The effect on mortality of antipyretics in the treatment of influenza infection: systematic review and meta-analysis

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DECLARATIONS

Competing interests
The Medical Research Institute of New Zealand and the University of Otago have received research funding from GlaxoSmithKline, one of the manufacturers of paracetamol. RB has received fees for consulting and speaking from GlaxoSmithKline

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Ethical approval
Not applicable

Summary

Objective To determine whether antipyretic treatment for influenza infection influences the risk of mortality in animal models and humans.

Design A systematic search of Medline, Embase and the Cochrane Register of Controlled Trials was undertaken to identify randomized placebo-controlled trials of antipyretic use in influenza infection in animal models or humans that reported mortality. A quantitative meta-analysis of the risk of death using Peto's one step odds ratio with calculation of the pooled risk of death and standard evaluation of heterogeneity was undertaken.

Setting Not applicable.

Participants Not applicable.

Main outcome measures Risk of mortality associated with antipyretic use in influenza infection.

Results Eight studies from three publications met the inclusion criteria. No human studies were identified. The risk of mortality was increased by antipyretic use in influenza-infected animals with a fixed effects pooled odds ratio of 1.34 (95% CI 1.04–1.73). An increased risk was observed with aspirin, paracetamol and diclofenac.

Conclusion In animal models, treatment with antipyretics for influenza infection increases the risk of mortality. There are no randomized placebo-controlled trials of antipyretic use in influenza infection in humans that reported data on mortality and a paucity of clinical data by which to assess their efficacy. We suggest that randomized placebo-controlled trials of antipyretic use in human influenza infection are urgently required, and that these are sufficiently powered to investigate a potential effect on mortality.

Guarantor
SE

Contributorship
SE undertook the systematic review and together with PS, KP and RB extracted the data for the meta-analysis which was undertaken by MW. All authors contributed to drafting the manuscript

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Introduction

In response to the recent global influenza pandemic, guidelines recommend that paracetamol or ibuprofen be used to treat fever and systemic symptoms of influenza, in both children and adults.[1,2] These recommendations are qualified by the acknowledgement that there is little scientific evidence for this approach, but that experience suggests that it may help and is unlikely to cause harm.[2] However, there is evidence that fever is a phylogenetically ancient host response to infection that may result in survival benefit and that treatment with antipyretics may adversely influence the course of infectious diseases.[3,4] In non-human mammals, antipyretic treatment increases the risk of mortality due to different bacterial, viral and parasitic infections.[5] In humans, the use of paracetamol for severe sepsis may increase the risk of mortality,[6] although the evidence regarding ibuprofen in severe sepsis is uncertain, with one study showing a non-significant 1.8-fold increased risk[7] and another larger study reporting similar rates of mortality in ibuprofen and placebo groups.[8] Paracetamol and/or aspirin increase the duration of viral shedding in rhinovirus infection,[9,10] prolong parasitemia in malaria[11] and delay healing of skin lesions in chickenpox.[12]

Comparable data for influenza infection in humans are limited to a retrospective review of six clinical trials which reported that paracetamol or aspirin use was associated with an increased duration of illness in experimental influenza A infection.[13] The evidence for this association is of modest strength as in the reviewed trials treatment was not randomized and antipyretic use may have been confounded by illness severity.[13] Non-experimental studies suggest an association between the use of aspirin and Reye's syndrome in febrile illnesses (including influenza) in children,[14,15] however, the nature of the association has been debated.[16] The purpose of this systematic review is to identify randomized placebo-controlled studies of antipyretic use for influenza infection in animal models or humans and to investigate whether antipyretic use influences the risk of mortality in influenza infection.

Methods

Eligibility and search strategy

A search of Medline was conducted from January 1950 to June 2009; of EMBASE from January 1947 to June 2009 and of the Cochrane Central Register of Controlled Trials in the second quarter of 2009. Studies were searched for using the keywords 'paracetamol', 'acetaminophen', 'NSAID*', 'non-steroidal anti-inflammatory*', 'aspirin', 'ASA' or 'antipyre*' and combined with the keyword 'influenza' (not 'review*', 'letter*', 'editorial*' or 'conference*'). Relevant studies written in foreign languages were translated. The reference lists of the relevant studies were examined.

Studies that met the following criteria were included in the meta-analysis:

- An *in vivo* animal study, or randomized controlled trial in humans of influenza virus infection;
- Treatment with the antipyretics aspirin, or paracetamol, or a non-steroidal anti-inflammatory drug (NSAID), and the dose of antipyretic was below the potentially lethal range;
- A placebo or control group was used in comparison to the antipyretic;
- Mortality data were reported;
- The study was not an influenza vaccine trial.

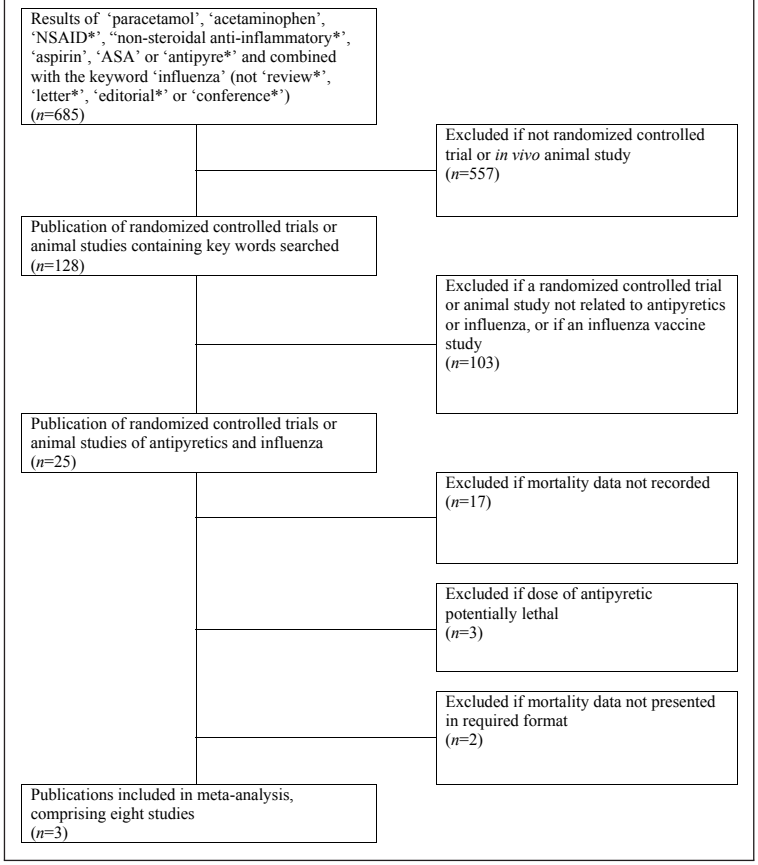
Data abstraction

One author (SE) examined each paper's title and abstract, and the full paper if necessary, to determine if eligible for inclusion. Four authors (SE, RB, KP and PS) independently extracted data from selected studies including animal type and number, antipyretic used, strain of influenza, Reye's syndrome model used and results (mortality outcome).

Statistical methods

Meta-analysis was by Peto's one step odds ratio, carried out according the formulae given by Bradburn and colleagues.[17] Publication bias was explored by both formal statistical tests and a funnel plot. Heterogeneity of study estimates was tested by standard methods with the intention of performing a meta-regression based on study level characteristics.[18] These were pre-specified as the type of animals, whether more than one specific type of antipyretic was compared with the control treatment, whether infection was with influenza A or B and whether the study used an experi-

Figure 1
QUOROM statement showing the flow of study selection



mental model of Reye's syndrome. One of the identified papers (Crocker *et al.*)[19] had sufficiently detailed data to be re-analysed by logistic regression to estimate the difference in mortality between aspirin and paracetamol in mouse models of influenza infection.

Results

The outcome of the search strategy is shown in Figure 1. Three publications reported eight trials that met the inclusion criteria (Table 1).[19–21] All studies involved animal models: mice (n=7), chickens (n=1). In two studies influenza A was used and in six studies influenza B was used. In two studies a chemical (the surfactant toximul) model of Reye's syndrome was used. The specific antipyretic used was aspirin in eight studies, paracetamol in four studies, and diclofenac in two studies. In six studies more than one antipyretic was compared with control treatment. In total, 1116 animals were studied with 697 in the antipyretic and 419 in the control treatment groups.

In the studies of Crocker *et al.*,[19] the effects of aspirin and paracetamol on mortality due to influenza B infection were investigated in neonatal and weanling mice, as well as the effects following pre-treatment with low doses of the industrial surfactant Toximul MP8, which produces many of the features of Reye's syndrome. *In vitro* studies were also undertaken which demonstrated that both aspirin and paracetamol caused a dose-dependent reduction in interferon-induced antiviral responses. In the studies of Davis *et al.*,[20] the effects of single or multiple doses of aspirin on mortality due to influenza B infection were investigated in three-week-old mice. Additional experiments were also undertaken in which influenza infection was shown to interfere with aspirin metabolism, markedly increasing the aspirin blood levels. In the studies of Sunden *et al.*,[21] the effects of aspirin and diclofenac on mortality due to influenza A infection were investigated in murine and poultry models of influenza encephalitis. Histology and immunohistochemistry were also undertaken, demonstrating that antipyretic treatment aggravated the haematogenous spread of influenza virus to the central nervous system in chicks.

Two additional studies in animal models were excluded as mortality data could not be derived. In a Romanian study of influenza A infection in which mice were treated with acetylsalicylic acid, the protective effect (expressed as the protective index), was 50% at Day 6, but by the end of the 11-day observation period, mortality was increased by 82%.[22] In a Polish study of influenza A infection, mice treated with indomethacin or aspirin had a 90% and 75% mortality at the end of the 12-day observation period, respectively, compared with a 50% mortality rate in both placebo groups, representing an 80% and 50% increased risk of mortality associated with indomethacin and aspirin, respectively.[23]

Three additional studies of influenza infection in ferrets were excluded as the dose of antipyretics administered was potentially lethal. In the study of Linnemann *et al.*,[24] 10 of 19 (53%) ferrets infected with influenza A or B receiving aspirin died, compared with none of the 11 (0%) untreated ferrets infected with influenza A or B (odds ratio 9.9, 95%

Table 1
Characteristics of studies included in meta-analysis

Study		*Animal type*	*Animal numbers*[*]	*Antipyretic*	*Strain of influenza*	*Model*[†]
Number	*Reference*					
1	Crocker *et al.*[19]	Newborn mice	T=155 C=98	Aspirin or paracetamol	B	–
2	Crocker *et al.*[19]	Weanling mice	T=114 C=74	Aspirin or paracetamol	B	–
3	Davis *et al.*[20]	Mice	T=61 C=60	Single dose aspirin	B	–
4	Davis *et al.*[20]	Mice	T=30 C=25	Multiple dose aspirin	B	–
5	Sunden *et al.*[21]	Mice	T=16 C=4	Aspirin or diclofenac	A	Encephalitis
6	Sunden *et al.*[21]	Chicks	T=24 C=6	Aspirin or diclofenac	A	Encephalitis
7	Crocker *et al.*[19]	Newborn mice	T=149 C=88	Aspirin or paracetamol	B	Reye's syndrome[†]
8	Crocker *et al.*[19]	Weanling mice	T=148 C=64	Aspirin or paracetamol	B	Reye's syndrome[†]

[*] T = Treatment group; C = Control group
[†] Reye's syndrome model: chemical (the surfactant toximul)

CI 2.1–46.4); 6 of 13 (46%) ferrets given only aspirin died. In the study of Deshmukh *et al.*,[25] which used an arginine-deficient dietary model of Reye's syndrome, 12 of 16 (75%) ferrets infected with influenza receiving aspirin died, compared with 1 of 5 (20%) untreated ferrets infected with influenza B (odds ratio 9.2, 95% CI 1.2–69.4); 2 of 6 (33%) ferrets given only aspirin died. In the study of Mukhopadhyay *et al.*,[26] which used an arginine-deficient dietary model of Reye's syndrome, 4 of 7 (57%) ferrets infected with influenza B receiving ibuprofen died, compared with 0 of 5 (0%) untreated ferrets infected with influenza B (odds ratio 10.6, 95% CI 1.0–108.6); 1 of 5 (20%) ferrets given only ibuprofen died.

No randomized placebo-controlled trials of antipyretics in influenza infection in human participants reported mortality outcomes. Three randomized placebo-controlled trials were identified that investigated the effects of antipyretics in the treatment of suspected influenza.[27–29] In these studies antipyretic treatment reduced fever and symptoms compared with placebo, however, in none was influenza positively diagnosed by virologic means. There were four randomized controlled trials of antipyretics in diagnosed influenza, but none included a placebo control group.[30–33] Three of these trials compared amantadine or rimantidine with various antipyretics;[30–32] one showed amantadine was more effective than aspirin in reducing signs and symptoms of influenza,[30] another reported that rimantadine reduced fever and symptoms of influenza early in the course of the illness but resulted in greater viral shedding late compared with paracetamol,[31] while the third found no difference in the clinical course of influenza between rimantadine and paracetamol groups, although rimantadine resulted in reduced viral shedding early in the course of the illness.[32]

There was one publication which included data from six clinical trials of intranasal challenge with influenza A, in which aspirin or paracetamol were offered for symptomatic relief. Influenza infected subjects who received antipyretics were sick on average 3.5 days longer than those not receiving such treatment. In a multivariate analysis which considered clinical variables such as maximum temperature and maximum number of symptoms, only antipyretic therapy exhibited a statistically significant relationship with duration of illness. However, antipyretic treatment was not

Table 2
Risk of mortality associated with antipyretic treatment in individual studies

Study		*Mortality/Total (%)*		*OR (95% CI)*
Number	*Reference*	*Antipyretic*	*Control*	
1	Crocker *et al.*[19]	70/155(45.2)	33/98(33.6)	1.6(1.0–2.7)
2	Crocker *et al.*[19]	32/114(28.1)	19/74(25.7)	1.1(0.6–2.2)
3	Davis *et al.*[20]	40/61(65.6)	32/60(53.3)	1.7(0.8–3.4)
4	Davis *et al.*[20]	12/30(40.0)	12/25(48.0)	0.7(0.3–2.1)
5	Sunden *et al.*[21]	3/16(16.7)	0/4(0)	4.0(0.2–80.5)
6	Sunden *et al.*[21]	4/24(16.7)	0/6(0)	4.0(0.3–53.6)
7	Crocker *et al.*[19]	80/149(53.7)	36/88(40.9)	1.7(1.0–2.8)
8	Crocker *et al.*[19]	50/148(33.8)	24/64(37.5)	0.9(0.5–1.6)

randomized, and subjects treated with antipyretics had higher maximum temperatures and more marked symptoms, suggesting that the association is likely to have been confounded by the severity of the illness.[13]

Table 2 shows the mortality for each of the arms of the trials together with the calculated odds ratio and 95% confidence intervals for mortality with antipyretics versus placebo. Table 3 shows the pooled estimates of mortality risk together with the heterogeneity analysis of these trials. Both the fixed and random effects estimates show that antipyretic use is associated with an increased risk of mortality in influenza infection in animal models. There was no statistical evidence of heterogeneity, and as a result we were unable to explore whether there was any difference in effect by animal age, which was suggested by the studies of Crocker *et al.*,[19] in which the increase in mortality with antipyretics appeared to preferentially occur in newborn rather than weanling mice. Figure 2 shows the forest plot for these estimates. Formal tests of publication bias were not statistically significant and the funnel plot (not shown) did not suggest publication bias.

In the study by Crocker *et al.*[19] the mortality risk for paracetamol versus aspirin could be calculated and there was no difference in mortality comparing these two different antipyretics, odds ratio for death: 1.0 (95% CI 0.7–1.5).

Table 3
Pooled odds ratios and I^2 statistic relating risk of death and antipyretic use in influenza-infected animals

Type of estimate	*OR (95% CI)*	
Fixed effects	1.34 (1.04 to 1.73)	
Random effects	1.34 (1.04 to 1.73)	
Heterogeneity		
Chi-square statistic	Degrees of freedom	*P* value
6.34	7	0.50
I-squared statistic (95% CI)	0(0–64.2)	

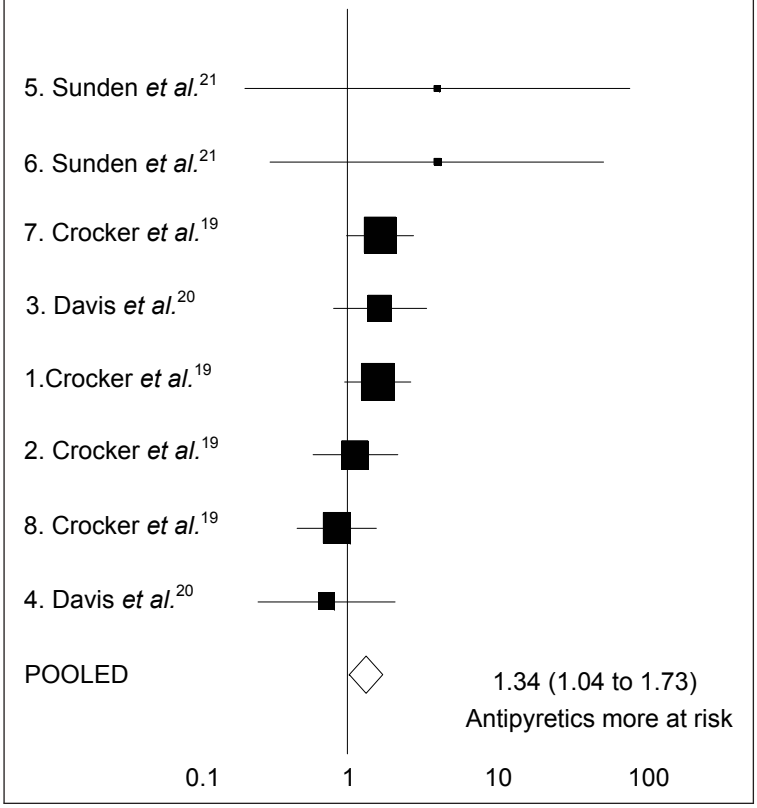
Discussion

This systematic review and meta-analysis has shown that antipyretic treatment increases the risk of mortality in animal models of influenza infection. No randomized placebo-controlled trials of antipyretic use in influenza infection in humans reported data on mortality. We suggest that randomized placebo-controlled trials of the effect of antipyretic use on the risk of mortality with human influenza infection are required.

An increased risk of mortality in animals was reported in studies of aspirin, paracetamol and diclofenac. We were not able to compare the effect of different antipyretics with each other apart from a re-analysis of a single trial which presented sufficiently detailed data.[19] For this analysis there was no difference in mortality comparing paracetamol with aspirin. As a result, the data are consistent with the effect on mortality as a class effect of antipyretics.

Not only were there no randomized controlled trials of antipyretic use in influenza infection in humans that reported data on mortality, but there was also a paucity of clinical data by which to assess their efficacy. The human studies identified

Figure 2
The forest plot shows the point estimates for each trial (in the centre of the shaded box) and its confidence interval (the ends of the lines). The size of the box is inversely proportional to the individual study variance. Studies with high variance have smaller boxes. The studies with small variance (large boxes) contribute more to the pooled study estimate, represented by the diamond. The odds ratios for risk of mortality are presented on the logarithm scale and the trials are ranked in order of the size of the odds ratio. The pooled estimate presented is the fixed effect estimate



either lacked a placebo group,[30–33] a virologic diagnosis of influenza,[27–29] or randomization of antipyretic treatment[13] which made interpretation of results difficult. There was also little uniformity of outcomes, with factors such as length of illness, amount of viral shedding and disease complications often not recorded. As a result, there is little evidence on which to assess the effect of antipyretics on the severity and/or duration of influenza infection in humans.

There are a number of potential mechanisms whereby treatment with antipyretics may increase the risk of mortality in influenza infection. The first is that human-tropic influenza viruses replicate in the upper respiratory tract at 33–37°C and that most naturally occurring influenza A strains that infect humans are temperature-sensitive, with inhibition of replication at high temperatures within the physiological range of 38–41°C.[34–37] The resulting low infectivity is likely to be due to various molecular defects, including reduced matrix protein, which is important for maintaining the structural integrity of influenza virus particles.[37,38] Human influenza A virus genome RNA synthesis is inhibited at temperatures of 41°C, with failure of replication despite transcriptional activity being maintained,[39] and impaired assembly of the viral components into infectious virus.[37] The degree of temperature sensitivity is also a characteristic that determines virulence, such that strains with a shut-off temperature of 38°C or lower cause mild symptoms, whereas influenza strains with a shut-off temperature of 39°C or more cause severe symptoms.[34] As a result, it is likely that antipyretic use leads to a reduction of the physiological febrile response which would otherwise inhibit replication. Furthermore, it suggests that the most virulent strains are those most liable to thrive with antipyretic use, as the high shut-off temperature may not be reached or sustained if an antipyretic is used and thus the virus will replicate without temperature-induced inhibition.

Temperature elevation is also associated with a wide range of immunological effects relevant to the host defence against influenza infection.[40–43] These include a greater proliferative response of lymphocytes, and increased production and activity of cytokines such as interferon. Whether reducing the physiological fever with antipyretics modifies these immunological responses, and thereby influences clinical outcomes, remains uncertain.[40] However, it is of interest that Crocker *et al.* reported that both aspirin and paracetamol decreased the interferon-induced antiviral responses of cultured mammalian cells.[19]

It is also possible that the increased mortality risk with NSAID and paracetamol treatments may have been partially due to their immunological or anti-inflammatory effects, unrelated to antipyretic activity. This is suggested by the recent study which showed that the reduction in antibody response to vaccination with paracetamol treatment occurred in children with or without febrile responses.[44] As temperature responses were not

measured in the studies included in this meta-analysis, this issue could not be addressed in this review.

There is also evidence from animal models that antipyretics may impair the response in bacterial pneumonia which may complicate influenza illness. Similar to influenza virus, many strains of *Streptococcus pneumoniae* are temperature-sensitive, with thermal death points of between 40–41°C.[45–47] Likewise, the capacity to grow at 41°C is a prerequisite, but not the sole factor in determining the virulence of *Streptococcus pneumoniae* in animal models.[46,47] It has also been demonstrated that treatment with antipyretics may increase the risk of mortality in experimental *Streptococcus pneumoniae* in animals.[48] In mice, treatment with aspirin prior to or immediately after *Streptococcus pneumoniae* inoculation increased mortality rates two to three-fold.[48] Furthermore, an elevated temperature within the physiologic range increases antibiotic bactericidal capacity against *Streptococcus pneumoniae*.[49] These findings are potentially relevant to the use of antipyretics for human influenza infection prior to the development of a secondary bacterial pneumonia, and their use during such secondary infections.

There were a number of methodological issues considered in the design and interpretation of the meta-analysis. The first is whether the systematic review identified all relevant studies. We are confident that in our comprehensive search strategy we have identified the eligible published studies including those not written in English, and the funnel plot suggested no publication bias. Two studies in animals were excluded as the actual number of deaths in each treatment group were not reported.[25,26] However, both these studies reported an increased risk of mortality with antipyretic use, of between 1.5 and 1.8-fold, consistent with our calculated pooled estimate of risk. Three additional studies were excluded as potentially lethal doses of antipyretics were administered, which explained in part the 9- to 11-fold increased risk of mortality observed with antipyretics in the setting of influenza infection. With these exclusions, there were eight studies involving 1116 animals, which resulted in the study findings being limited by the low power.

The second issue is the use of the Peto's one step as the meta-analytic method to determine the odds ratio for risk of mortality. This method was chosen as it has the best performance of a number of meta-analytic techniques for studies with zero cell counts.[17]

Another consideration is the generalizability of the findings from animal studies to human influenza infection. The influenza viruses were laboratory-adapted for virulence to achieve a high mortality rate in the animal models used. The markedly lower mortality rate in human influenza infection also meant that the potential effect on mortality could not be examined in the few small randomized controlled clinical trials that have been undertaken of antipyretics in influenza infection. However, if the increased risk of mortality of the magnitude present in animals applies to the antipyretic treatment of influenza infection in humans, then this would be of considerable public health importance due to the widespread use of antipyretics for seasonal and pandemic influenza.

Finally, most of the animal studies included in this review utilized mouse models, which generally have a fall in body temperature with influenza infection.[50] This difference limits the generalizability of the study findings, particularly in regard to the potential mechanisms of the effect observed.

In conclusion, this systematic review and meta-analysis has shown an increased mortality rate in animals treated with antipyretics during infection with influenza A or B, with no informative randomized placebo-controlled trials in humans. We propose that randomized placebo-controlled trials of antipyretic use in pandemic and seasonal influenza in humans are urgently needed in order to establish appropriate evidence-based management guidelines.

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