolization experiments, the explanatory fixed effects were temperature (categorized into 7 to 12°C, 17 to <27°C, and ≥27°C) and relative humidity (RH) (categorized into <30%, 30-70%, ≥70%). Two random effects were included; an interaction term between study and temperature and one between study and RH (Equation 4).

$$y_{ijkl} = \mu + temperature_i + RH_j + study_k + study_k*temperature_i + study_k*RH_j + \varepsilon_{ijkl}$$

(Equation 4)

For the analysis evaluating virus log $t_{1/2}$ in water experiments, the fixed effects were water source (3 level categorical variable, distilled, buffered or lake), temperature (categorized to 2 to 12°C, 17 to <27°C, and ≥27°C), pH (categorized as normal (pH 6 to 8) or extreme (< pH 6 or ≥ pH 9)) and salinity (categorized into 0 to 1ppm, ≥1 to 30ppm, ≥30ppm)(46). Like temperature, pH and salinity were rounded to the nearest whole number before categorization. Five random effects were included in the water model: study and the interaction between study and each main effect (i.e. study*water source, study*temperature, study*salinity, study*pH) (Equation 5).

$$y_{ijklmn} = \mu + water\ source_i + temperature_j + salinity_k + pH_l + study_m + study_m*water\ source_i$$

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$+ study_m*temperature_j + study_m*salinity_k + study_m*pH_l + \varepsilon_{ijklmn}$

(Equation 5)

Results

Literature search and relevance screening

The cutoff date for citation searching was January 25, 2008. After de-duplication by matrix, 2118, 8114, 8288 citations remained in the air, soil (includes feces and fomites), and water searches respectively. After de-duplicated, 9760 references were available for relevance screening. Four citations were identified by hand searching (Figure 1). One hundred and thirty two citations passed first relevance screening. Reasons for exclusion are included in Appendix A5. Of the 132 citations 92 were excluded at the 2nd relevance level after retrieving the articles, primarily due to lack of environmental sampling or reporting only discovery, rather than persistence of the influenza virus. Other citations were excluded as they reported virus stability in laboratory techniques (1,30,49,51), disinfection (12,16,43,50,70,82), persistence in eggs, meat or carcasses (1,3,32,37,48,58), transmission rather than persistence (34,45,69) or, only one sampling time (17,20,35,55,80).

Quality assessment and data extraction

Forty studies were identified that contained 122 experiments of which 77 were relevant and evaluated for quality assessment. Fifteen studies reported persistence of influenza in air, 15 in water, 10 in soil or feces and five on fomites (several studies included multiple matrices). Twelve studies published prior to 1970 (11,15,21,22,25,31,33,39,47,56,60,79) reported influenza persistence in air, while the remaining three were published between 1970 and 1990 (28,38,53). Five studies reporting persistence in water were published prior to 1970 (19,41,72,73,75), 3 between 1970 and 1990 (54,77,83), and seven from 1990 to January 2008 (8,29,34,66,67,69,84). Two studies reporting persistence in either feces, wastewater, soil or compost were published prior to 1970 (63,75), one between 1970 and 1990 (77) and seven were published since 1990 (13,20,35,36,57,64,80) . Influenza persistence on fomites was investigated twice prior to 1970 (14,75), once between 1970 and 1990 (3) and twice since 1990 (44,71).

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Of the 77 relevant experiments within the 40 studies, 56 did not describe the key features recommended by the content experts. Ultimately only 19 studies contained at least one experiment which included the quality criteria. The most common feature missing was a description of virus concentration at two time points. Six of the 15 aerosol studies were excluded because none of the experiments reported results in viral concentration(15,31,33,38,60,79), and two studies reported mean persistence in all experiments rather than persistence over time (22,28). Of the 15 water studies, four studies failed to report virus concentration adequately in all experiments(35,42,72,75), three studies contained experiments which reported mean persistence time at multiple pH measurements (17,54,83), several experiments reported only a final persistence time when virus was determined undetectable(20,80,83) and one reported all results as persistence over freeze-thaw cycles rather than time (19). No study with experiments reporting on virus persistence in wastewater, soil, compost or under UV light passed quality assessment (13,27,36,57,75,80).

Data analysis and evidence summation

Twenty one relevant experiments contained within 19 studies passed quality assessment review. The detailed characteristics of the 19 studies are provided in Appendix A6.

Table 1 describes the number of times it was possible to calculate the virus half-life for each combination of virus and matrix from the 21 experiments. It is notable that no reporting of variation could be performed at the observation level as none was reported in any experiment evaluated. The observations (converted to half-lives (days)) extracted from the 21 experiments of the 19 studies are depicted in Figure 2, categorized by matrix, grouped by temperature (low= 2-12°C, moderate=17-26°C and warm = $\geq$27°C) and identified by varied parameter (e.g. categories of relative humidity, or water source, salinity or pH). The majority of half-life observations (127/191) were available from experiments evaluating persistence in water. Table 2 describes the frequency of half-life observations in air, water and feces evaluated from the 21 experiments. The most common temperature evaluated in aerosol experiments (22/28 half-life observations) evaluated virus persistence at temperatures 17-<27°C. The most common humidity evaluated in aerosol experiments (13/28 half-life

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observations) was 30-70%. Most water experiments evaluated low pathogenicity viruses, in buffered, filtered water at fresh water salinity (0-1ppm), and normal pH (6-8). Twenty-eight independent observations of influenza half-life on fomites were extracted from the four relevant experiments of 3 studies. Numerous fomites were represented only in a single study, therefore a half-life table and reported conditions for each experiment are provided in Table 3 and no summary analysis was attempted for these data. Similarly the number of studies (n=2), experiments (n=4) and virus half-life observations (n=28) that evaluated feces or diluted feces matrices were limited, therefore the raw data, estimated half-life, and conditions of each experiment were reported in Table 4.

Neither standard deviations nor errors were reported at the experiment level; therefore it was not possible to assess variance at the experiment or study level, nor between studies. With this in mind, the following models were constructed based on the available summary observations relayed in each experiment. The results of the overall linear mixed model showed both main effects in the model were significant, matrix ($p<0.02$) and temperature ($p=0.034$). The pair-wise comparisons are presented in Table 5. The half-life of influenza virus was predicted to be significantly longer in water than air, however the confidence interval after Tukey's adjustment for multiple comparisons was vast ($10^{\Delta}_{\text{water vs. air}} = 27$ times longer, 95% CI: 2.22 to 336 times). Increasing temperature was associated with a shorter virus half-life, though a significant difference ($p=0.031$) was only found between low temperatures (2-12°C) and elevated temperatures ($\geq$27°C) ($10^{\Delta}_{\text{low vs. elevated}} = 11.6$ times longer, 95% CI: 1.28 to 105 times (Table 5)). No other matrix or temperature comparison was significant (Tukey-Kramer test p value > 0.05). The quad contrast for temperature did not identify significant quadratic influence to any model, nor did the quad contrast for salinity or relative humidity for the water or air models respectively. The covariance parameter estimates for the random effects, study nested within matrix, study(matrix)*temperature and the residual error were 0.17, 0.20 and 0.12 respectively. Although the study(matrix)*temperature component comprised 41% of the variance, the biological significance of this is not clear. We hypothesize it is related to the diversity of the temperature parameters investigated between the studies in that temperature was the single

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parameter measured across matrices. It is plausible that although temperature would preferably have been studied as a continuous variable, the extracted data necessitated broad categories to be used instead, possibly causing observations which otherwise would have been spread out, to be coalesced into groups.

Seven studies containing seven relevant experiments reported persistence of influenza in aerosols. Table 1 and Table 2 illustrate the diversity evaluated by the 28 observations within those seven experiments passing quality assessment. The main effects for the aerosol model were temperature (p=0.003) and relative humidity (RH) (p=0.15). The pair-wise comparison suggested the half-life of influenza decreased as temperature increased (Table 6). For example, virus half life was predicted to be approximately 16.5 times longer at temperatures between 7 $^{\circ}$C and 12 $^{\circ}$C compared to temperatures $\geq$ 27 $^{\circ}$C (95% CI : 4.88 to 56 times). The covariance parameter estimates for the random effects, study, study*temperature, study*RH and the residual error, were 0.33, 0.007, 0.10 and 0.08 respectively.

Seven studies with eight relevant experiments described influenza persistence in water. The main effects for the water model were water source (p=0.37), temperature (p=0.12), salinity (p=<0.0001) and pH (p=0.04). Increased salinity was a significant deterrent to influenza persistence, with both fresh water (0-1ppm) (having the longest persistence), and brackish water (>1-30ppm), significantly longer than salt water ($\geq$30ppm); 2.31 times longer (p<0.0001) and 1.49 times longer (p=0.006), respectively. Table 7 provides the pair-wise comparison for salinity. pH was also a significant main effect, where influenza persisted an estimated 6.89 times longer (95% CI: 1.12 to 42.2 times) in pH 6-8 when compared to extreme pH (<6 and $\geq$9). The covariance estimates for the random effects of study, study*water source, study*temperature, study*salinity, study*pH and residuals were 0, 0.087, 0.064, 0, 0.049 and 0.043 respectively.

Discussion

The aim of this review was to summarize the findings from experiments that report persistence of influenza in the environment. The motivation for the review was to provide better science-based information to inform policies that will impact livestock producers and

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surrounding communities. For example, to establish that a production site is free of influenza prior to re-population it may be necessary to sample the premises. The available literature should be able to inform which environmental matrices are associated with longer persistence and therefore should be targeted for testing for influenza virus. Recent outbreaks of avian influenza as well as the interest in the novel H1N1 influenza virus suggest that the need for high quality information about the persistence of influenza virus in livestock environments will only increase.

The data, although limited, suggest the half-life of influenza is significantly shorter in air compared to other matrices and that in air, as in other matrices, persistence of influenza is longer at lower temperatures. Theoretically this information and the accompanying estimates of virus half-life could be combined with estimates of virus concentration to predict aerosol dispersion between facilities. Such approaches have been used to predict aerosol transmission of other livestock pathogens such as foot and mouth disease and PRRS virus (4,5,23,29). However, although general associations can be described from the data, the estimates obtained from the review of virus half-life have wide confidence intervals (Table 5, Table 6, Table 7). This limitation highlights the need for more applicable primary research into the feasibility of facility-to-facility transmission of influenza.

The data summation also suggests that influenza has an increased half-life in water compared with feces and fomites (Table 5), and that persistence may be longer in cool clean water than buffered or lake water ($p=0.0015$). The application of this information is that in a depopulation situation, to understand whether influenza remains in a barn, water testing would appear to be the more sensitive evaluation, and sampling water from clean water sources such as troughs or nipples would be better then testing manure, waste or contaminated water in the barn. Weber et al.(76) also concluded that water might be considered a reservoir for influenza, given the similar data evaluated.

These conclusions are consistent with others (59) regarding prolonged persistence at low temperatures and shortened persistence at extreme pHs and salinities. However, other studies have not previously tried to quantitatively summarize magnitude of differences across

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multiple studies. More recent studies have continued to demonstrate this as well (9,18). Weber et al. (76) also conducted a review of influenza virus and commented on the short duration of persistence of influenza in the airborne state, particularly in low to moderate temperatures and low RH, although this statement was based on human transmission models, which may not be appropriate to apply to airborne persistence in the field between barns of pigs or poultry.

One potential source of bias in our summarized analysis was the number of studies ultimately evaluated, which may have resulted in correlations between results of the same study. The use of a nested random effect was incorporated to adjust for this issue however statistical adjustment post hoc is likely a poor substitute for more studies with greater variation. This particularly applies to the water dataset, where, after adjusting for the between study variation in the random effect (i.e. study*temperature), temperature was no longer a significant variable, likely due to the large discrepancy between observation contribution from each study (e.g. one of the 7 water studies alone contributed 63 to the total 127 observations) (Appendix A6). For the water model, if study was included as a main effect along with water source, temperature, salinity and pH, all main effects but water source became significant at $p<0.0001$. Another source of potential bias was the diversity in measurements of viral concentration (i.e. $TCID_{50}$, EID_{50}, ELD_{50}, CFU, MP_{50}). We used conversion of all assays to viral half-life as a method to obtain a specific assay independent measure of persistence; however there was little overlap between measurement units even within the same matrix, unless an author provided continuity between papers (8,66,67). Unless the research community agrees upon a standard method for quantification of virus, this issue will continue to arise for those needing to summarize results across studies. In addition, minimal statistics were performed in many studies, therefore standard errors were frequently not reported and variance could not be determined for the outcomes, which also relates to the variance between units of measure. Similarly, for environmental conditions such as temperature, pH, salinity, or relative humidity that did not vary during the experiment, the baseline level of the variable was often not reported or described in vague terms. To incorporate these results into the cumulative dataset, terms such as room

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temperature or fresh water were interpreted and estimates were assumed, because of lack of specifics. Likewise, experiments which only portrayed data graphically were interpreted and estimated to enable their inclusion in the review and this estimation would not be as accurate as data extracted from experiments presenting numerical results.

Potentially, the most significant findings of the review were ancillary findings about data quantity and quality. The review documents the paucity of experiments reporting quantitative assays to assess the persistence of influenza in environmental matrices found in livestock facilities, a finding similarly determined by Stallknecht et al(65). The application of systematic review principles to reviewing literature is not as widespread in the bench sciences as clinical sciences, however others have applied similar approaches to the evaluation of the information about influenza virus and reached similar conclusions about the paucity of data and quality of reporting (76). Similarly, Shahid et al. (59) who investigated inactivation rather than virus persistence in a narrative discussion, noted the aim of their review was to add evidence to the "scant …. information" available for biosecurity recommendations for poultry facilities. We had anticipated that persistence of influenza on surfaces and in feces and feces-like matrixes would have generated more primary research, however statistical synthesis of virus half-life in feces and fomites was not possible as so few observations were available (Table 4, Appendix A6). Similarly, since no soil or compost study reported key features of a persistence study it was not possible to report on the persistence of influenza in common methods of livestock mortality removal. More recent work has evaluated the persistence of avian influenza in land disposal (18).

The lack of data may partly be a function of the systematic review methodology which uses pre-determined parameters and criteria for the evaluation of citations for relevance and these criteria are followed sequentially and strictly. As a consequence of this approach relevant experiments would not be considered if the title or abstract did not discuss the pertinent topic of the persistence of influenza, or were not evidently primary research. However, the potential for this bias seems unlikely as few relevant studies were identified outside the electronic search and the search was comprehensive.

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Further, in the experiments conducted, the variation in parameters assessed was narrow. Illustrative of the lack of range assessed is that only 30 observations in water, three observations for feces or diluted feces and three observations in air were available at or below 12°C. This lack of data is particularly relevant as low temperatures may occur in livestock facilities or manure storage units. Data on the persistence of influenza at extreme values of pH or salinity are of less importance since it is likely the range of pH and salinity observed in livestock facilities is narrow.

The study designs and methods of reporting were also extremely heterogeneous and often limiting. Several studies were performed "at room temperature", as well, the sensitivity of the equipment was uniformly absent, therefore there was significant interpretation necessary regarding the parameter values reported. Because of this, it was necessary to categorize naturally continuous variables like temperature, salinity, pH and relative humidity. The continuous nature of these parameters may impact viral half-life in a progressive manner, and this could have been lost by our wide groupings. Likewise, even within the categories, there was insufficient representation to examine interactions between temperature and humidity, or temperature and pH for example, and these are common questions about influenza.

Another ancillary finding of the review was the failure by authors to report sufficient information to understand the experiment design, execution and results. Many descriptions of methods and outcomes were unclear or incomplete, and it was often difficult to determine if key features were present. For example, there was minimal reporting of limits of detection for the viral assays used, which clearly affects the ability to synthesize data between studies. In addition, the absence of variance reporting was consistent over all experiments evaluated in this review. In fact, there was no error reporting of any kind. Of similar concern, it was uncommon to report the number of replicates or even state the use of multiple replicates or samplings per time interval. Because of this, the uncertainty within studies clearly impacts the uncertainty when synthesizing information between studies. Key areas that require considerable improvement in reporting are the descriptions of environmental conditions and the statistical methods. These concerns along with other key features identified by the authors stimulated a follow-up study evaluating the comprehensiveness of study reporting in the 19

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studies described (Irwin and O'Connor).

Ultimately this review revealed that, though there is a significant amount of published literature regarding influenza, there are very few studies that can be used to support decision-making and policy formation. Although this study was comprehensive, the resultant data extracted for this synthesis leaves a great deal of uncertainty for field application or management decisions, and is outdated for certain matrices. Future work should use improved reporting of study designs and outcomes, to enable a more thorough and robust meta-analysis of environmental persistence of influenza.

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Tables and Figures

Figure 1. Flow chart of literature search, relevance screening and quality assessment process for influenza persistence in environmental matrices.

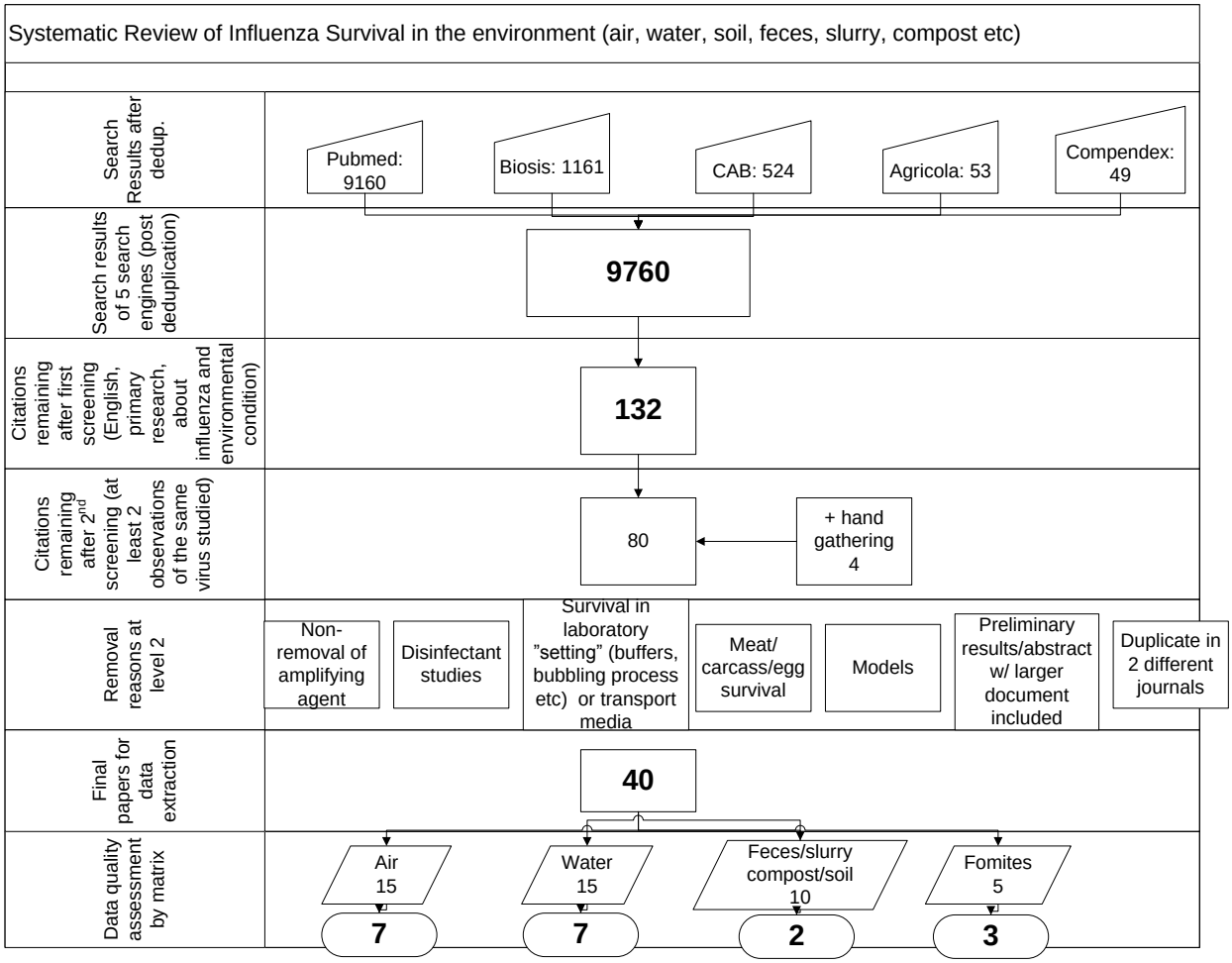

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Figure 2. The 191 observations (converted to $t_{1/2}$ (days)) sorted by matrix, separated by temperature and differentiated by varying parameter*

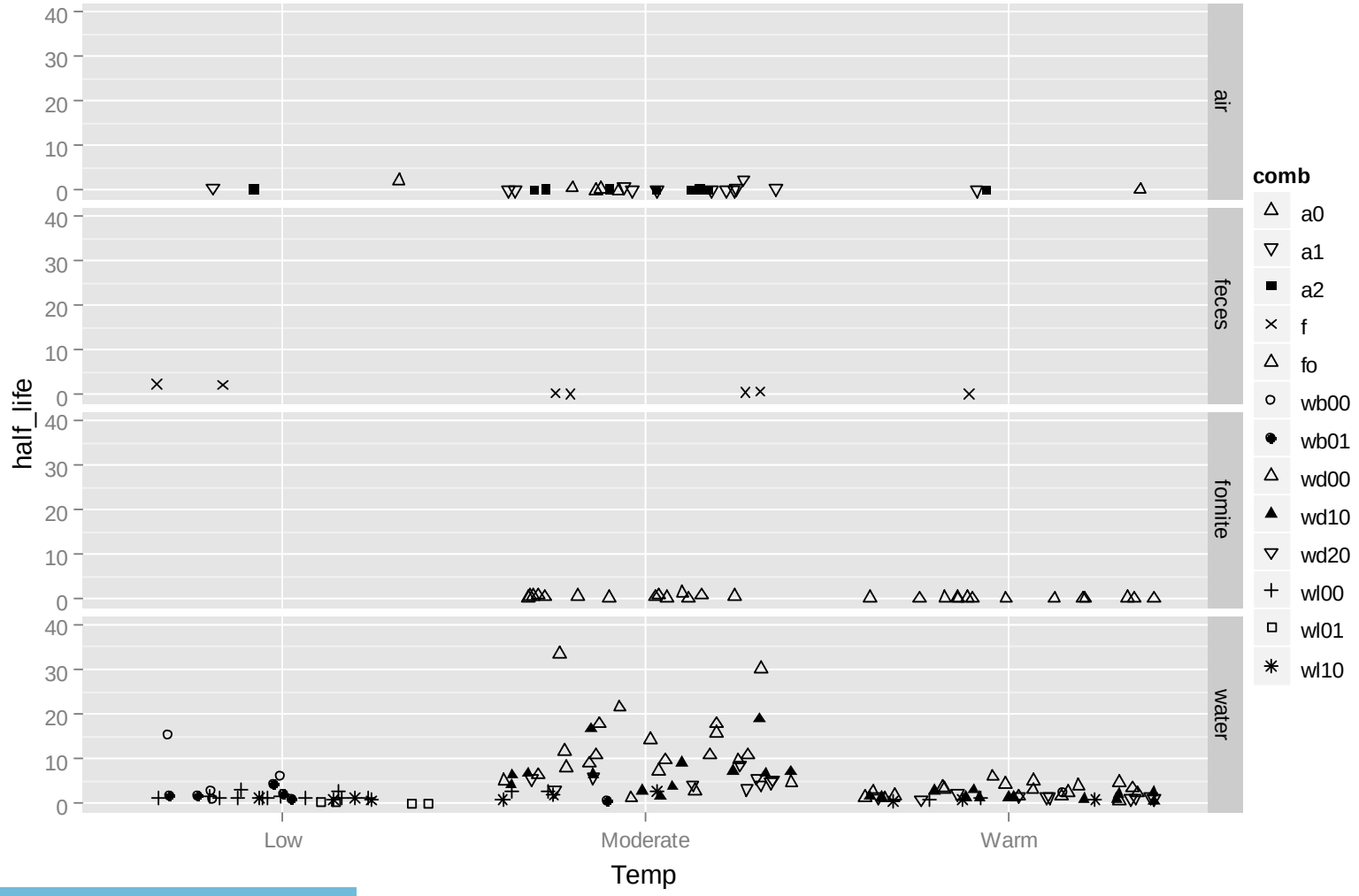

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Figure 2. (continued) Key

A0: Air, RH <30%	**Wb00**:Buffered water, 0-1ppm, norm pH	**Wl00**: Lake water, 0-1ppm, norm pH
A1: Air, RH 30-70%	**Wb01**:Buffered water, 0-1ppm, extreme pH	**Wl01**: Lake water, 0-1ppm, extreme pH
A2: Air, RH ≥70%	**Wd00**: Distilled water, 0-1ppm, norm pH	**Wl10**: Lake water, 1-<30ppm, norm pH
F: Feces	**Wd10**: Distilled water, 1-<30ppm, norm pH	
Fo: Fomite	**Wd20**: Distilled water, ≥30ppm, norm pH	

*For better graphic visualization, data points of $t_{1/2}$ =75days in water (low temperature category), and $t_{1/2}$ =120 days in feces (low temperature category) were excluded

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Table 1. Frequency of viral strain and matrix observations from 19 experiments studying the persistence of influenza in the environment.

Matrix	Species	Number of virus half-life estimates
Air	H1N1	26
Air	H2N2	1
Air	H4N6	1
Water	H3N8	2
Water	H4N6	2
Water	H5N1	12
Water	H5N2	9
Water	H5N3	6
Water	H5N7	6
Water	H5N8	6
Water	H6N2	34
Water	H7N1	1
Water	H7N3	20
Water	H7N4	6
Water	H7N7	8
Water	H10N7	3
Water	H11N6	10
Water	H12N5	2
Feces	H11N9	4
Feces	H5N1	4
Fomites	H1N1	11
Fomites	H12 N7	11
Fomites	Influenza B/ Illinois	6

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Table 2. Frequency of matrix conditions from 19 experiments studying the persistence of influenza in the environment.

Matrix	Variables reported	Measures reported	Number of virus half-life estimates
Air	Temperature (°C)	7-12°C	3
		17-<27°C	22
		≥27°C	3
	Relative Humidity	<30%	6
		30-70%	13
		≥70%	9
Water	Water type	Buffered	86
		Distilled	11
		Lake	30
	Temperature	2-12°C	30
		17-<27°C	50
		≥27°C	47
	Salinity	0-1ppm	71
		>1 to <30ppm	36
		≥30ppm	20
	pH	Normal (pH 6-8)	117
		Extreme (<6 and ≥9)	10
	Water clarity	Filtered	106
		Unfiltered	10
		Not described	11
Feces	Feces type	Dried	1
		Moist	5

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	In river water	2
Temperature (°C)	4-12°C	3
	17-<27°C	4
	≥27°C	1

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Table 3. Virus type, environmental conditions, number of observations and predicted half-life in days (T ½) of influenza virus in fomites matrix

Strain	Fomite	Temperature	Relative humidity	t ½	Ref.
H1N1	paper tissue transfer to hand	27.8-28.3	35-40%	0.008	(3)
H1N1	Copper	22	50-60%	0.021	(44)
H1N1	Cotton	27.8-28.3	35-40%	0.027	(3)
Influenza B/Illinois	Cotton	26.7-28.9	55-56%	0.04	(3)
Influenza B/Illinois	Paper tissue	26.7-28.9	55-56%	0.049	(3)
H1N1	Paper tissue	27.8-28.3	35-40%	0.055	(3)
Influenza B/Illinois	Magazine	26.7-28.9	55-56%	0.058	(3)
Influenza B/Illinois	Cotton	26.7-28.9	55-56%	0.084	(3)
H1N1	Magazine	27.8-28.3	35-40%	0.126	(3)
H1N1	Steel transfer to hand	27.8-28.3	35-40%	0.130	(3)
H1N1	Cotton	27.8-28.3	35-40%	0.170	(3)
Influenza B/Illinois	Plastic	26.7-28.9	55-56%	0.197	(3)
H1N1	Polyester	“room”	nd*	0.208	(71)
H1N1	Plastic	27.8-28.3	35-40%	0.222	(3)
Influenza B/Illinois	Steel	26.7-28.9	55-56%	0.248	(3)
H1N1	Cardboard	“room”	nd	0.263	(71)
H1N1	Cotton	“room”	nd	0.309	(71)
H1N1	Steel	27.8-28.3	35-40%	0.333	(3)
H1N1	Rubber boot	“room”	nd	0.495	(71)
H1N1	Tile	“room’	nd	0.507	(71)

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H1N1	Tire	“room”	nd	0.518	(71)
H1N1	Plastic	“room”	nd	0.604	(71)
H1N1	Wood	“room”	nd	0.667	(71)
H1N1	Feather	“room”	nd	0.705	(71)
H1N1	Steel	“room”	nd	0.742	(71)
H1N1	Steel	22	50-60%	0.750	(44)
H1N1	Egg shell	“room”	nd	0.853	(71)
H1N1	Latex	“room”	nd	1.391	(71)

*nd = not defined

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Table 4. Virus type, environmental conditions, number of observations and predicted half-life in days (T ½) of influenza virus in feces matrix.

Strain	Feces consistency	Temperature (C)	N	t 1/2	Ref.
H5N1	Dried feces	25	1	0.0669	(64)
H5N1	Feces in water	35	1	0.1338	(64)
H11N9	Feces in lake water	22	1	0.2606	(77)
H11N9	Normal feces	22	1	0.5114	(77)
H5N1	Feces in water	25	1	0.6740	(64)
H11N9	Normal feces	4	1	2.2053	(77)
H11N9	Feces in lake water	4	1	2.2482	(77)
H5N1	Feces in water	4	1	120.41	(64)

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Table 5. Multivariate, multiple comparison adjusted estimates of association between environmental conditions and influenza virus half-life ($\log_{10}$ t ½) (n=191)

Multiple comparison	Point estimate of the difference (Δ)	Half-life ratio(10Δ)	95% confidence interval of 10Δ*	Adj. p-value
Matrix: water vs. aerosol	1.44	27.3	2.22 to 336	0.010
Matrix: feces vs. aerosol	1.04	11.0	0.43 to285	0.18
Matrix: fomite vs. aerosol	0.63	4.22	0.19 to 92	0.52
Matrix: water vs. fomite	0.81	6.46	0.30 to 139	0.31
Matrix: water vs. feces	0.39	2.48	0.12 to 52.7	0.81
Matrix: feces vs. fomite	0.42	2.61	0.06 to 109	0.87
Temperature 2-12°C vs. > 27°C	1.06	11.6	1.28 to 105	0.03
Temperature 2-12°C vs. 17-<27°C	0.79	6.12	0.73 to 51.1	0.09
Temperature 17-<27°C vs. > 27°C	0.28	1.90	0.28 to 13.1	0.63

Full model: $\log_{10}t_{1/2} = \mu$ + matrix + temperature + study (matrix) + study(matrix)*temperature

*95% Confidence Intervals which include 1 show no significance α=0.05.

†P values from Tukey-Kramer adjustment for multiple comparisons

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Table 6. Pairwise adjusted† estimates of the change of virus half-life ($\log_{10}$ t ½) and environmental conditions in air (n=28)

Multiple comparison	Point estimate of the difference (Δ)	Half-life ratio(10Δ)	95% confidence interval of 10Δ	Adj. p-value†
Temperature: 7-12°C vs. 17-<27°C	0.70	4.99	1.59 to 15.67	0.02
Temperature: 7-12°C vs. > 27°C	1.22	16.5	4.88 to 55.96	0.0002
Temperature: 17-<27°C vs. > 27°C	0.52	3.31	1.05 to 10.39	0.099

Full model: $\log_{10}t_{1/2} = \mu$ + temperature + RH + study + study*temperature + study*RH

*95% Confidence Intervals which include 1 show no significance α=0.05.

†P values from Tukey-Kramer adjustment for multiple comparisons

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Table 7. Adjusted† estimates of the change of virus half-life ($\log_{10}$ t ½) and environmental conditions of water (n=127)

Multiple comparison	Point estimate of the difference (Δ)	Half-life ratio(10Δ)	95% confidence interval of 10Δ*	Adj. p-value†
Salinity: 0-1ppm vs. >1-30ppm	0.19	1.55	1.19 to 2.01	0.0004
Salinity: >1-30ppm vs. >30ppm	0.17	1.49	1.06 to 2.09	0.016
Salinity: 0-1ppm vs. > 30ppm	0.36	2.31	1.66 to 3.22	<0.0001
pH: 6-8 vs. <6 or >9	0.84	6.89	1.12-42.2	0.043

Full model: $\log_{10}t_{1/2}$ = μ + water source + temperature + salinity + pH + study + study*water source + study*temperature + study*salinity + study*pH.

*95% Confidence Intervals which include 1 show no significance α=0.05.

†P values from Tukey-Kramer adjustment for multiple comparison

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Appendix

Appendix A1. Combination of search phrases and terms used search to identify the articles in the CAB and Agricola electronic data.

Population	Survivability	Matrix		
		Air:	Soil	Water
influenza influenzavirus Orthomyxoviridae Influenzavirus C influenza C Influenza A Influenzavirus A H1N1 H2n2 H3N2 H3N8 H2N3 H5N2 H7N7 H9N2 (Influenza in Birds) Influenza B influenzavirus B (Hemagglutinin Glycoproteins) Human influenza	(virus or viral or microbial or microbe) and (pathogenicity survivability survival stability infectivity infection infective "infective dose" infect viability "environmental stability" inactivation transmission)	air aerobiology aerosol “air movement” “air microbiology” “particulate matter” Dust “UV intensity” “ultraviolet light intensity” droplet “droplet nuclei” humidity moisture weather temperature season sunshine sunlight irradiation	Soil dirt earth mud “soil microbiology” “geologic sediments” pH “mineral content “ “soil composition” soil mineral* kaolin Clay sand manure	water or wetland* or waterway or watershed or pH or manure or feces or faeces or faecal shedding or fecal shedding or wastewater or effluent or irrigation or drying or desiccation or desiccating or lyophilization or lyophilized or water microbiology

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Appendix A2. Combination of search phrases and terms used search to identify the articles in the PUBMED electronic data.

Population	Outcome	Matrix		
		Air	Water	Soil
influenza OR influenzavirus OR (influenza A) OR (influenzavirus A) OR (influenza B) OR (influenzavirus B) OR (influenza C) OR (influenzavirus C) OR "Orthomyxoviridae"[Mesh] OR "Influenzavirus C"[Mesh] OR "Influenza A virus, H1N1 Subtype"[Mesh] OR "Influenza A virus, H2N2 Subtype"[Mesh] OR "Influenza A virus, H3N2 Subtype"[Mesh] OR "Influenza A Virus, H5N2 Subtype"[Mesh] OR "Influenza A Virus, H7N7 Subtype"[Mesh] OR "Influenza A Virus, H9N2 Subtype"[Mesh] OR "Influenza in Birds"[Mesh] OR "Influenza B virus"[Mesh] OR "Influenza A Virus, H3N8 Subtype"[Mesh] OR "Influenza A Virus"[Mesh] or H2N3 OR	(Virus or viruses or viral or microbial or microbe) and (pathogenicity OR survivability OR survival OR stability OR infectivity OR infection OR infective OR "infective dose" OR infect OR viability OR "environmental stability" or inactivation or transmission)	air OR aerobiology OR aerosol OR (air movement) OR (air microbiology) OR (particulate matter) OR ("Dust"[Mesh]) OR (UV intensity) OR (ultraviolet light intensity) OR droplet OR (droplet nuclei) OR humidity OR moisture OR weather OR temperature OR season OR sunshine OR sunlight OR irradiation	water or watershed or waterway or wetland* or pH OR manure or feces or faeces or faecal shedding or fecal shedding or slurry OR drying OR desiccation OR desiccate* OR lyophilization OR lyophilize* OR wastewater OR effluent OR irrigation	soil OR dirt OR earth OR mud OR ("Soil Microbiology"[Mesh]) OR ("Geologic Sediments"[Mesh]) OR pH OR (mineral content) OR (soil composition) OR (soil mineral*) OR kaolin OR clay OR sand OR manure

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"Hemagglutinin Glycoproteins, Influenza Virus"[Mesh] OR "Influenza, Human"[Mesh]				

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Appendix A3. Combination of search phrases and terms used search to identify the articles in the Compendex electronic data.

Population	Outcome	Matix		
		Air	Soil	Water
influenza or influenzavirus or Orthomyxoviridae or Influenzavirus C or influenza C or Influenza A or H1N1 or H2n2 or H3N2 or H3N8 or H2N3 or H5n2 or H7N7 or H9N2 or (Influenza in Birds) or Influenza B or Hemagglutinin Glycoproteins or Human influenza or flu	microbial viability or virus stability or virus survivability or virus survival or environmental stability or virus concentration or infective or infectivity or infective dose or infect or pathogen survival or environmental degradation or environmental inactivation or environmental conditions or transmission	air or aerobiology or aerosol or air movement or air microbiology or particulate matter or Dust or UV intensity or ultraviolet light intensity or droplet or droplet nuclei or humidity or moisture or weather or temperature or season or sunshine or sunlight or irradiation	soil or dirt or earth or mud or "Soil Microbiology" or "geologic sediments" or pH or mineral content or soil composition or soil mineral* or kaolin or clay or sand or manure	(water or wetland* or watershed or waterway or pH or manure or feces or faeces or faecal shedding or fecal shedding or wastewater or effluent or irrigation or drying or desiccation or desiccating or inactivation or lyophilization or lyophilized or water microbiology)

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Appendix A4. Combination of search phrases and terms used search to identify the articles in the Biosis electronic data.

Population	Survival	Matrix		
		Air:	Soil:	Water:
TS= (influenza or influenzavirus or Orthomyxoviridae or Influenzavirus C or influenza C or Influenza A or H1N1 or H2n2 or H3N2 or H3N8 or H2N3 or H5N2 OR H7N7 or H9N2 or Influenza in Birds or Influenza B or Hemagglutinin Glycoproteins or Human influenza)	TS= (microbial viability or virus stability or virus survivability or virus survival or environmental stability or virus concentration or infective or infectivity or infective dose or infect or transmission)	TS=(air OR aerobiology OR aerosol or air movement OR air microbiology OR particulate matter OR "Dust"[Mesh] OR UV intensity OR ultraviolet light intensity OR droplet or droplet nuclei or humidity OR moisture OR weather OR temperature OR season or sunshine or sunlight or irradiation)	TS=(soil OR dirt OR earth OR mud OR "Soil Microbiology" OR Geologic Sediments OR pH OR mineral content OR soil composition OR soil mineral* or kaolin or clay or sand or manure)	TS=(water or watershed or waterway or wetland or wetlands or pH or manure or feces or faeces or faecal shedding or fecal shedding or slurry or wastewater or effluent or irrigation or drying or desiccation or desiccating or lyophilization or lyophilized or water microbiology)

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Appendix A5. Reasons for exclusion of 9578 references from 9760 titles and abstracts screened for relevance in a review of persistence of influenza in environmental matrices.

Reason for exclusion	Number of studies excluded†	Number of primary exclusions
Non-English research	1296	1296
Publication was a survey/testimonial/editorial	600	493
Publication was a review/report	460	444
Publication was a simulation model	171	169
Type of publication could not be determined	108	74
The article not about influenza	3474	2920
Focus microbe could not be determined	89	9
Article does not involve environmental sampling	7779	3680
Environmental sampling cannot be determined	69	5
Does not provide 2 observations of the same virus	16	3

†Studies may have multiple reasons for exclusion

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Appendix A6: Study description of influenza persistence primary research passing relevance and quality criteria described in this systematic review.

Study	Pub. year	Matrix	Virus	Host species	Condition	Methods of calculating virus titer	Duration of study	Outcome measured	Replicates per studied condition documented	Quality experiments out of relevant experiments	Tot # obs toward model
Moses et al.	1947	Water (buffered virus solution)	Dutch East Indies strain of Fowl Plague Virus (H7N7) and variant virus (Strain 4395) from FPV	avian	pH stability (2-12)	Reciprocal of the log of the dilution lethal for embryos	1 hour to 1 week	ELD_{50}	3	1/1	8
Scholtissek	1985	Water (buffered virus solution)	A/FPV/Rostock/34 (H7N1)	avian	pH= 5.2	PFU an HA units	30min	PFU/ml (log 10)	3	1/3	1
Stallknecht et al	1990	Water (buffered virus solution)	A/mottled duck/LA/38M/87 (H6N2), A/blue-winged teal/LA/44B/87 (H4N6), A/green-winged teal/LA/169GW/88 (H10N7)	avian	pH (5.8, 6.2, 6.6, 7.0, 7.2, 7.4, 7.8, 8.2, salinity (0,5,10,15, 20, 30ppm) temperature (17, 28C)	Microtiter endpoint confirmed w/CPE	Varied per trial, up to 19d	Linear regression of the log transformat. of $TCID_{50}$	1? (ns)	2/4	22
Stallknecht et al	1990	Water (buffered virus solution)	A/Gadwall/LA/17G/87 (H3N8), A/Blue-winged teal/LA/44B/87 (H4N6); a/mottled duck/LA/38M/87 (H6N2), A/Blue-winged teal/LA/188B/87	avian	Temperature (17 and 28C, and one virus was tested at 4C)	Microtiter endpoint confirmed w/CPE	60d (one study continued for 90d)	Linear regression of the log transformat. of $TCID_{50}$	2? (ns)	1/1	11

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			(H12N5) and A/green-winged teal/:A/169GW/88 (H10N7)								
Zarkov	2006	Lake water	H6N2 (Bulgaria) and A/duck/England/56 (H11N6)	avian	pH, salinity, temperature	Reed & Muench	0-18d	EID_{50}	1, 2ml sample from original water each sampling time, but used 6 9d old CE for each re-isolation and viral dilution	1/1	20
Lucio-Forster et al	2006	water	H5N2	avian	37 and 4°C	Reed & Muench	48hr	EID_{50}	3 replicate samples at each time point, each inoculating 4, 9-11 day old CE w/ 0.1ml for each sample, positives were titrated into 4, 9-11d CE.	1/3	2
Brown et al.	2007	Water	MN/98, MN/00, NJ/01, NJ/01, MN/98, TX/02, DE/00, DE02, Mongolia/05, Anyang/01	avian	Salinity (0,15,30ppm) at 17C and 28C	Microtiter endpoint confirmed w/CPE	60d until 2 consecutive weeks of non-detection of virus	$TCID_{50}$	1? ns	1/1	63
Shortridge et al.	1998	Feces	H5N1	avian	Temperature (dry and 25°C, moist at 4, 25	Infectivity titer: Embryonated	Up to 40 days	EID_{50}	ns	1/1	4

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					and 35°C)	egg titration					
Webster et al	1978	feces	Duck/Memphis/546/74 (H11N9)	avian	Temperature (Feces (pH 7.68) and feces in Miss. river water (6.8) at 4 and 22°C)	Infectivity titer: Embryonated egg titration	32 days	EID_{50}	ns	1/2	4
Noyce et al.	2007	Fomite (copper and stls steel)	A/PR/8/34 (H1N1)	human	Sterile Cu and stnls steel inoc. at 22 +/-2°C and 50-60% RH	Epiflouresc. imaging	24 hr	Virus particles	3	1/1	2
Bean et al.	1982	fomites	A/Brazil/11/78-like (H1N1) and influenza B/Illinois/1/79-like	human	6 materials and hand transfer of both Influenza A: temperature 27.8-28.3°C, RH at 35-40%, and influenza B: temperature 26.7-28.9, RH 55-56%	Karber (expressed in)	72hr	$TCID_{50}$	6	2/3	14
Tiwari et al.	2006	fomites	A/Herring gull/Delaware 471/86 (H13N7)	human	Pieces of 12 material inoc. and dried (30-40min) and incubated at room temp (and RH)	Reed & Muenchen	9days	$TCID_{50}$	1	1/2	12
Schaffer et al.	1976	Aerosol	WSN (H1N1)	human	Wells refluxing atomizer: each run = 2 aerosols held	Modification of Kilbourne's plaque assay	60min (sampled at 1, 15, 30 and	PFU; half-life given for 1-15min and 15-60min	11	1/3	2

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					simult. at 21C in 208L dual stirred settling chamber. Ea. run employed either a single virus prep at 2 diff RH, or diff. prep or additive at a single RH.	technique (no tracer)	60min)	intervals			
Mitchell et al.	1968	Aerosol	PR8/34 (H1N1), FM1 (H1N1), Duck Czech/56 (H4N6)	Human and avian	Rotating drum at 70F, virus aerosolized until internal humidity reached RH = 75%, samples removed for 3min using Shipe impinger (bacterial tracer and Anderson sampler were used for testing uniform delivery)	Not stated, used bacterial tracer	Tables say 36hr	"Viral particles"; concentration determined by EID and HA titer	"several"	1/2	3
Schulman	1967	aerosol	Jap 305 (H2N2), and NWS (H1N1)	human	Serial dilutions of allantoic fluid seed virus nebulized into a closed chamber, sampled using Shipe impinge,	Not stated (no tracer)	60min	EID_{50}	2?	1/2	2

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					room air temp and RH						
Hood	1963	aerosol	PR8/34 (H1N1) and Asian (Singapore, type A2)	human	Stainless steel drum (500L); Temp= 22-26.6C and either 15-21% RH, 52-55%RHor 78-85%RH	Egg-membrane piece technique (MP50) Fazekas de St. Groth & White (no tracer)	Up to 20hr	MP_{50}	9 (2 different suspensions) replicates at 15RN, 4 (2 dif. susp) replicates at 52RH and 8 (2 dif. susp) replicates at 78RH	1/4	3
Buckland et al.	1962	Aerosol	WS and Swine influenza A (H1N1 and H1N1)	Human and swine	Dried on glass slide	Droplets dried on glass slide	2.5hr	ID_{50}	2-3 replicates	1/1	4
Harper	1961	Aerosol	PR8	human	Temperature (7.0-8.0, 20.5-24.0 and 32.0) and RH (20-25, ~35, ~50, ~65, ~80)	Egg-membrane piece technique (MP50) Fazekas de St. Groth & White (^{32}P tracer)	23hr	MP_{50} (%)	At least 3/tem:RH combination (2 with 4 and 5 replicates)	1/1	11
Parker et al.	1944	Aerosol	Melbourne	human	Air/dry	Glass tubing (no tracer)	Up to 22 days	ELD_{50}	7 mucin (only 3 replicates with concentration reported), 3 talc (0 reported concentration), 21 air current (0 reported concentration)	1/3	3

ns: not stated ?: questionable

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Appendix A7: SAS code from the models included in the review

Code used for the full matrix model

```
Proc Mixed data = overall;
class matrix tempcat REF_ID ;
model LOGT1_2= tempcat matrix /ddfm=kr solution;
random REF_ID(matrix) REF_ID(matrix)*tempcat;
LSMeans matrix tempcat /pdiff cl adjust=tukey;
run;
```

Code for air matrix model

```
Proc mixed data= air:
class Rhcat tempcat REF_ID;
model LOGT1_2= tempcat Rhcat /ddfm=kr solution;
random REF_ID REF_ID*tempcat REF_ID*Rhcat;
LSMeans tempcat Rhcat/pdiff cl adjust=tukey;
run;
```

Code used for the water matrix model

```
Proc Mixed data-water
class water_source tempcat REF_ID sal extrph;
model LOGT1_2= tempcat water_source sal extrph /ddfm=kr solution;
random REF_ID REF_ID*water_source REF_ID*tempcat REF_ID*sal REF_ID*extrph;
LSMeans water_source tempcat sal extrph /pdiff cl adjust=tukey;
run;
```

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CHAPTER 3. AN OBSERVATIONAL STUDY EVALUATING THE COMPREHENSIVENESS OF REPORTING: AN EXAMPLE USING ENVIRONMENTAL PERSISTENCE OF INFLUENZA

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CK Irwin, AM O'Connor

Abstract

This study uses a systematic review of published studies on influenza persistence in environmental matrices (11), to assess the quality of current literature on influenza environmental persistence. Expectations from experts in disease ecology, virology and environmental studies in addition to expectations identified as gaps in the influenza systematic review and current, accepted guidelines for performing clinical trials, were used to create a set of considerations for creating and reporting experimental studies of viral persistence. The 19 studies passing minimal quality criteria in Irwin et al. (11) were reviewed using these considerations and the results were tabulated. Significant findings included: all studies described the virus assay used for detection, but only 21% described the assay limits of detection; only 37% of studies described the virus propagation method and the number of virus passages; 79% of studies described the baseline experimental (non-manipulated) parameters; although 84% described the investigator manipulated parameters, no study provided the sensitivity of the equipment used to measure the manipulated conditions; 58% of studies described the duration of the study in the methods, and only 37% described the sampling interval in the methods; only two studies clearly reported the number of replicates used and both used multiple replicates; no study reported how summary information was obtained for samples and replicates; only 4 studies reported methods used to summarized overall outcomes between replicates; no study provided descriptive results that included variance; although one study calculated half-life values using influenza persistence

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outcomes, no study provided overall outcomes that included variance.

Introduction

In laboratory sciences, as in clinical sciences, material sciences, and mathematical sciences, a key principle of publication of research findings is that the publication provides a reproducible description of the work conducted. A reproducible description serves many purposes including: enabling other researchers to replicate the study and evaluate if the results are repeatable rather than due to chance; allowing assessment of the potential biases in the conduct of the study that may provide an alternative explanation for the outcome; and, enabling the incorporation of the results into research synthesis methods such as meta analysis, systematic reviews or risk assessment.

In clinical research there has been an increased focus in recent years on the quality of reporting and how closely reports adhere to the concept of reproducibility. Many studies have provided empirical evidence that clinical trials and observational studies frequently fail to report sufficient information for reproduction, assessment of bias, and research synthesis (5,9,12,19,20). Consequently, guidelines for how to report biomedical and clinical study designs have been developed and adopted by clinical journals (3,16,17,29). These guidelines are designed to encourage reporting of key study design aspects that enable readers to assess internal bias, external validity and enable data extraction.

Similar evaluations of the comprehensiveness of research reports in the laboratory sciences seem rare (18). Articles or editorials have described poor reporting of statistical methods but otherwise there appears to be little empirical evaluation of the quality of reporting in the laboratory sciences. However, the motivations for reproducible reporting are as applicable to laboratory science research as clinical sciences. With this motivation, our aim was to describe how closely a group of laboratory science studies adhered to the concept of a reproducible description by tabulating the frequency of which studies reported key study design features. The study population for the evaluation was a series of studies reporting the persistence of influenza in the environment. We chose this study population given our interest in this area, and are unaware of other studies that have evaluated the

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comprehensiveness of reporting design features in laboratory science studies.

Materials and Methods

Approach to identifying the literature for evaluation

Nineteen studies, used for a systematic review and meta-analysis evaluating the persistence of influenza in environmental matrices were used for this study (11). The methods of identifying the literature, conducting the review, summarizing the data and the conclusions of that review are reported elsewhere (11).

Identifying key features of study design for evaluation

To assess the comprehensiveness of reporting in a group of studies it is necessary to identify key study features required for a reproducible document. For many study designs such as randomized controlled trials, diagnostic test evaluations and observational studies, published guidelines provide this information and are readily available (3,7,8,13-16,22,25,27-31). For the laboratory sciences, guidelines for comprehensive reporting were not readily available; therefore the key features required for evaluation were determined using a two-step process.

In the first step, content experts in virology, environmental sciences, and disease ecology were consulted in a series of group and individual meetings and asked to identify the features required in an experiment to assess virus persistence in environmental matrices. This process was part of the systematic review (11). The content experts concluded that each experiment should describe the influenza subtype, including the number of virus passages prior to the experiment, the environmental matrix, the method of spiking the environmental matrix with the virus, the study duration and sampling intervals, the environmental parameters (i.e. temperature, relative humidity, salinity, pH) under which the experiment was run, measurement of the virus using a quantifiable concentration assay and at least two sample periods where virus continued to be detected. The rationale for the last two features was to enable determination of virus decay.

The second step in the identification of key design features for assessment occurred at the conclusion of the original systematic review. In a debriefing about the review, previously

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omitted key features associated with the reproducibility of the studies, the ability to assess bias or the ability to extract data were identified. These related mainly to a description of the study protocol and the methods of data handling and analysis. Using these sources of information a list of 17 key reporting features to be evaluated in the studies was developed (Table 1). Of the 17 key reporting features evaluated, 15 were methodological features and two related to descriptions of the results. The 15 methodological features were subdivided into attributes about the study organism, study setting, study protocol and data handling. The last two concerned data analysis. The features and rationale are reported in Table 1.

Assessing the presence of key features

The unit of concern for the evaluation of reporting was the study. For each of the 19 studies, the presence or absence of the feature in the appropriate section of the manuscript was evaluated. Evaluation for features was conducted by one reviewer (CI), who consulted with the experts or the co-author when the information was unclear. Possible responses for the 17 items were yes or no. No judgment was made about the correctness of the approach reported. For example, a study reporting the detection limit for the virus quantification assay received a yes for response regardless of the level of detection, and no, if the detection limit was not mentioned. If a study referred the reader to another citation for a method, the response for that feature was presumed yes, although additional investigation was not pursued. Experimental settings and conditions were expected to be described clearly. Descriptions such as "grown in eggs", "serial passage", "in a drawer at room temperature" or "room humidity" were considered insufficient for replication, and resulted in a negative response. Further, the feature was expected to be present in the appropriate section of the manuscript. For example, if un-manipulated or manipulated experimental parameters were not stated in the methods section of the manuscript the response for that feature in this review was no, even if graphs or narration in the results section provided this information. When multiple aspects were required for a complete description of a checklist items, the item was marked yes only when all aspects of the description were present. For example, checklist item 1 required both a description of the concentration units of the assay and a description of the detection limits for an affirmative response.

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Results

Description of the study population

Nineteen studies were included in the study population as they described experiments that evaluated persistence of influenza virus in the environment (11). Twenty-one relevant experiments were described in the 19 studies. The detailed characteristics of the 19 studies are provided elsewhere (11).

Methods assessment and evaluation

Figure 1 and Table 2 describe the frequency of reporting of the 17 checklist items in the 19 studies, as well the frequency of reporting by matrix (air, water, feces and fomites) and publication year category (<1970, 1970-1990 and ≥1990).

Checklist items 1-3: Attributes of the virus

For checklist item 1, although all 19 studies described the virus assay, including the units of concentration, only 21% (4/19) provided the limit of detection for that assay, prior to reporting the results.

For item 2, 11 of 19 studies (58%) provided complete descriptions of the influenza virus. Six of eight studies with incomplete descriptions were published prior to 1977 and these studies provided descriptions, which included colloquial terms (e.g. PR8, Melbourne strain, Dutch East Indies Fowl Plague Virus) but no H or N information. All studies published prior to 1970 also lacked H and N characterization, suggesting this issue was related to the time of publication. Indeed, we found a WHO memorandum released in 1971 (1,6), recommending revisions to the methods of influenza nomenclature to include the H (Hemagglutinin) and N (Neuraminidase) antigenic characteristics of influenza viruses, which explains this observation.

For Item 3, the majority of studies reported the method of virus propagation (16/19), but only 37% (7/19) detailed the propagation method and described virus passages.

Checklist items 4-9: Attributes of the setting

All studies provided a complete description of the matrix (item 4).

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Fifteen of the nineteen studies described the experimental baseline data (item 5) i.e. the non-manipulated conditions of the laboratory. Four of seven studies published from 1970-1990 contained the item 5 information, however 2 of the 8 published ≥1990 failed to include it.

Sixteen of nineteen studies provided the specific details of the investigator manipulated parameters however none described the sensitivity of the equipment (i.e. the sensitivity of sensors for relative humidity, salinity or temperature). Therefore no study met item 6 criteria.

Items 7 and 8 were consistently well reported.

The majority of studies (14/19) reported the concentration of the replicate post inoculation (item 9), and often this was the first sampling time (or series of samplings i.e. aerosol studies), but it was sometimes unclear in resultant graphs whether the author intentionally included equilibration time as part of the decay curve.

Checklist items 10-13: Study Protocol

Although the duration of a study (item 10) could often be determined by looking at tabulated or graphical results, only 11 of the 19 studies described the study duration in the methods of scientific manuscripts.

Similarly, a description of the sampling intervals (item 11) was infrequently present in the methods section (7 of the 19 studies), though 11 of the 12 which failed to discuss the sampling intervals in the methods did have them reported in result tables or graphs.

Only two studies clearly stated the number of true replicates used in the study (1 of 3 fomite studies and 1 of 7 water studies) (Item 12). Of these 2 studies, one was published after 1990, the other, between 1970 and 1990. Both stated multiple replicates (item 12a). Seven other studies provided either a range of replicates used in the experiments or pictorially described 2 presumable replicates in graphed or tabulated results, but because the descriptions required interpretation they did not meet the criteria for reproducibility.

In seven of the 19, the number of samples per replicate was stated (item 13), or it was

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interpreted that the sample equaled the replicate, using terms like, "… aliquots were removed each time period…", "…each time [a] sample was removed…". Of these seven, only three reported >1 samples per replicate.

Checklist items 14 and 15: Attributes of data handling and analysis

No study completely addressed item 14, because none included all three components of item 14 criteria: a description of the statistics used to summarize the data from sampling intervals (mean and standard deviation or range); a description of the statistics used to summarize the replicates; and the methods describing any necessary transformation of data. One study did state the mean was the summary statistic (10), however this study did not provide measures of variation for the mean, nor did it state the number of replicates or samples taken per replicate (items 12 and 13), therefore there was no description of statistics used to summarize data for either samples or replicates. Another study reported the summary result as, "The best fit was estimated by eye" (21) but again contained insufficient information about the number of replicates or samples per replicate the study used. Neither of the two studies with multiple replicates (item 12a) described the method of summarizing replicate data, though Bean et al. (2) did describe the statistical procedures used to summarize the final outcomes by fomite. Eight studies did not log transform data because their results remained in virus titers or were percent recovery. Seven studies did transform data according to graphs in the results, but did not mention the transformation in the methods. Only 4 studies stated some type of transformation of outcomes was performed for results reporting, and one was Schaffer et al. (21), where the visual estimate of percent recovery was transformed to half-lives. Only one other study mentioned half life calculations (24).

Four of the 19 studies reported the statistical methods used to assess the outcomes (item 15).

Checklist items 16 and 17: Reporting attributes of data analysis

All 19 studies provided descriptive results, typically in graphic or tabular form. However, none of the summarized outcomes also provided estimates of variance (item 16) therefore no study met item 16 criteria. It is noteworthy that the preliminary experiment of Bean et al. provided confidence intervals for recovered concentrations of virus immediately post matrix

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inoculation, however no additional reporting of variance in the following persistence experiments was stated.

Three studies created univariate linear regression models for overall persistence at each investigator manipulated environmental parameter by influenza subtype (4,23,24), however none described the variation within the slope estimates of each of those models (i.e. confidence intervals), nor model fit (item 17). Only one calculated half-lives from the persistence outcomes, but without variance (24).

Discussion

The evaluation of reporting quality is not as widespread in the bench sciences as clinical sciences, but it is useful to identify strengths and weaknesses in study reporting. The results of this study can draw attention to potential needs for improved reporting of design and methods in the current scientific literature concerning influenza, as well, may be extrapolated to other fields of bench science. Similar studies showing empirical evidence of poor reporting have provided the motivation for reporting guidelines in other areas of scientific research (3,16,25,26,29).

Although this study identified several aspects of reporting are consistently well executed (i.e. description of virus assay, experimental matrix and method of inoculation of the matrix and replicates), there appears to be room for improvement. Attention should focus on reporting of the baseline (un-manipulated) environmental parameters (item 5) as well as the manipulated parameters and descriptions of the standard limits of error for the tools or equipment used to measure any of those varied parameters (i.e. thermometers, relative humidity sensors etc) (item 6). These features are critical for attempts to reproduce the study and for combined studies in meta-analyses. It was surprising that many studies used apparent sampling intervals in resultant graphs or tables but failed to report study durations (item 10) and sampling intervals (item 11) in the methods section of studies (42 and 63% respectively). As reviewers, we argue readers should understand the design of a study before reading results.

There may be some aspects of this review which highlight the need for alternative

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approaches to study design. As mentioned, this review did not judge the appropriateness of the methods reported, however one area does require comment. Many studies failed to report the number of replicates used for each observation summary (11%, item 12) as well as the number of samples analyzed at each study time point (37%, item 13). For the majority of studies this information was not discernable (item 12). Studies that did report the number of samples often reported evaluating only a single set of virus concentration samples (over the sampling duration) as an appropriate descriptor of the outcome i.e. N (replicate)=n (sample)=1, therefore there was no summary method to describe for sample reporting. Conclusions from these studies were essentially based on non-replicated measurements. Because of the lack of sampling variance, and the lack of reporting standard variation if it existed, the published persistence results should be couched with significant uncertainty. The rationale for using only one observation as basis for inference in most areas of research is rarely justified. In this study population, it was common not to report replication, or to infer different samples at varying sampling times were replicates. It may be of interest for others to evaluate whether this approach to study design is a characteristic of studies reviewed here or a characteristic of many bench science studies.

The description of the results of an experiment should include the number of samples taken at each sampling time point per treatment of that experiment. This information enables understanding of the stability of summarized estimates and the role of chance in the outcome. When possible, multiple replicates should be used. An inoculated suspension is typically used to create replicates, which are then sampled at various time intervals over the duration of the study. If a single suspension creates a single replicate (N=1), which is sampled singly (n=1) over time, it is not possible to assess variance inherent in the virus and the experimental procedure. If multiple replicates were made from the single inoculated suspension (pseudo-replication), variance could be determined, but it would refer to the variance within the original suspension. A preferable experimental design would be to have multiple inoculated suspensions to create multiple and different replicates, enabling the repeated sampling method to assess variance for both the within sample and between sample measurements (Figure 2).

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Regardless of sampling method, the number of samples tested should be reported with the observed data or in the summary statistics with the relevant measure of variation. None were reported in summary statistics in the studies evaluated in this review (Figure 1, Table 2, item 14). Care should also be taken to describe any transformations and statistical methods used to assess the outcome, as evidenced in our review, only 3 of 19 studies described statistical tests to any detail (4,23,24).

It was unexpected to find so few studies reporting results as decay rates or half-lives of the virus. Virus titer, percent virus remaining and duration of persistence are not easily applicable to the field as they can only be useful when exact starting concentrations are repeated. Alternatively, results reported as decay rates or half-lives have significantly more utility, as they can be applied to any starting concentration, and therefore are able to be used in existing environmental settings and can be applied to any known starting concentration of virus.

This review suggests that, as has been documented in other fields, the reporting of these studies may be less than ideal to meet the requirements for a reproducible description of an executed study. Further, beyond looking at the reporting methods this review identified a common flaw in design execution. Our methodological assessment confirms the need for additional but significantly improved studies regarding influenza persistence in the environment; the need for more transparency, with more focus on detailed reporting within sampling; and the need for attention to replication, to provide more robust outcome information to support decision-making and policy formation. It is currently unclear if the issues highlighted are specific to the test base or indicative of larger concerns. Additional studies of this nature on different topics would be required to understand if a systemic problem exists. In the areas where guidelines have been published, evidence of systemic problems with design execution and reporting in multiple fields led to guideline development. This area of study reporting evaluation is in its infancy in the bench sciences, but deserves continued, aggressive attention to improve the information available for field application as well as decision making.

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Tables and Figures

Table 1. The seventeen key reporting features evaluated in studies reporting the persistence of a virus in the environment.

	Design feature	Elaboration	Rationale for feature
Attributes of the virus			
1	Virus detection assay	Provided a description of the assay including the virus concentration units of the studied suspension (fluid/air), and the limits of detection for the assay.	Quantitative assays are required to calculate a virus decay rate. The limit of the detection of the assay is needed to interpret "not detected".
2	Influenza subtype	Stated the virus subtype based on standardized Hemagglutinin and Neuraminidase classification (taxonomy), and the organism from which the virus was initially recovered.	Subtype and species of interest should be stated to allow for proper statistical assessment if subtype appears pertinent to the model.
3	Virus propagation method	Provided a description of the propagation method and passage number for virus amplification in the experiment.	The dynamics and behavior of a virus can change with increased numbers of passages and among culture cell types.

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Table 1. Continued

	Design feature	Elaboration	Rationale for feature
Attributes of the setting			
4	Matrix	Provided a complete description of matrix including characteristics such moisture content, particulate matter, and source.	A complete description of the matrix is needed to assess the validity of the matrix to field application, and to make an experiment reproducible. For example, the observations and inference obtained from persistence measures in buffered water, distilled water or lake water vary, as will the inference obtained from persistence measures derived from fresh moist feces compared to dry feces.
5	Experimental baseline data	Provided quantifiable descriptions of the non-manipulated parameters in the study. Issues that may be relevant depend upon the matrix but may include room temperature, altitude, and relative humidity.	Terms such as room temperature, tap water, normal pH and sunlight fail to convey the study settings accurately. Quantifiable descriptions are needed. For research synthesis application such as meta-analysis and risk assessment, baseline data can become study level observations. Further, baseline data may represent effect modifiers that enable understanding of results from different studies because they were conducted at different baseline settings such as temperature or relative humidity.
6	Investigator manipulated parameters	Provided quantifiable descriptions of the parameters manipulated by the investigator e.g., pH, salinity, mineral content, relative humidity, temperature or ultraviolet intensity. Include a description of the sensitivity of the equipment used to measure the investigator manipulated parameters of interest.	Specific details of the manipulated conditions enable reproduction, assessment of the external validity of the study and comparison between studies. Terms such as room temperature, tap water, normal pH and sunlight fail to convey the study accurately and are not reproducible. Quantifiable descriptions are needed.

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Table 1. Continued

	Design feature	Elaboration	Rationale for feature
Attributes of the setting			
7	Method of inoculating the matrix: Equipment	Described the equipment used to perform the study and how was it used.	The sensitivity of equipment and standard errors can vary significantly therefore the equipment or tools must be described completely. For example, it is likely inaccurate to compare outcomes from aerosol studies using an enclosed room and single circulating fan with a study using a rotating drum and a nebulizer.
8	Inoculated suspensions	Described whether the study used a single inoculated suspension or several independently inoculated suspensions.	For understanding the inference from the study and the appropriateness of the data analysis, knowledge of the number of independent suspensions studied is critical. This information combined with the number of replicates (N) and samples (n) provides the basis for data analysis. This aspect of a study should be documented, since it would add unaccounted error in future meta-analyses.
9	Starting concentration in the matrix	Described the concentration post-matrix equilibration at the initiation of the experiment, and described time for equilibrium.	There is a time interval at the beginning of all experiments in which the inoculums equilibrate to the media (dilution effect) and virus is lost (e.g. to adherence to glass, precipitation due to large droplet size). Therefore the starting concentration might not be solely a function of dilution in the matrix. The concentration of the virus in the matrix should be measured after a biologically sensible equilibration time.

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Table 1. Continued

	Design feature	Elaboration	Rationale for feature
Study protocol			
10	Study duration	Described the total duration of the study in hours or days – inoculation to endpoint, as well as any stopping rules.	Clearly stating study duration enables comparison of study design with duration of study reported. In addition, it allows incorporation into future meta-analyses.
11	Sampling interval	Described the exact day of sampling i.e. Day 0,2,3,4. Used specifics rather than terms such as "every 2nd day" which could lead to confusion if the start day was not clear.	Intervals should be of the smallest increment to ensure recovery of live virus within multiple samplings. The interval should be based on previous work. The closer the intervals of sampling, the more can be learned about the persistence of the virus. The first sample should be taken after a biologically sensible equilibration
12	Number of replicates	Stated the number of replicates.	The replicate is the unit made from the inoculating suspension. Replicates (N) and samples (n) taken per replicate per time interval form the basis of a variance estimate of the virus concentration (figure 2).
12a	Multiple replicates		Variation within a population is normal. Therefore multiple observations are required to describe a population. Studies with a single observation cannot assess variation and are of limited value for inference to the population.
13	Number of samples per replicate per time point	Described the sample unit studied from each replicate. Stated the number of samples taken per replicate per time interval.	As noted above, replicates (N) and samples (n) taken per replicate per time interval for the basis of the variance estimate of the virus concentration (figure 2).

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Table 1. Continued

	Design feature	Elaboration	Rationale for feature
Data handling and analysis			
14	Method to summarize samples and replicates at each interval	Described the statistic (i.e. mean, standard deviation, range) used to summarize data at each sampling interval. Describe whether the data will be transformed prior to summarization or after.	Understanding how data is summarized and transformed is necessary for accurate interpretation, repeatability and future meta-analyses. Therefore the method used should be clear to the reader. For example, a frequent error is failure to report clearly, log transformation of the original data and subsequent calculation of the mean of the log transformed values, i.e. the geometric mean.
15	Statistical method used to estimate outcome (s)	Stated the outcomes assessed and described the methods of calculating a persistence or virus decay rate, including the statistical model and model assumptions used to estimate decay.	The description of statistical methods allows reproducibility, assessment of the validity of the model and enables future assessments to compile results from multiple studies. Virus persistence should be reported as a rate, rather than a specific time, since the exact length of persistence depends on the starting amount of virus in a given setting.

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Table 1. Continued

	Design feature	Elaboration	Rationale for feature
Results			
16	Descriptive results	Provided the summary outcome and variance of the measurements at each time point.	Specifying a mean value should include the n of each sampling as well as the variance of results around that mean. Standard error is not a useful summary method for variance, as it pools often unrelated samples. Whether the study intends to analyze a decay rate or persistence, reporting data in a manner so information can be replicated or used in future analyses enhances the value of the results. Virus persistence should be reported as a rate, rather than a specific time, since the exact length of persistence depends on the starting amount of virus in a given setting.
17	Results of statistical test (s) used to estimate virus decay (Regression models)	Provided the estimate of decay rate (based on the methods proposed in item 15) and an estimate of the variance of the decay rate.	Transparency of the model and estimates of the fixed variables in the model is critical for external validity. Confidence intervals provide insight to the variance of the estimates as well as the utility of the model. Likelihood testing as well as model fit should be assessed and reported.

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Figure 1. Frequency of reporting key design features by item (Table 1, 2) in studies reporting the persistence of influenza in the environment (11)

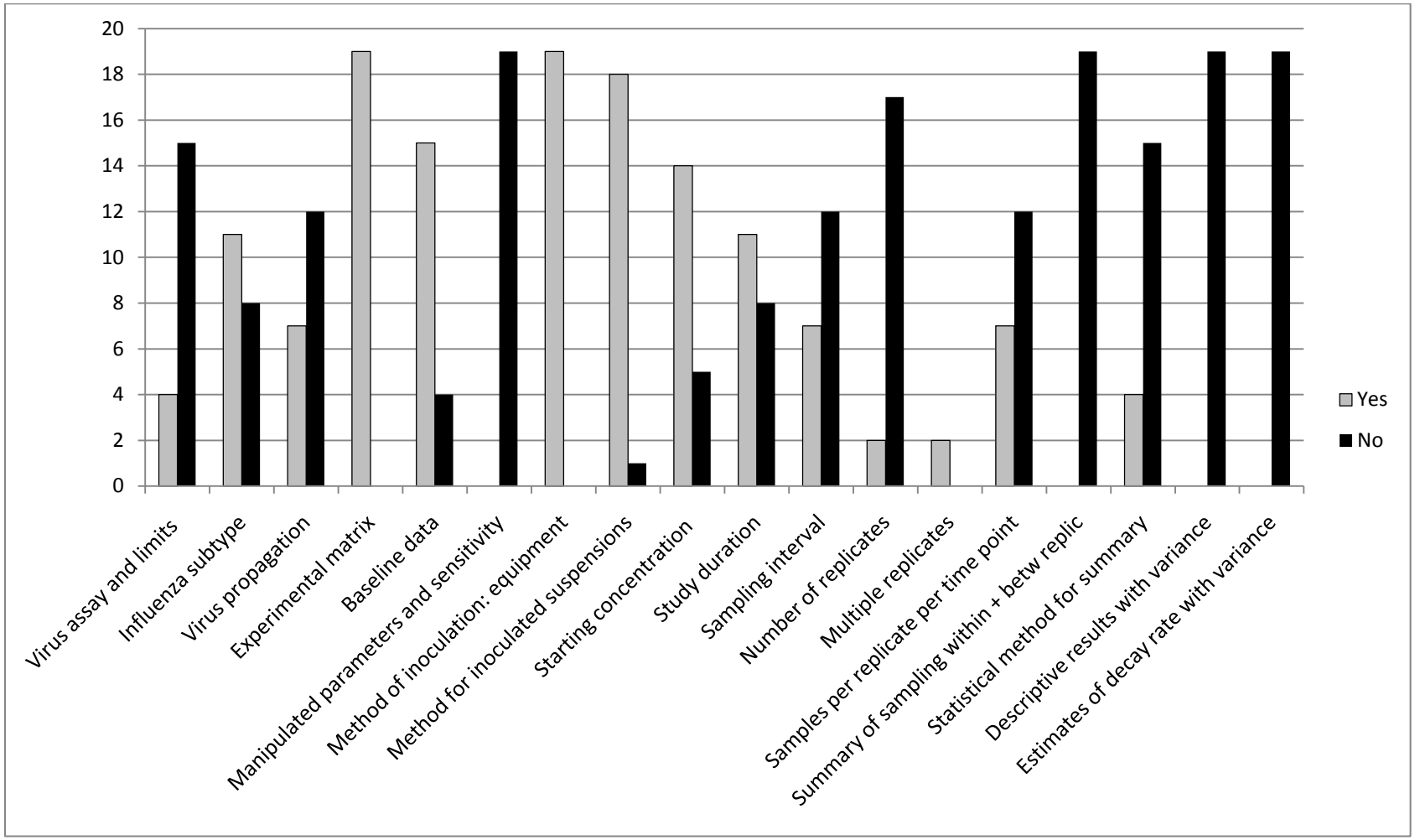

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Table 2. Checklist of frequency of reporting key design features by matrix and year of publication in studies reporting the persistence of influenza in the environment (11)

		Number positive/total possible						
	Design feature	Air	Water	Feces	Fomites	<1970	1970-1990	≥1990
1	Described virus assay and limits of detection	1/7	2/7	0/2	1/3	1/7	0/4	3/8
2	Described the influenza subtype, including H and N components	0/7	6/7	2/2	3/3	0/7	3/4	8/8
3	Described virus propagation method and passage	2/7	3/7	0/2	2/3	1/7	2/4	4/8
4	Described experimental matrix	7/7	7/7	2/2	3/3	7/7	4/4	8/8
5	Described experimental baseline data	5/7	7/7	1/2	2/3	5/7	4/4	6/8
6	Described investigator manipulated parameters and sensitivity of the equipment used	0/7	0/7	0/2	0/3	0/7	0/4	0/8
7	Described method of inoculating matrix: equipment	7/7	7/7	2/2	3/3	7/7	4/4	8/8
8	Described method for inoculated suspensions	7/7	7/7	1/2	3/3	7/7	4/4	7/8
9	Described starting concentration in the matrix	5/7	6/7	0/2	3/3	5/7	2/4	7/8
10	Described study duration in methods	3/7	6/7	0/2	2/3	3/7	1/4	7/8
11	Described sampling interval in methods	2/7	4/7	0/2	1/3	2/7	1/4	4/8

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Table 2. Continued

		Number positive/total possible						
	Design feature	Air	Water	Feces	Fomites	<1970	1970-1990	≥1990
12	Described the number of replicates	0/7	1/7	0/2	1/3	0/7	1/4	1/8
	12a) Multiple replicates	0/7	1/7	0/2	1/3	0/7	1/4	1/8
13	Described the number of samples per replicate per time point	1/7	3/7	0/2	3/3	1/7	1/4	5/8
14	Described summary of sampling within and between replicates, as well as transformed data	0/7	0/7	0/2	0/3	0/7	0/4	0/8
15	Described statistical method used to summarize outcome (s)	0/7	3/7	0/2	1/3	0/7	1/4	3/8
16	Provided descriptive results with variance	0/7	0/7	0/2	0/3	0/7	0/4	0/8
17	Provided estimates of decay rate with variance	0/7	3/7	0/2	0/3	0/7	0/4	0/8

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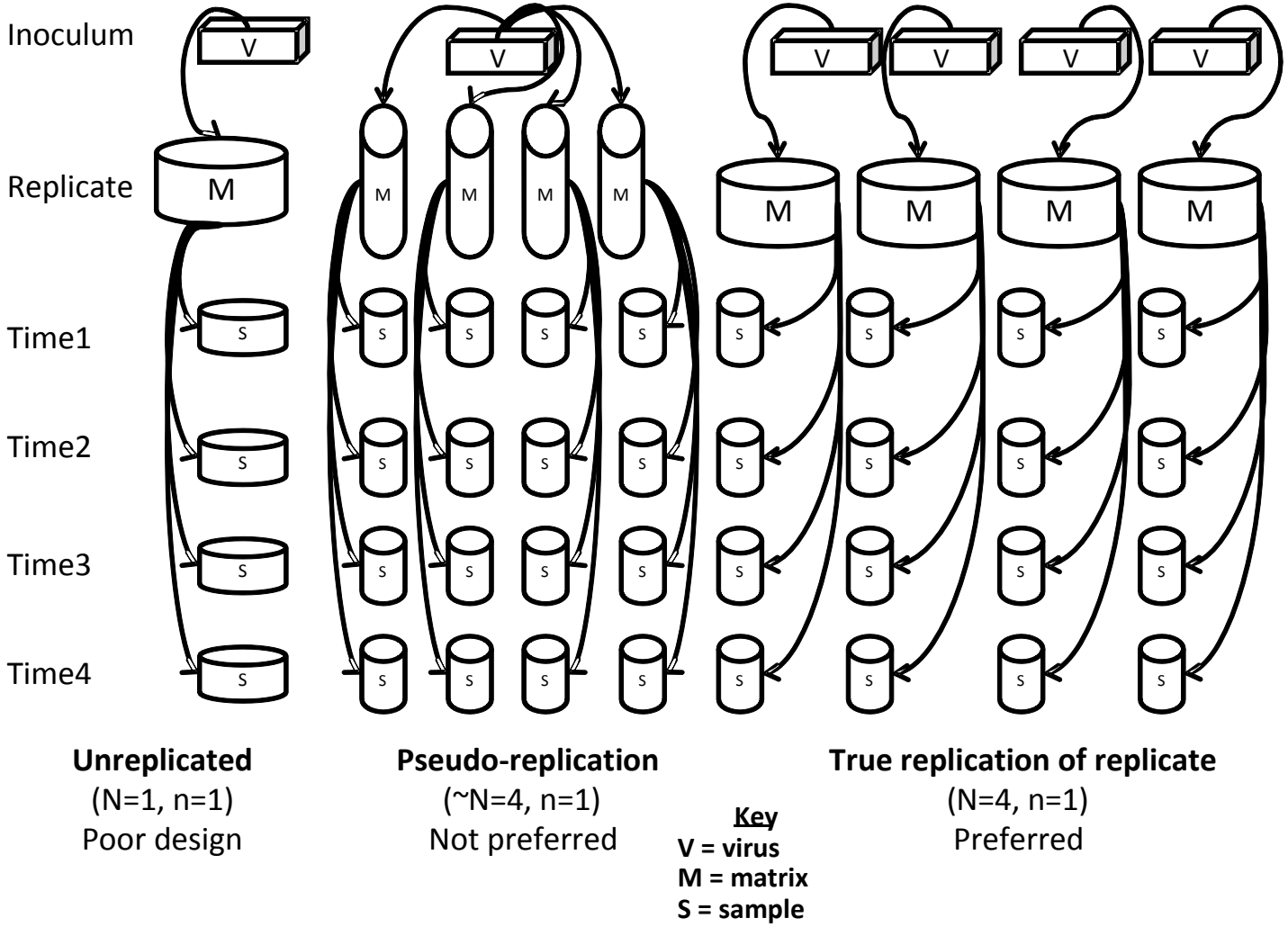
Figure 2. Comparison of methods for multiple replicates and sampling to achieve time interval point estimates.



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CHAPTER 4. CONCLUSIONS ON SYSTEMATIC REVIEWS IN AGRICULTURE FOOD SCIENCE

Summary

After performing a systematic review on the persistence of influenza in the environment, and identifying and quantifying the gaps in both knowledge and reporting in the literature, this thesis has not only shown alignment with current systematic reviews in veterinary medicine, identifying gaps in literature and study design and reporting, it confirmed the need for implementation of more systematic reviews in the bench sciences, as these sciences are fundamental to the veterinary field. Still, there are key issues which need to be dealt with to apply systematic reviews to the laboratory sciences.

Indicators of quality reporting in microbial research

As discussed in Chapter 1, guidelines for quality reporting in randomized controlled trials have been created by the CONSORT and readily adopted and implemented in the field of human medicine. Similar guidelines have evolved from this template when the need has been identified (typically by systematic reviews), for example the guidelines for reporting observational studies STROBE (15,16), guidelines reporting of meta-analyses the QUORUM (4) and MOOSE (14) statements, and PRISMA (5), for reporting systematic reviews. Guidelines for less dichotomous qualification methods, such as diagnostic test evaluations are understandably more difficult to create, but are in process (STARD statement (1)) given the need and critical role diagnostic tests or test evaluations play in fundamental scientific discovery. Once implemented, reporting guidelines allow for a more objective evaluation of the validity of a manuscript, by ensuring the transparency of the design and analysis, and therefore enabling an assessment for bias as well. As guidelines become the accepted and adopted standards for reporting of future work, it will raise the bar for research reporting.

Chapter 3 identified there are currently no guidelines for bench science reporting, and there is significant need to improve the reporting, reduce the heterogeneous designs and measures, and analyze outcomes with adequate statistics, in the field of microbiology. Because much

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of microbiology is ultimately applied to the field, particularly when involving livestock diseases, creating guidelines for the reporting of microbiologic studies would benefit not only the discipline of microbiology and the scientists themselves, as described in Chapter 1, but also production veterinary medicine, public health, decision making government personnel and the livestock industry. The recommendations outlined in Chapter 3 for laboratory science reporting evolved from the observation of repeatedly missing information in the studies assessed in the systematic review on the environmental persistence of influenza. They are comprehensive and systematic enough to provide a template for the construction of a true set of laboratory science guidelines in the near future. A hopeful and natural sequelae of these guidelines is that editors and reviewers for publication will begin to evaluate manuscripts using them, which will provide the additional impetus for adoption, as well, inherently improve the quality of the published literature.

To be clear, reporting guidelines will improve the quality of studies and their reporting, facilitating evaluation for bias and study repeatability. Quality assessments relate more to the field of study, as well the question of interest of the research, and typically involve experts in the field, who assist in developing criteria which are then used for the assessment. For example, using the influenza persistence study, reporting guidelines would recommend the specific un-manipulated and manipulated parameters be documented, as well as the method and measurement units for the outcome of virus persistence. To evaluate the quality of a study for the investigation of persistence, on the other hand, inclusion criteria from experts in the field mandate only studies reporting virus persistence using concentration units (i.e. EID_{50} $TCID_{50}$ etc) and virus recovery at multiple time intervals be considered "quality" studies. Studies which provide only a single summary value for the duration of persistence, or state virus detection using egg embryo death or survival for example, contain insufficient information to evaluate true persistence or virus decay over time.

Indicators of sources of bias in microbial primary research

Given the utility and understanding of what a systematic review can provide a particular field of medicine, it is noteworthy that they are virtually absent in the bench sciences, which provide the foundation for applied studies in livestock medicine. There are several aspects of

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a systematic review that make it very applicable to the laboratory sciences. Systematic reviews must be transparent and repeatable - both of these attributes should be fundamental to reporting the results of laboratory research. Transparency and comprehensive reporting not only allow scientists to better evaluate a study and repeat it if desired, but they allow the design of a study to be judged for the potential of bias and help create a foundation for consistency of future work. O'Connor et al (7) identified a study reporting on the community health effects of animal feeding operations, where subjects for inclusion to the cohort were identified by local activists, as persons who were "distressed about the effects of the nearby hog farms"(2). Because of the transparency of the study, the potential for selection bias of the subjects was clearly evident. Similarly, when studies are more clearly and completely reported, the meta-analysis of the work should be not only more precise, but more valid. For example, in an environmental persistence study of a highly transmissible virus, if a temperature setting of an experiment was described as "outdoor", it should be considered unacceptable as this term is not specific and cannot be recreated in another study. If, however, this study was included in a meta-analysis of virus persistence it could lead to misclassification bias of the data, since "outdoor" would need to be interpreted because the true temperature was unknown. Several examples like this were uncovered through the systematic review of Chapter 2, identifying why guidelines for reporting would be so appropriate to the field of microbiology.

Just as transparency (or quality reporting) allows for the evaluation of bias in a study, it helps assure the statistical assessment of a study is described and performed clearly also. As previously stated, the information learned in the laboratory is directly applied to the field at some point, however if no statistical assessment is performed, expected outcomes in the field are uncertain at best. An important quality reporting (as well as assessment) criterion that is particularly applicable to the bench sciences is replication. Replication allows for the determination of variance, and variance allows for better understanding of normal variation around determined estimates. Variance, or standard deviation, can only be determined when multiple replicates are used, and if the data is summarized such that there are sample and replicate summary statistics inclusive of demonstrated error, rather than a single mean value.

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Historically, statistical assessments in the bench sciences literature have been poor, which limits the applicability of the information discovered. These items are critical for livestock medicine, since, as stated previously, the laboratory is the source for information that is quickly adopted and expected to be useful to the field. With such a lack of validity, or support of validity, it is unsettling to see current studies adopted so readily, for not only animal health, but also policy and decision making endeavors.

Systematic reviews will identify current gaps in the bench science literature as they have in the medical and food safety arena, to the benefit of the research community. The outcome will be not only better scientific investigations due to better reporting of methods, but also improved utility to the field, and the ability to create meta-analyses on data that is more unified in parameters and measurements. This advancement will ultimately promote true progress in the scientific community, the food animal veterinary field and the public at large.

The critical component of a systematic review is the systematic and transparent nature of the process. When the fundamental comprehensiveness or methodologic nature of the systematic review is not adhered to, admittedly outcomes from such reviews will be guilty of the same bias as a study which was not transparent on their reporting (6,11). The field of systematic reviews is incipient in veterinary science, therefore it is necessary to focus on the continuous improvement of this relatively new technique as this tool will be a significant resource for applicable, summarized data for veterinarians and decision makers in the near future.

Still, systematic reviews, as with any critiquing literature, have been criticized on several points themselves. A common criticism is that systematic reviews are so refined in their scope, that the results may not be applicable or the quality criteria are too narrow that rarely can studies measure up for inclusion. Critics challenge that the review outcome in these circumstances of, “more and better research is needed” is ultimately self fulfilling, and the review provides limited utility to guide practice or policy (9). There are additional criticisms of the review process, in that although there are a significant number of citations at the outset of all reviews, because the screening process is so cursory (just reviewing the title and abstract of each citation) it is plausible that there are citations that are missed through

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oversight. This hypothesis is countered, however, with the facts that the screening process should be performed by at least 2 independent reviewers to reduce selection bias, and hand gathering of citations is a pertinent step of the process, to find articles cited by other reviews which may not have been found in the search engines (9). Potts et al (10) discuss that often RCTs (and therefore syntheses of RCTs) are over-rated particularly where resources are scarce (naming oral fluid treatment of childhood diarrhea, circumcision and HIV transmission and misoprostol treatment of postpartum hemorrhage), however the underlying assumption for this concern is that systematic reviews only value RCT, another unfounded criticism (8). These concerns are more tenable than the original parachute argument by Smith (13), where the concept of lack of evidence is taken to absurdity, with the claim that there are no RCT supporting the use/value of parachutes to prevent trauma due to "gravitational challenge", therefore the statement that parachutes are a valuable intervention for skydiving is unfounded and full of uncertainty. Truthfully, observational studies are more common in veterinary medicine and microbiology, and they provide very applicable and useful information. In systematic reviews, it is the transparency of reporting, not the type of study, that should be adhered to in order to enable detection of potential sources of bias and assess validity.

Admittedly, systematic reviews take time, and their ability to remain current with the available literature has been questioned (3,12). Shojania et al (12) found that depending on the field of study, it was possible that a systematic review could be outdated before or at publication, particularly in the field of cardiovascular medicine, but the median time before a signal (indicator for information re-review) was 5.5 years (CI 4.6-7.6yr) in this particular review of 100 systematic reviews.

The future

Ultimately, in addition to significantly improved reporting and study designs, there needs to be more and better synergy between the laboratory sciences and field application. Often studies are run in a "vacuum"-type setting, and outcomes are not practical for field application to livestock production medicine, if there is no plan for follow up studies. Given the current public awareness and interest in agriculture, as well the sensitivity to animal well-

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being, it should become routine and expected to form partnerships or collaborations between the scientific and agricultural communities, to apply laboratory learning to the field more routinely or have follow-up corroboration studies. To illustrate, influenza persistence studies using distilled water, or water buffered with PBS provide limited insight to real world settings, therefore, after discovering foundation knowledge in the laboratory, studies involving the same virus, but in settings of tap water from animal drinkers, water troughs themselves as well as lagoon or pit waters that may be re-cycled for flushing of waste in confinement operations should be executed, as they are more realistic and concerning matrices for influenza persistence in livestock settings. Likewise, humidity and temperature values that are collected and monitored in the barn, water, lagoon and air sources should be used as environmental settings of interest for future influenza persistence studies.

In this way, when systematic reviews gather a body of evidence which has been reported well, with consistency and transparency, the meta-analysis that is synthesized will not only have strong external validity, it will provide solid estimates to answer questions, in this case, about the duration of influenza persistence in environmental settings. It will report variation, equally important to understanding normal or expected variability around the mean under given conditions. And, it will be more valuable and enduring for both veterinarians and decision makers creating policies, given the high quality of the resultant information and robustness of the data. The systematic review methodology can be an indispensable tool for the advancement of the laboratory sciences, given the continuing focus from veterinarians, public health officials and policy makers on critical reporting of the essential components of study designs. The measures and assessments for study quality will enhance the scientific foundation of livestock medicine, thereby promoting the development of synergies between the laboratory and the field for succeeding studies in the future.

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