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# Effect of Vitamin D supplementation to reduce respiratory infections in children and adolescents in Vietnam: a randomized controlled trial

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Running head: Vitamin D for respiratory infection

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**Background:** It is uncertain whether Vitamin D can reduce respiratory infection.

**Objective:** To determine if vitamin D supplementation reduces influenza and other upper viral respiratory tract infections.

**Methods:** 1,300 healthy children and adolescents between the ages of 3 and 17 years were randomized to vitamin D (14,000U weekly) or placebo for eight months in Vietnam. The primary outcome was **reverse transcriptase (RT**) -PCR confirmed influenza infection and the co-primary outcome was multiplex PCR confirmed non-influenza respiratory viruses. Participants, care givers, and those assessing outcomes were blinded to group assignment.

**Results:** 650 children and adolescents were randomly assigned to vitamin D and 650 to placebo. The mean baseline serum 25-hydroxyvitamin D levels were 65.7 nmol/L and 65.2 nmol/L in the intervention and placebo groups respectively, with an increase to 91.8 nmol/L in the vitamin D group and no increase, 64.5 nmol/L, in the placebo group. All 1,300 participants randomized contributed to the analysis. We observed RT-PCR confirmed influenza A or B occurred in 50 children (7.7%) in the vitamin D group and in 43 (6.6%) in the placebo group (hazard ratio [HR] 1.18, 95%CI 0.79 to 1.78). RT-PCR confirmed non-

influenza respiratory virus infection occurred in 146 (22.5%) in the vitamin D group and in 185 (28.5%) in the placebo group (hazard ratio [HR] 0.76, 95% CI 0.61 to 0.94). **When considering all respiratory viruses, including influenza, the effect of vitamin D in reducing infection was significant, HR 0.81, 95% CI 0.66 to 0.99.**

**Conclusion:** Vitamin D supplementation did not reduce the incidence of influenza but moderately reduced non-influenza respiratory viral infection.

**Key words:** Influenza, respiratory viruses, vitamin D, randomized trial

**Trial registration:** This study is registered with ClinicalTrials.gov, number NCT01705314.

## Introduction

Influenza and other respiratory viruses account for substantial morbidity in children (1-5). The prime strategy for preventing respiratory infection is vaccination against influenza. Protection against influenza with vaccines is approximately 60% effective, but may be lower, particularly when there is a mismatch between antigens in the vaccine and the circulating influenza strains (6). In the absence of available vaccines against other respiratory viruses, strategies in addition to influenza vaccination may be of clinical benefit to children.

It has been proposed that vitamin D, ingested as cholecalciferol (vitamin D) or ergocalciferol (vitamin $D_2$), can reduce viral respiratory infection, possibly by stimulating expression of antimicrobial peptides, such as the defensin retrocyclin-2 (7, 8). Observational studies in children have demonstrated an association between vitamin D levels and respiratory infection, but have been inconsistent (9-14). A recent systematic review and meta-analysis of

individual participant data of vitamin D clinical trials of children and adults reported a reduced risk of acute respiratory infection (odds ratio 0.88, 95%CI 0.81 to 0.96) (15). Important limitations were that definitions of respiratory infection in children varied considerably (e.g. including pneumonia (16), otitis media (17), and exacerbation of asthma (18, 19)) and the vast majority of trials (23 of 25) did not include any laboratory confirmation of respiratory infection (15, 20).

Conducting randomized controlled trials of vitamin D supplementation to prevent influenza and other respiratory infections can be challenging in settings where vitamin D deficiency may not be prevalent and uptake of influenza vaccination is relatively high (21, 22). In contrast, in most low and middle income countries, such as Vietnam, children are not routinely vaccinated against influenza and vitamin D deficiency in children has been reported to be more prevalent (23).

We conducted a randomized trial of vitamin D in children in Vietnam to assess its effectiveness in reducing laboratory-confirmed influenza and non-influenza viral respiratory tract infections. We hypothesized that vitamin D would reduce both laboratory confirmed influenza and non-influenza respiratory viral infection compared to placebo.

## Methods

### Study design

A placebo-blinded randomized controlled trial. The study was conducted in two phases. In the first phase, we enrolled participants from one commune, Thanh Ha (pop. 9,699), in the Thanh Liem district of Ha Nam Province. In the second phase of the trial, we enrolled

participants from another commune, Kien Khe Town (pop. 9,832) also in Thanh Liem district. A screening and enrollment log was used to record potential and enrolled study participants. Demographic information, past medical history, use of prescription medications were recorded. Ethics approval was obtained at McMaster University, Hamilton, Ontario, Canada and at National Institute of Hygiene and Epidemiology, Ministry of Health, Vietnam.

Participants

We enrolled children and adolescents between the ages of 3 and 17 years in Thanh Liem District of Vietnam. Thanh Liem is a rural district of Ha Nam Province, part of the Red River Delta region of Vietnam, located 50 kilometers south of Hanoi. Children born prematurely at gestational age <32 weeks, children with any chronic illness (except asthma), children with impaired vitamin D metabolism (e.g. anti-seizure medications) and children with a sibling participating in the study (to reduce clustering effects) were excluded. For all participants aged 7 to 17 years, an assent form was obtained. Signed parental consent was required for all participants.

Randomization and blinding

Participants were assigned at random to one of the two study groups (vitamin D or placebo), in a 1:1 ratio. Computer generated randomization was performed by an external research organization, with treatment assignments made in random permuted blocks of 4. The placebo liquid, fractionated coconut oil, was identical in appearance and taste to the vitamin D liquid. All study medication was packaged in identical appearing bottles. The appropriate bottle of study medication, which was stored at the commune health centers at the study sites, was labeled with the participant identification number and that individual received the contents of

the pre-labeled bottle with the participant identification. Participants were randomized in sequential order as they were enrolled. All participants and their parents, research field staff, study investigators, and research staff at McMaster University and at National Institute of Hygiene and Epidemiology were blinded.

Procedures

Children and adolescents randomized to Vitamin D received 7 drops (0.028ml per drop) of D-drops (Drdrops Company, Woodbridge, Ontario) (14,000U/week of vitamin D) weekly for eight months while those randomized to placebo received 7 drops (0.028ml per drop) of placebo drops for eight months. The study medication was dispensed directly onto the participant's tongue. The study medication was given weekly under direct observation at the commune health center and hamlet culture houses, missed doses were recorded.

All participants were assessed for signs and symptoms of influenza twice weekly by trained hamlet health workers over a 12-month influenza surveillance period. This extended 4 months beyond the 8-month period of weekly study medication. Participants were visited at home, contacted by phone, or seen at the commune health center. Each enrolled participant received a pocketed folder containing participant diary sheets and they or their parents received instruction on how to complete these. The participant, or their parents or care providers, were responsible for completing the daily checklist which served as a memory aid. The twice weekly contact took place on non-consecutive study days and optimally 3 days apart. This began after participants had been allocated to vitamin D or placebo. A standardized questionnaire was used by the trained field staff to record symptoms or signs of respiratory infection. Health workers obtained an oropharyngeal swab from participants with > 1 symptoms or signs of respiratory infection including fever (temperature ≥38.0 degrees

Celcius), cough, nasal congestion, sore throat, headache, sinus problems, muscle aches, fatigue, earache, ear infection (physician-diagnosed), or chills. Use of an oropharyngeal swab, as opposed to nasal, was based on a high rate of positivity obtained in the pilot study with repeated specimens and perceived greater participant compliance by field staff. The specimens were stored in a refrigerator at 2 to 8 degrees Celcius until delivery to the local laboratory which typically was within 3 hours of collection.

Serum 25(OH)D levels was assayed using the DiaSorin vitamin D TOTAL competitive chemiluminescent immunoassay on an automated LIAISON analyzer (Stillwater, MN). A blood specimen was obtained at baseline **and at 8 months after randomization**. The local laboratory shipped the specimens to the laboratory at National Institute of Hygiene and Epidemiology within 24 hours of collection where the specimens were stored at ≤ -70 degrees Celcius. These specimens were batched and then sent to McMaster University.

Outcomes

The primary outcome of this study was reverse transcriptase (RT) PCR-confirmed influenza infection using multiplex PCR for influenza A and influenza B (using modified CDC primers for matrix A gene). The co-primary outcome, non-influenza respiratory viral infection, was measured using a separate multiplex PCR for parainfluenza 1, 2, 3, metapneumovirus, RSV, Entero-rhinovirus, and adenovirus.

Secondary outcomes included the following: influenza-like illness, defined as cough and fever of ≥ 38.0 degrees Celsius, receipt of antibiotics, use of over the counter medication for respiratory symptoms, pharmacy visits, private medical clinic visits, medical center or hospital visits. These outcomes were measured through parental report with confirmation through review of commune health center records when possible.

Research staff monitored for signs and symptoms of toxicity using check lists for symptoms. The major potential toxicity was hypercalcemia which could present as kidney stones. Data on toxicity were recorded monthly.

Statistical Analysis

We calculated our sample size based on the results of the first phase of the study. We had enrolled and randomized 400 participants and followed them over 12 months. Without unblinding this first phase of the trial, we reviewed the results of samples from 260 participants who we had tested by RT-PCR. The results showed that 81 of the 260 (31%) participants had at least one respiratory virus detected and 34 (13%) had influenza detected. We reasoned that by expanding the sample size by another 900 participants for a total of 1,300 (650 participants in each group), we would have 80% power to detect a 40% risk reduction for influenza and 80% power to detect a 25% risk reduction due to vitamin D for all other respiratory viruses.

For PCR confirmed influenza and non-influenza respiratory infection, we conducted a time to event analysis using Cox proportional hazards for laboratory-confirmed influenza and for non-influenza respiratory viruses. To avoid lack of independence associated with counting multiple outcomes, each of these outcomes in a participant was only counted once.

The secondary outcomes were treated as being dichotomous i.e. the occurrence or receipt of ≥ 1 of the following: influenza-like illness, a course of antibiotics, pharmacy visit, over the counter medication for respiratory symptoms, private medical clinic visits, hospital or medical center visits. For each of these secondary dichotomous outcomes, we estimated the absolute risk difference of the vitamin D effect.

We planned to analyze participants in the group to which they were assigned in case of cross-overs. *P* values and 95% confidence intervals were calculated with 2-tailed tests. Since the type 1 error was split between the two primary outcomes, differences with $P < .025$ for 2-tailed tests were considered significant for these outcomes. For all other outcomes differences with $P < .05$ were considered significant. Statistical analyses were conducted using SAS version 9.2 (SAS Institute, Cary, North Carolina) and R version 3.2.

### Patient involvement

No patients were involved in the design of the research question, study design, or the outcome measures. Because of patient preference, we did use oropharyngeal as opposed to nasopharyngeal swabs. A summary of the vitamin D levels was disseminated to participants.

## Results

### Participants

There were 1,641 children and adolescents assessed for eligibility in two communes in Vietnam (Thanh Ha and Kien Khe, both in Thanh Liem district, Ha Nam province). Potentially eligible participants were pre-screened using household demographic information from local population records. Of those assessed, 341 did not participate, 326 of whom were not interested and 15 did not meet eligibility criteria (Figure 1). A total of 1,300 were enrolled with 650 randomized to Vitamin D and 650 to placebo. There were 400 participants (200 to each group) enrolled from Thanh Ha and followed from December 8, 2013 and ended December 14, 2014. During the second year of the study, 900 new participants were enrolled from Kien Khe and randomized (450 to each group) and followed from June 2015 until June 2016 (Figure 1).

Characteristics of enrolled participants are shown in Table 1. The mean age of participants was 8.5 years (standard deviation [SD] 4.0 years); 52.2 % were female, both figures were similar between the two study groups (Table 1). Of the 1,300 participants, 1073 (82.5%) had baseline and follow up sera for vitamin D collected. 54.8% of the participants had baseline vitamin D levels between 50 nmol/L and 74 nmol/L, while 16% had levels from 25 nmol/L to 49 nmol/L. Less than 1% had levels < 25 nmol/L. There was an increase in the mean vitamin D level to 91.8 nmol/L (SD 23.6 nmol/L) in the vitamin D group and no increase in the placebo group, 64.5 nmol/L (SD 17.5 nmol/L). In the vitamin D group, 83.5% (543/650) of participants completed all of their weekly doses during follow up compared to 82% (533/650) in the placebo group. Of the weekly doses missed, a total of 162 were missed in 107 participants in the vitamin D group compared to 222 missed doses in 117 placebo group participants.

Mean (SD) duration of follow-up was similar between groups: 331.9 (87.3) days in the vitamin D group and 332.9 (85.1) days in the placebo group. Of enrolled participants, 1189 (91.5%) completed follow up over the 12-month period. Of the 111 participants who withdrew from the study, 56 were in the vitamin D group and 55 in the placebo group. None of these withdrawals were due to adverse events. Reasons for withdrawal are shown in Figure 1. There were 1,697 oropharyngeal specimens obtained (854 in the vitamin D group and 843 in the placebo group).

Outcomes

We observed RT-PCR confirmed influenza A or B in 50 children (7.7%) in the vitamin D group and in 43 children (6.6%) in the placebo group (Table 2). Of these, 67 (72.0%) had influenza A and 24 (28.8 %) had influenza B and 2 (2.2%) had both influenza A and B. Non-

influenza respiratory virus infection occurred in 146 (22.5%) in the vitamin D group and in 185 (28.5%) in the placebo group (Table 3). There was a total of 177 (27.2%) influenza and non-influenza respiratory viruses in the vitamin D group and 209 (32.2%) in the placebo group.

We found no significant difference between vitamin D and placebo groups for RT-PCR confirmed influenza, hazard ratio [HR] 1.18, 95% CI 0.79 to 1.77 (Figure 2). We found that vitamin D significantly reduced non-influenza respiratory viral infection, HR 0.76, 95% CI 0.61 to 0.94 (P=0.011). Although the attack rates differed between the two stages of the study, the relationship between the two groups was similar (Table 2). When considering all respiratory viruses, including influenza, the effect of vitamin D in reducing infection was significant, HR 0.81, 95% CI 0.66 to 0.99.

When we compared the vitamin D group to the placebo group for secondary outcomes we found the following: 50 (7.7%) versus 62 (9.5%) with ≥ 1 episodes of influenza-like illness (absolute difference -1.8 %, 95%CI -4.9% to 1.2%), 270 (41.5 %) versus 282 (43.4 %) who received ≥ 1 course of antibiotics (absolute difference -1.85%, 95%CI -7.2% to 3.5%), 245 (37.7%) versus 251 (38.6%) that visited a pharmacy because of respiratory infection (absolute difference -0.92%, 95%CI -6.2% to 4.4%), 338 (52.0%) versus 361 (55.5%) that used over the counter medications for respiratory infection (absolute difference -3.5%, 95%CI -9.0 to 1.9%). No significant difference in these outcomes between groups was found. Only two participants in each group visited a private clinic, only one in the vitamin D group and two in the placebo visited a hospital or medical center for respiratory symptoms. Only one serious adverse event was reported, which was not related to the study, a hospitalization for a scheduled tonsillectomy.

**We conducted a post-hoc sub-group analysis limited to participants who at baseline had 25OHD levels less than 50 nmol/L. In this cohort, there were only 14 cases of influenza, the hazard ratio was 2.5 (5%CI 0.78 to 7.9), P=0.12. When we conducted the analysis using other respiratory viruses as the outcome, there were 46 events, HR 0.92, 95%CI 0.52 to 1.66, P=0.8.**

## Discussion

We found that vitamin D supplementation of 14,000U/week for 8 months had no significant effect on confirmed influenza infection in healthy children and adolescents between the ages of 3 and 17 years in Vietnam. However, supplementation significantly reduced RT-PCR confirmed non-influenza respiratory viral infections by about 25%.

### Strengths and limitations of study

Strengths of the study was that it was a randomized placebo-controlled trial with laboratory confirmed respiratory viral infection as the main outcome and measurement of vitamin both at baseline and at follow up.

One possible limitation was the use of oropharyngeal specimens instead of nasopharyneal specimens. This was based on adherence to testing and we reasoned that an increase in the number of swabs would make up for any reduction in sensitivity. The attack rate for all respiratory viruses in our study was 35%, which is comparable to other studies that have used RT-PCR to detect infection in children (24-26). A study in the same population in Vietnam reported similar attack rates as ours (23). Also, detection of the types of viruses and their relative frequency was similar to previous reports. The most common non-influenza respiratory virus we detected was entero-rhinovirus, consistent with other studies (24-26). In

our study, the ratio of attack rates for vitamin D to placebo for entero-rhinovirus, 0.83, represented the median effect for non-influenza respiratory viruses, which ranged from 0.47 for human meta-pneumo virus to 1.17 for parainfluenza viruses 1 to 3.

Only 17% of participants in our study had vitamin D levels < 50 nmol/L, in contrast to a previous study from Vietnam where > 50% children had insufficiency or deficiency (23). The relatively high vitamin D levels in our study may have reduced the effectiveness of vitamin D supplementation in preventing influenza. In contrast, vitamin D supplementation demonstrated effectiveness in reducing non-influenza respiratory viral infection. Although vitamin D may have variable immunomodulatory effect on different respiratory viruses (30), we are unaware of a specific mechanism that would explain the differential effect we observed. The lower confidence interval for the effect of vitamin D on influenza, 0.79, along with the point estimate of 1.18, suggests that these data are unlikely to be compatible with a large protective effect of influenza.

Comparison with other studies

Two previous trials of vitamin D in children reported either an effect of vitamin D on all respiratory viruses or no effect on all influenza (27, 28). These studies had limitations such as lack of RT-PCR testing (27, 28), survey-reported symptom outcomes at the end of the study (27), and the lack of criteria for ascertaining outcomes (28) that limit inferences and comparisons to our trial. A randomized trial of high-dose versus standard-dose vitamin D supplementation in Canadian young children did not demonstrate a reduction of overall wintertime upper respiratory tract infections (29). Our study was conducted in a middle-income country where children and adolescents are not routinely vaccinated against influenza. Vitamin D levels, while not as low as anticipated, may still be lower in this

population than in many settings in developed countries. Therefore, our results may apply to children and adolescents in other low and middle income countries.

Conclusion

Our results show that vitamin D supplementation does not reduce influenza but can reduce non-influenza respiratory infections in children and adolescents aged 3 to 17 years in a low and middle income country. Our findings imply that vitamin D supplementation can play a moderate role in reducing illness caused by respiratory viruses.

## Footnotes

Contributors: ML, DDA, MS, JM conceived and designed the study. VDT, VT, NBN, TTMH, LMT, SE were responsible for acquisition of data. ML, BW, PS, EP were responsible for the analysis and interpretation of data. ML produced a first draft of the manuscript and all authors provided intellectual input. ML is the guarantor.

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Competing interests:

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: all authors had support from Infectious Diseases Research at McMaster University that funded the research and the Ddrops Company that provided the vitamin D for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work

in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.



Ethical approval: The study was approved by the research ethics board of McMaster university and that of the Ministry of Health in Vietnam.

Data sharing: Patient level data and statistical code are available upon request from the corresponding author at loebm@mcmaster.ca.

Transparency: lead author (ML) affirms that the manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

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**Table 1**. Baseline characteristics of 1,300 enrolled participants aged 3 to 17 years in Vietnam

| **Variable** | **Vitamin D** | **Placebo** |
|---|---|---|
| | N=650 | N=650 |
| **Mean years of age (SD)** [a] | 8.6 (3.9) | 8.4 (4.0) |
| 3 to 6 (%) | 242 (37.2%) | 245 (37.7%) |
| 7 to 9 (%) | 171 (26.3%) | 146 (22.5%) |
| 10 to 12 (%) | 107 (16.5%) | 127 (19.5%) |
| 13 to 17 (%) | 130 (20.0%) | 132 (20.3%) |
| **Sex (Female)** | 325 (50.0%) | 354 (54.5%) |
| **Mean Baseline Vitamin D level (SD)**[b] | 65.73 (16.72) | 65.21 (16.89) |
| < 25 | 3 (0.54%) | 3 (0.55%) |
| 25 to 49 | 92 (16.64%) | 86 (15.87%) |
| 50 to 74 | 300 (54.25%) | 296 (54.61%) |
| ≥ 75 | 158 (28.57%) | 157 (28.97%) |

[a] Standard deviation

[b] Based on 553/650 (85.1%) samples from intervention group and 542/650 (83.4%) in the placebo group.

**Table 2**. Effectiveness of vitamin D supplementation on reducing RT-PCR confirmed influenza and non-influenza respiratory infections.[a]

| Outcome | RT-PCR-Confirmed Infection | | HR (95% CI) |
|---|---|---|---|
| | Vitamin D | Placebo | |
| **Non-influenza virus infection** | | | |
| All Years | 146/650 (22.5%) | 185/650 (28.5%) | 0.76 (0.61 to 0.94) |
| Year 1 | 54/200 (27.0%) | 75/200 (37.50%) | 0.65 (0.46 to 0.93) |
| Year 2 | 92/450 (20.4%) | 110/450 (24.4%) | 0.82 (0.62 to 1.08) |
| **Influenza virus infection** | | | |
| All Years | 50/650 (7.7%) | 43/650 (6.6%) | 1.18 (0.79 to 1.77) |
| Year 1 | 25/200 (12.5%) | 29/200 (14.5%) | 0.85 (0.50 to 1.46) |
| Year 2 | 25/450 (5.6%) | 14/450 (3.1%) | 1.82 (0.95 to 3.52) |
| **All viral infections** | | | |
| All Years | 177/650 (27.2%) | 209/650 (32.2%) | 0.81 (0.67 to 0.99) |
| Year 1 | 72/200 (36.0%) | 90/200 (45.0%) | 0.73 (0.53 to 0.99) |
| Year 2 | 105/450 (23.3%) | 119/450 (26.4%) | 0.87 (0.67 to 1.13) |

[a] All hazard ratios were calculated using the participants' first infection with the virus.

**Table 3**. The distribution of RT-PCR confirmed respiratory viral infection in participants by vitamin D group and placebo group. [a]

| Variable | Vitamin D | Placebo |
|---|---|---|
| | N=650 | N=650 |
| ≥ 1 virus | 177 (27.2%) | 209 (32.2%) |
| Influenza A or B | 50 (7.7%) | 43 (6.6%) |
| Influenza A | 40 (6.2%) | 29 (4.5%) |
| Influenza B | 11 (1.7%) | 15 (2.3%) |
| Adenovirus | 10 (1.5%) | 11 (1.7%) |
| Entero-rhino | 130 (20.0%) | 159 (24.5%) |
| MPV | 12 (1.8%) | 25 (3.8%) |
| Parainfluenza 1 | 1 (0.2%) | 0 (0.0%) |
| Parainfluenza 2 | 0 (0.0%) | 2 (0.3%) |
| Parainfluenza 3 | 12 (1.8%) | 9 (1.4%) |
| RSV | 7 (1.1%) | 10 (1.5%) |

[a] The sum of participants with confirmed infection with the different types of viruses is greater than the number for ≥ 1 virus because of those participants infected with more than one type of virus.

**Figure 1.** Flow of study participants: vitamin D supplementation versus placebo in children and adolescents in Vietnam.

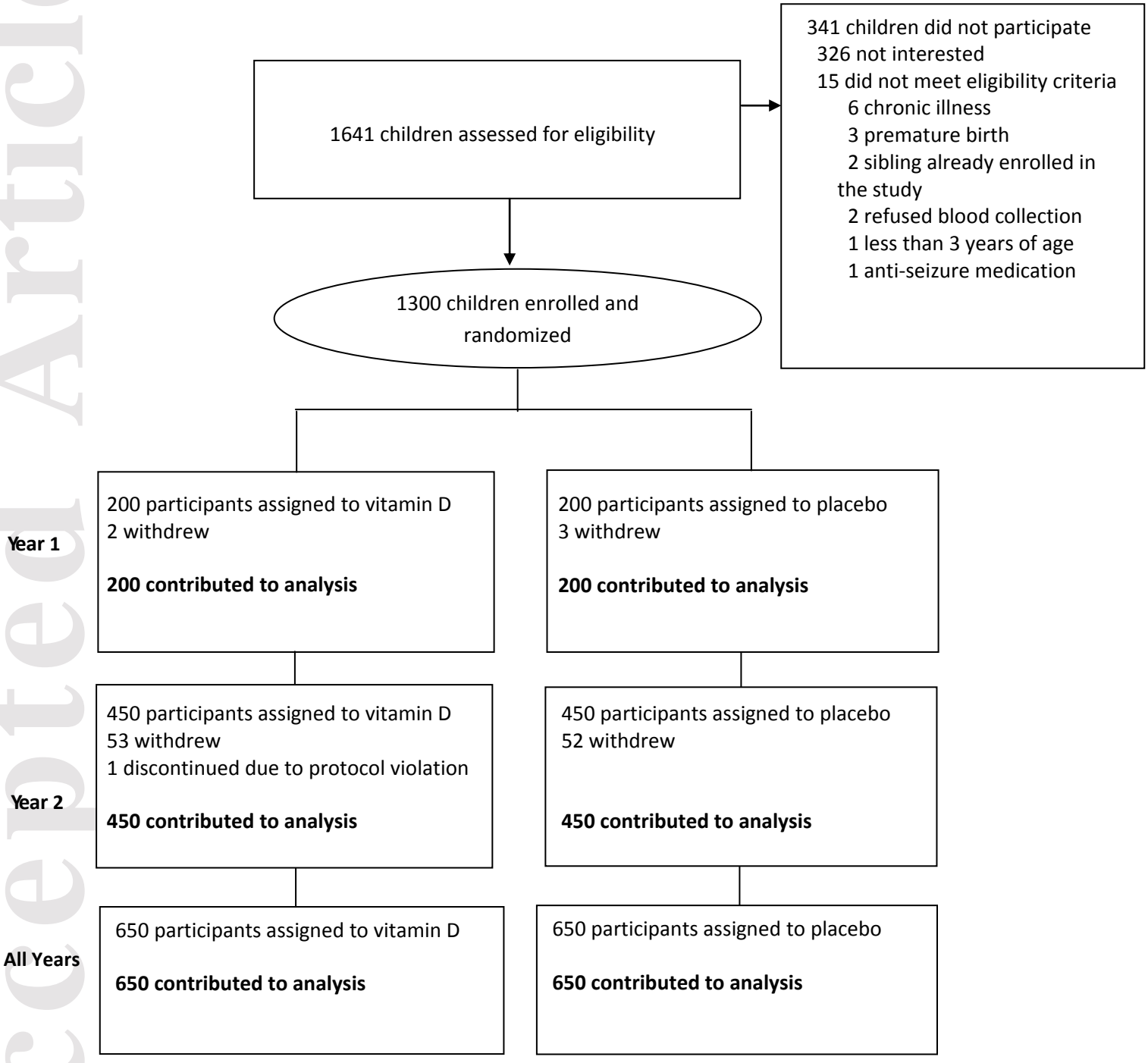
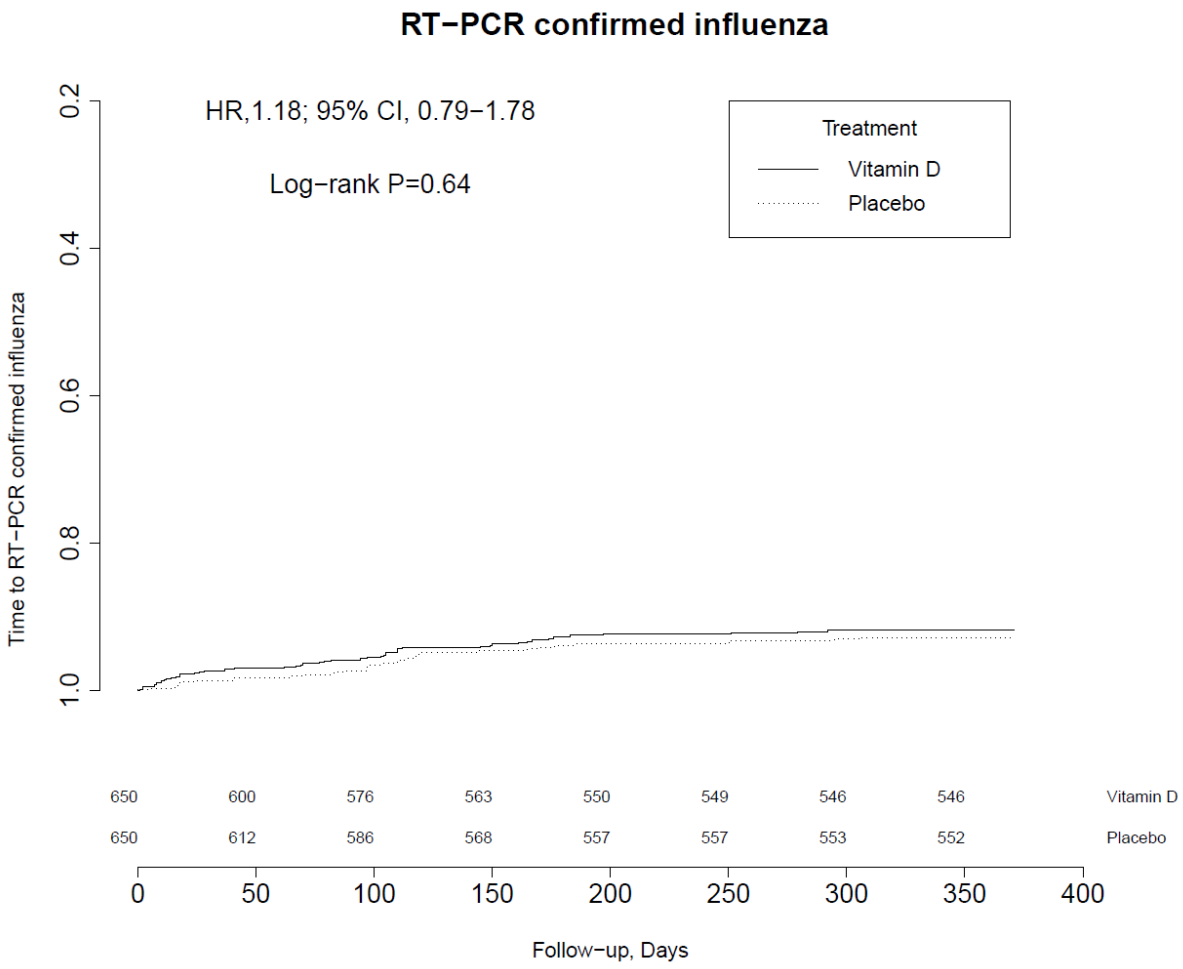


Figure 2. Kaplan-Meier curve of time to first RT-PCR confirmed A. influenza A or B infection, B. Non-influenza respiratory viruses.

A.



B.