REVIEW

Protection of children against influenza: Emerging problems

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ABSTRACT

Influenza is a common disease and in children it can be severe enough to lead to hospitalization and death. Protection of all children against influenza, particularly the youngest, is strongly recommended by most health authorities. However, available vaccines cannot be used in the first 6 months of age, a period of life characterized by the highest risk of influenza-related complications. Maternal immunization is an attractive possibility to overcome this problem. For years, protection against influenza has been pursued by administering the trivalent inactivated vaccine given intramuscularly (IIV3). More recently, a trivalent live attenuated influenza vaccine (LAIV3) administered intranasally was licensed and adopted in a number of countries as an alternative to IIV3. In recent years, to increase protection and include a second B strain, quadrivalent inactivated (IIV4) and live attenuated vaccines (LAIV4) were prepared and licensed. However, during the 2015–2016 season the effectiveness of LAIVs was debated, and they were withdrawn from the list of recommended influenza vaccines in the USA. This review presents an update on the evidence related to the protection of infants against influenza through maternal immunization and the effectiveness of LAIV. Available data indicates that despite maternal immunization, a number of children have no protective antibody levels at birth, and in the majority of children with antibody protection, it is limited to the first 8 weeks of the postnatal period. Moreover, data on LAIV effectiveness in the pediatric population must be clarified because this vaccine can significantly improve vaccination coverage in children.

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Introduction

Influenza is a common disease. Up to 30% of children, with the highest prevalence among the youngest, are infected by influenza viruses every winter season.¹ Most of the disease cases are mild and spontaneously resolve; however, influenza in children can be severe enough to lead to hospitalization and death.² Lafond et al. estimated that worldwide, influenza is the cause of approximately 374,000 hospitalizations in children <1 year of age (of which 228,000 occur in children <6 months of age) and 870,000 hospitalizations in children <5 years of age annually.^{3,4} In the last four influenza seasons in the USA, a total of 515 influenza-associated pediatric deaths have occurred.⁵ However, it is highly likely that the true impact of influenza infection in pediatrics is significantly higher than what is reported in epidemiological studies and official statistics. This is because a great number of patients who are hospitalized and die from severe respiratory problems because of influenza infection are not tested for influenza viruses. Moreover, influenza infection is often not reported as the contributory cause of hospitalization or death when the main signs and symptoms of disease are strictly related to the worsening of an existing chronic underlying illness.⁶

For many years, it was thought that severe influenza cases were only common in children at high risk of influenza-related complications because of a chronic, severe, underlying disease.⁷

Consequently, the prevention of influenza through the use of available influenza vaccines was only recommended to these individuals. However, in recent years, several studies have shown that severe cases can occur in otherwise healthy children. In a study in the USA examining influenza seasons from October 2004 to September 2012, 830 pediatric influenza-associated deaths were reported, and 43% of the children who died had no high-risk medical conditions.⁸ This explains why some countries currently recommend universal influenza vaccinations in children. Because the highest risk of hospitalization and death in otherwise healthy children occurs in the first years of life, a number of countries limit vaccination recommendations to infants, toddlers and preschool children.⁹ In other cases, such as in the USA, influenza vaccine administration is recommended in all pediatric populations from 6 months to 17 years and also in adults of any age.¹⁰

For years, protection against influenza has been pursued by administering the trivalent inactivated vaccine given intramuscularly (IIV3).¹¹ More recently, a trivalent live attenuated influenza vaccine (LAIV3) administered intranasally was licensed and adopted in a number of countries as an alternative to IIV3.¹² In recent years, to increase protection and include a second B strain, quadrivalent inactivated (IIV4) and live attenuated vaccines (LAIV4) were prepared and licensed.¹³ However, because these preparations cannot be used in younger infants,

different immunization methods to protect these subjects have been pursued. Maternal immunization is one of these methods, although presently it is not adequately used.^{14,15} Moreover, during the last season the effectiveness of LAIV in the USA was debated, and the live vaccine was withdrawn from the list of recommended influenza vaccines in this country. This review presents an update on the evidence related to the protection of infants against influenza through maternal immunization and the effectiveness of LAIV.

Search strategy and selection criteria

References for this review were identified through two searches. In the first search, PubMed was used for articles published from January 2000 to September 2016 with the terms “influenza vaccine” or “influenza vaccination” and “pregnancy” or “maternal” and “infant” or “neonate.” In the second search, PubMed was used for articles published from January 2000 to September 2016 with the terms “live attenuated influenza vaccine” or “LAIV” and “effectiveness” or “efficacy” and “children” or “child” or “pediatric.” Relevant articles published before 2000 were identified through searches in Google Scholar and Springer Online Archives Collection. Only articles published in English were included.

Protection of the infant through maternal immunization

Children in the first months of life are at increased risk of severe influenza but cannot be protected by available influenza vaccines because these preparations are not licensed for use in children < 6 months of age. In infants, the immunogenicity of the available preparations is generally poor, and the safety and clinical efficacy of IIV3 and IIV4 have not been precisely defined.¹⁶ Moreover, LAIV3 and LAIV4 are not licensed for children < 2 years of age for safety reasons.¹² To overcome these problems and to assure protection against influenza to younger infants, several attempts have been made (Table 1). The use of adjuvant vaccines, intradermal injections and the administration of an increased dose of antigens have been evaluated in controlled clinical trials with good results.¹⁷ However, none of these measures has been definitively accepted by health authorities nor have they been approved for use in current clinical practice. In contrast, a potential preventive measure that has aroused much interest and has been adopted in a number of countries for infant protection is maternal immunization.

Although LAIV is not recommended for pregnant women because it is based on live viruses, the administration of IIV to pregnant women has long been recommended by many health authorities. Influenza can be more severe during pregnancy than in the general population, and IIV can evoke a significant

immune response leading to protection against infection and disease in a large number of pregnant women.^{18–24} Moreover, no contraindication to influenza vaccine administration exists; IIV is well tolerated and safe, it does not cause any relevant adverse effects in the mother, and it does not appear to interfere with the normal development of the fetus.^{25–28}

Evidence that the serum IgG antibodies of pregnant woman actively cross the placenta and reach the fetus²⁹ and that additional IgA antibodies are transferred via breast milk¹⁹ has further increased interest in maternal vaccination. A single administration is presumed to protect both the mother during pregnancy and the conceptus during foetal life and the first period of extra-uterine life. The administration of IIV to pregnant women is presently strongly recommended by the World Health Organization³⁰ and several health authorities worldwide for the protection of mothers and infants against influenza-related complications.^{31–33}

The relevance of maternal immunization for infant protection against influenza is evidenced by a number of studies, which reported that children born to mothers who had received IIV during pregnancy had significantly higher levels of antibodies against the vaccinated influenza viruses and a reduced risk of influenza infection and influenza-related hospitalization compared to children from unimmunized controls. Both the seasonal IIV3 and the monovalent influenza vaccine specifically prepared for the recent A/H1N12009 pandemic were found to be effective.^{19–23,29,34–37} However, the effectiveness of IIVs in infant protection was not precisely defined because the results of the various studies varied significantly, primarily because of the different methods used to evaluate the impact of vaccine administration. In several cases, only the incidence of influenza-like illness in vaccinated and in untreated populations was compared. In other studies, the surveillance of influenza infection was laboratory-confirmed with rapid tests or with molecular methods.³⁸

Despite the evidence that maternal immunization can protect the mother/child dyad, influenza vaccination coverage among pregnant women has remained significantly lower than desired in all the regions examined. In the USA, coverage for the 2015–2016 season was approximately 50%, and no increase has been reported since the 2012–2013 season.¹⁴ In Europe, where influenza vaccination coverage for pregnant women for the 2012–2013 season was reported by only seven member states (United Kingdom [England, Wales, Scotland, Northern Ireland], Germany, Ireland, Romania, Hungary, Slovenia, and Lithuania), influenza vaccination coverage ranged from 0.2% to 64.6%, with a median value of 25.5%. The highest coverage rates were reported by the United Kingdom and varied from 40.3% in England to 64.6% in Northern Ireland,¹⁵ although coverage rates did not significantly increase with time.³⁹

Several factors may explain the poor compliance with official recommendations (Table 2). Health care providers can influence the use in pregnant women who are cautious about vaccinations because they are assuming responsibility for their fetus.^{40,41} Moreover, misperceptions and lack of awareness regarding influenza vaccine efficacy and safety have been identified as barriers to vaccination among health care providers.⁴² It has been shown that more than one third of obstetrician-gynaecologists do not offer the vaccine in their

Table 1. Attempts to increase the efficacy of the influenza vaccine in infants.

Attempts
Use of adjuvant influenza vaccines
Intradermal injection of influenza vaccine
Administration of an increased dose of antigens

Table 2. Explanations for poor compliance with official recommendations for maternal immunization against influenza.

People	Reason
Pregnant women	Fear for their fetus
Health care providers	Misperceptions and lack of awareness regarding influenza vaccine efficacy and safety Doubts regarding the best timing of immunization and duration of protection
Everybody	Product information that limits or even contraindicates the use of influenza vaccines during pregnancy

practice.⁴³ Many obstetricians may not be completely convinced of the efficacy of the influenza vaccine owing to a series of problems strictly related to the influenza vaccine itself, such as the best timing of immunization, the real efficacy of IIV and the duration of protection, which have not been completely solved. Regarding the best time of immunization, it is obvious that if the main aim is to protect the mother, vaccine has to be administered in the first period of pregnancy, whereas protection of concept can be better achieved if immunization occurs later, when antibodies can cross the placenta. Serum antibody concentrations in the neonate at birth are proportional to the serum level of the mother at the end of pregnancy, and these levels strictly depend on the moment of maternal immunization. Vaccine administration too close to conception or delivery is probably poorly effective in conditioning protective levels in the child. Vaccine administration between the 32nd and 36th week has been suggested to be the optimal time period, but the problem has not been extensively studied and remains unsolved.²⁰ The need to reconcile opposing demands may explain why, with few exceptions,⁴⁴ in most of the official recommendations maternal immunization is suggested during pregnancy without any further detail. Regarding duration of protection, available data indicate that the prevalence of children born to vaccinated mothers protected from influenza is temporally limited, in accordance with the decline of serum influenza antibody concentrations. The proportion of neonates with protective ($\geq 1:40$) concentrations of haemagglutinin antibodies has been reported to be less than 80% for the A/H1N1 2009 and B/Victoria strains and less than 60% for the A/H3N2 strain.⁴⁵ Further controls have shown that the percentage of infants with protective antibody titres against all of the vaccine strains decreased from more than 56% in the first week of life to less than 40% at 16 weeks of age and less than 10.0% at 24 weeks of age.⁴⁵ As a consequence, the efficacy of IIV against laboratory-confirmed influenza illness was 85.6% (95% CI: 38.3%–98.4%) among infants 8 weeks of age or younger and decreased to 25.5% (95% CI: –67.9% to 67.8%) among infants 8 to 16 weeks of age and to 30.3% (95% CI: –154.9% to 82.6%) among infants 16 to 24 weeks of age. These findings suggest that to increase the influenza vaccination coverage and convince reluctant obstetricians, a more precise definition of the best timing for maternal immunization and a better understanding of transplacental antibody kinetics during pregnancy are needed. Moreover, the availability of more immunogenic vaccines could significantly improve physician compliance with official recommendations.⁴⁶

In addition to these problems, other barriers to maternal immunization to protect the child exist. Product information for several vaccines limits or even contraindicates the use of influenza vaccines in pregnant women. Proveaux et al. studied vaccination information available online, provided by the manufacturer and approved by the regulator regarding the use of influenza vaccines, including package inserts, package labels, package leaflets, patient information labels, product descriptions and summaries of patient characteristics.⁴⁷ A total of 96 vaccines on the market were evaluated. Only 10 of the vaccines had product information that suggested use during pregnancy, and among these, there was a wide variety of information regarding safety data and at what stage of pregnancy administration was preferred. The majority of leaflets contained precautionary language that could discourage use in pregnant or lactating women, and several included contradicting use information. Product information from 4 vaccines indicated that the influenza vaccine should not be used in pregnant women.

To overcome barriers and to increase influenza vaccination coverage in pregnant women, several attempts have been made. Provider-focused interventions and pregnant woman-focused interventions were carried out (Table 3). In the first case, common strategies included notifying providers about the influenza vaccination status of pregnant women, establishing standing orders authorizing nursing staff to administer the vaccine without a medical consultation, giving provider feedback by reporting the influenza vaccination rates of other institutions, and providing education to improve the knowledge and attitudes of healthcare workers toward influenza vaccination in pregnancy. In pregnant woman-focused interventions, the main intent was to disseminate education and the promotion of materials that target pregnant women by mass media campaigns via the Internet, posters and leaflets, lectures and workshops, and personalized reminders and recall systems. A good example of an intervention that targeted both physicians and pregnant women is given by the toolkit distributed by the American College of Obstetricians and Gynecologists.⁴⁸ The toolkit includes materials to help physicians and their staff communicate with patients regarding the importance of receiving the recommended immunizations and how to successfully incorporate immunizations into routine care. A recent systematic analysis of the effectiveness of these interventions reported discordant

Table 3. Interventions to improve maternal immunization coverage against influenza.

Target	Intervention
Provider-focused	Notifying providers about the influenza vaccination status of pregnant women Establishing standing orders authorizing nursing staff to administer the vaccine without a medical consultation Giving provider feedback by reporting influenza vaccination rates of other institutions Providing education to improve the knowledge and attitudes of healthcare workers toward influenza vaccination in pregnancy
Pregnant women-focused	Disseminating education and promoting mass media campaigns Educational activities on the Internet Preparation of posters and leaflets Lectures and workshops Personalized reminders and recall systems

results, with risk differences (RDs) ranging from -0.15 to 0.03 , although in most cases significant increases were observed in influenza vaccine uptake in pregnant women after the interventions.⁴⁹ Unfortunately, most of the studies with favorable results had significant methodologic limitations because they were non-randomized and frequently included a very low number of subjects. Consequently, it was not possible to define the best strategy to significantly improve coverage. However, one randomized controlled trial of moderate quality showed that providing an influenza pamphlet, with or without a verbalized benefit statement, improved the vaccination rate. Based on this finding, the authors recommended that clinicians must provide influenza pamphlets to pregnant women with a verbalized statement about the benefits of influenza vaccines to children.⁴⁹ However, further high-quality studies are needed to plan adequate and effective interventions to increase vaccination coverage among women.

Live attenuated influenza vaccine (LAIV) effectiveness

LAIV is a live attenuated influenza vaccine for administration by intranasal spray authorized for use in people 2–59 years of age (Table 4). The influenza strains used in LAIV are attenuated, cold-adapted, and temperature-sensitive, so they replicate in the nasal mucosa rather than in the lower respiratory tract and do not cause influenza.¹² LAIV was found to be effective in influenza prevention in several controlled trials conducted in children before the A/H1N12009 pandemic. Compared with conventional IIV3, LAIV was found to be superior in most studies.^{50–52} Superiority was more evident in children <6 years of age than in older children. Moreover, for children <6 years of age, the evidence for the superiority of LAIV was of higher quality. Based on these findings, the Advisory Committee on Immunization Practices (ACIP) of the USA included LAIV, initially as LAIV3 and later as LAIV4, on the list of influenza vaccines recommended for influenza prevention in children. Furthermore, in the USA in 2014, health authorities suggested the preferential use of LAIV, when immediately available, for healthy children 2–8 years old who did not have contraindications to the vaccine.^{53–55} Several other countries have licensed LAIV for use in children.^{53,54} In the UK, health authorities that had previously recommended IIV only for the prevention of influenza in at-risk children planned to initiate a universal childhood influenza vaccination program using LAIV because of its efficacy, safety and easy administration.⁵⁶ As expected,

LAIV efficacy estimates carried out in the USA during the 3 influenza seasons from 2010–2011 to 2012–2013 evidenced that the vaccine provided statistically significant protection with a reduction of medically attended, laboratory-confirmed influenza illness.⁵⁷ Influenza occurrence, independent of the viral strain, was similar for participants vaccinated with LAIV or IIV. However, beginning in 2013–2014, a year in which the A/H1N1pdm09 strain predominated worldwide, contrasting results of LAIV effectiveness were reported. All three test-negative studies carried out in the 2013–2014 influenza season in the USA reported low to negative and statistically non-significant vaccine effectiveness estimates for LAIV against any influenza strain and against A/H1N1pdm09.^{58,59} In particular, in the study by Caspard et al., in which a total of 1,033 children and adolescents were included, influenza was detected in 14% of all children, with 74% of the influenza cases due to A/H1N1pdm09 strains, 21% to influenza B, and 4% to influenza A/H3N2. LAIV did not show significant effectiveness against A/H1N1pdm09 (VE 13% [95% CI: -55 to 51]) but was effective against B/Yamagata strains (82% [95% CI: 12 – 96]).⁵⁹ In contrast, results from studies in children vaccinated with IIV revealed that vaccine effectiveness against A/H1N1pdm09 strains was 74% (95% CI: 50 – 86). Furthermore, effectiveness against B/Yamagata strains in children vaccinated with IIV was 70% (95% CI: 18 – 89). These results were surprising because in 2009–2010, when monovalent vaccines were administered during the A/H1N1pdm09 pandemic, the LAIV strain was shown to be effective in children 2–9 years of age, with a vaccine effectiveness of 82% (95% CI: 14 – 96) when cases that occurred within 7 days of vaccination were excluded, and 55% (95% CI: -270 to 95) when cases occurring within 14 days of vaccination were excluded.⁵⁹ However, 2013–2014 USA results varied from those of different geographic areas. Two studies conducted in Canada in 2013–2014 suggested that LAIV was effective, although estimates were based on small sample sizes with wide confidence intervals.^{60,61} Furthermore, a study carried out in the UK revealed a lower, although not statistically significant, incidence of influenza illness in communities in which school-aged children were vaccinated with LAIV.⁶²

The 2014–2015 influenza season was not only dominated by A/H1N1pdm09 but also by antigenically drifted A/H3N2 viruses. The data collected in the USA and in the UK revealed that both LAIV and IIV were poorly effective against all strains of influenza and influenza due to the dominating virus. In the USA, it was reported that LAIV and IIV effectiveness against any influenza was 3% and 15%, respectively, whereas that against A/H3N2 was -5% for LAIV and 13% for IIV.⁵⁷ In the UK, LAIV effectiveness against A/H3N2 was 35% (95% CI: -29.9 to 67.5), whereas for influenza B it was 100% (95% CI: 17.0 to 100.0). IIV was less effective against both viral strains, although differences did not reach statistical significance.⁶³

Different and conflicting results of surveillance studies were also reported for the 2015–2016 influenza season, in which the A/H1N1pdm09 virus predominated. Moderate and statistically significant LAIV effectiveness against any influenza infection (46%–58%) was observed among children and adolescents 2–17 years of age in two American studies,⁶⁵ in the UK,⁶⁶ in Canada,⁶⁷ and in Finland.⁶⁸ Compared to IIV, LAIV was slightly less effective, but differences did not reach statistical

Table 4. Main characteristics of live attenuated influenza vaccines.

Characteristics
Attenuated, cold-adapted, and temperature-sensitive influenza strains
Replication of influenza strains in the nasal mucosa rather than in the lower respiratory tract without causing influenza
Intranasal administration
Availability of trivalent and quadrivalent formulations
Authorized for use in people 2–59 years of age
Superior efficacy in influenza prevention compared to inactivated influenza vaccine in several controlled trials conducted in children before the A/H1N12009 pandemic
Optimal efficacy observed in children <6 years of age
Safety problems because of wheezing-related risks in children <2 years of age

significance. In contrast, in the same age group, the US Flu Vaccine Efficacy Network found very low, if any, LAIV effectiveness against any influenza (3%), with statistically significant differences compared to IIV, for which vaccine effectiveness was 63%.⁶⁹ Effectiveness against A/H1N1pdm09 was –15% for LAIV and 54% for IIV.

Data regarding LAIV effectiveness led to different conclusions by health authorities in different countries. In the USA, the authorities considered all of the data collected by the US Flu Vaccine Efficacy Network in the seasons 2013–2014, 2014–2015, and 2015–2016 and concluded that LAIV4 did not have any statistically significant benefit in preventing influenza (all 95% CIs spanned zero).⁷⁰ On the contrary, IIV was considered effective, although seasonal variations were observed. Children who received LAIV4 had a 2.5-fold greater chance of developing influenza, attributable to any virus, compared to children who received IIV. Consequently, during its June 2016 meeting, the American Advisory Committee on Immunization Practices (ACIP) recommended that LAIV should not be used during the 2016–2017 influenza season.⁷⁰ However, the US Food and Drug Administration decided that LAIV4 had to remain licensed for use in the USA⁷¹ because of the limitations of observational studies in estimating vaccine effectiveness. In contrast, Canada,⁵⁴ the UK,⁷² and Finland⁷³ have not withdrawn LAIV from the list of influenza vaccines recommended for use in children by health authorities because current evidence indicates a protective effect of LAIV, although it is lower than expected.

The low LAIV effectiveness during the 2014–2015 influenza season was evidenced in several countries and can be explained by several factors, including the presence of antigenically drifted A/H3N2 viruses. The effectiveness of IIV during that period was also shown to be low. In contrast, the poor vaccine effectiveness during the 2013–2014 and 2015–2016 influenza seasons in which A/H1N1pdm09 dominated and the discordant vaccine effectiveness estimates between studies carried out in different countries during this period are difficult to explain. These findings have been suggested to reflect biological mechanisms, methodological issues or both, including biases in the design of observational studies and statistical considerations, which limit the precision of vaccine effectiveness estimates.⁵⁷ In 2013–2014, the test negative design was used in the USA⁵⁹ and in Canada,⁶¹ but opposite results were obtained. The two studies carried out in Canada in the same influenza season used different methods but led to the same conclusions.^{60,61} Poor LAIV effectiveness against A/H1N1pdm09 may depend, as previously observed with IIV,⁷⁴ on reduced immunogenicity of the vaccine as a result of a more highly vaccinated population in recent years compared with populations of earlier studies, in which a higher proportion of children were vaccine-naïve. The importance of this problem is not defined, but the use of LAIV in countries that have recently introduced this preparation and have maintained its use despite the ACIP decision may offer adequate data to inform this question. Potential interference among viruses included in the LAIV4 cannot be excluded but is not consistent with the results reported when the immunogenicity of LAIV4 was compared with that of LAIV3.⁷⁵ Heat susceptibility may have played a role, at least for the poor vaccine effectiveness in 2013–2014. During that

Table 5. Possible reasons for reduced quadrivalent live attenuated influenza vaccine (LAIV4) effectiveness.

Possible reasons
Reduced immune response against A/H1N1pdm09 as a result of a more highly vaccinated population during the influenza seasons 2013–2014 and 2015–2016
Antigenically drifted A/H3N2 viruses during the 2014–2015 influenza season
Biological mechanisms
Methodological issues in studies (i.e., biases in the design, statistical limits)
Potential interference among viruses included in the LAIV4
Heat susceptibility

influenza season, LAIV was prepared with the A/California/7/2009pdm09 strain. This virus has an increased susceptibility to degradation compared with other live viruses, although degradation occurs at a temperature well above what is recommended for storage and handling (2–8°C).^{76,77} A study carried out in the USA regarding the association between vaccine shipping conditions and LAIV lot effectiveness revealed that from 2013 to 2014, the proportion of LAIV recipients who tested positive for the A/H1N1/2009 pandemic virus among children who received a lot released between August 1 and September 15, 2013 was significantly higher than that in children who received a lot shipped either earlier or later (21% vs. 4%; $p < 0.01$).⁷⁸ A linear relationship was observed between the proportion of positive subjects and outdoor temperature during truck unloading at distributor locations. The importance of outdoor temperature on the effectiveness of LAIV was further supported by evidence that a reduction similar to that found in 2013 against the A/H1N12009 pandemic virus was reported in the 2010–2011 season when most LAIV doses were shipped before mid-September. In contrast, significant effectiveness was observed in 2009–2010 when LAIV was shipped in the USA after mid-September and in 2013–2014 in Canada when the vaccine was shipped starting in November. However, heat susceptibility cannot explain the poor vaccine effectiveness found in 2015–2016 because in that season, the A/California/7/2009pdm09 strain was substituted in LAIV by the A/Bolivia/559/2013/H1N1 strain, a virus that is significantly more stable. Unfortunately, the real immunogenicity of this strain has not been specifically evaluated in randomized controlled trials, and it cannot be excluded that poor vaccine effectiveness may be linked to the relatively reduced immunogenicity due to reduced replicative fitness of both viral strains.^{78,79} However, differences among studies carried out in 2015–2016 do not appear to support this hypothesis. Table 5 summarizes possible reasons for reduced LAIV4 effectiveness.

Conclusions

Protection of all children against influenza, particularly the youngest, is mandatory for most health authorities. However, how this protection can be achieved has not been defined. Currently available vaccines cannot be used in the youngest children, a group of subjects at the highest risk of influenza-related complications. Maternal immunization is an attractive possibility to overcome this problem. However, several problems concerning the best time of immunization, the number of children protected and the duration of protection have not been solved.

Available data indicate that despite maternal immunization, a number of children have no protective antibody levels at birth and that in most cases the duration of protection is limited to the first 8 weeks of the postnatal period. Therefore, infants remain unprotected for an extended period before vaccines can be administered. The availability of more immunogenic and effective preparations may evoke a stronger immune response in the mother, resulting in a longer efficacy period in the infant. Worldwide, the influenza vaccination coverage of pregnant women is very low, and only strong initiatives to improve coverage will favor infant protection.

Moreover, the best influenza vaccine to use in children has not been precisely defined. LAIV has raised interest because it avoids intramuscular injection. However, it is licensed only for children ≥ 2 years of age, and its real efficacy is debated. The ACIP of the USA has recently decided that for the 2016–2017 influenza season, LAIV could not be used because it was found to be ineffective in the previous 3 influenza seasons. This decision was not accepted by several other health authorities because LAIV vaccine effectiveness was considered adequate in their countries. The reasons for the differences in vaccine effectiveness are unknown. However, data on LAIV effectiveness must be clarified because LAIV can significantly improve vaccination coverage in children. Parents are reluctant to accept immunization by intramuscular injection, and the availability of an intranasally administered, effective and safe vaccine may significantly improve compliance with recommendations. Further studies are needed to improve our knowledge and solve all the problems to assure the best protection of children against influenza, a common frequently under evaluated disease.

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No potential conflicts of interest were disclosed.

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References

- [1] Antonova EN, Rycroft CE, Ambrose CS, Heikkinen T, Principi N. Burden of paediatric influenza in Western Europe: a systematic review. *BMC Public Health* 2012; 12:968; PMID:23146107; <https://doi.org/10.1186/1471-2458-12-968>
- [2] Principi N, Esposito S. Severe influenza in children: incidence and risk factors. *Expert Rev Anti Infect Ther* 2016; 14:961-8; PMID:27560100; <https://doi.org/10.1080/14787210.2016.1227701>
- [3] Lafond KE, Nair H, Rasooly MH, Valente F, Booy R, Rahman M, Kitsutani P, Yu H, Guzman G, Coulibaly D, et al. Global role and burden of influenza in pediatric respiratory hospitalizations, 1982–2012: a systematic analysis. *PLoS Med* 2016; 13:e1001977; PMID:27011229; <https://doi.org/10.1371/journal.pmed.1001977>
- [4] Heikkinen T, Silvennoinen H, Heinonen S, Vuorinen T. Clinical and socioeconomic impact of moderate-to-severe versus mild influenza in children. *Eur J Clin Microbiol Infect Dis* 2016; 35:1107-13; PMID:27086364; <https://doi.org/10.1007/s10096-016-2641-9>
- [5] Centers for Disease Control and Prevention (CDC). Weekly U.S. Influenza Surveillance Report. Available at: <http://www.cdc.gov/flu/weekly/index.htm> Accessed on: September 30, 2016.
- [6] Centers for Disease Control and Prevention (CDC). Estimating seasonal influenza-associated deaths in the United States: CDC study confirms variability of flu. Available at: http://www.cdc.gov/flu/about/disease/us_flu-related_deaths.htm Accessed on September 30, 2016).
- [7] Principi N, Esposito S. Are we ready for universal influenza vaccination in paediatrics? *Lancet Infect Dis* 2004; 4:75-83; PMID:14871631; [https://doi.org/10.1016/S1473-3099\(04\)00926-0](https://doi.org/10.1016/S1473-3099(04)00926-0)
- [8] Wong KK, Jain S, Blanton L, Dhara R, Brammer L, Fry AM. Influenza-associated pediatric deaths in the United States, 2004–2012. *Pediatrics* 2013; 132:796-804; PMID:24167165; <https://doi.org/10.1542/peds.2013-1493>
- [9] European Centre for Disease Prevention and Control. Recommended immunization for influenza. Available at: <http://vaccine-schedule.ecdc.europa.eu/Pages/Scheduler.aspx> Accessed on September 30, 2016.
- [10] Grohskopf LA, Sokolow LZ, Broder KR, Olsen SJ, Karron RA, Jernigan DB, Bresee JS. Prevention and control of seasonal influenza with vaccines. *MMWR Recomm Rep* 2016; 65:1-54; PMID:27560619; <https://doi.org/10.15585/mmwr.rr6505a1>
- [11] Esposito S, Principi N. Different influenza vaccine formulations and adjuvants for childhood influenza vaccination. *Vaccine* 2011; 29:7535-41; PMID:21820478; <https://doi.org/10.1016/j.vaccine.2011.08.012>
- [12] Esposito S, Montinaro V, Groppali E, Tenconi R, Semino M, Principi N. Live attenuated intranasal influenza vaccine. *Hum Vaccin Immunother* 2012; 8:76-80; PMID:22251995; <https://doi.org/10.4161/hv.8.1.18809>
- [13] Ambrose CS, Levin MJ. The rationale for quadrivalent influenza vaccines. *Hum Vaccin Immunother* 2012; 8:81-8; PMID:22252006; <https://doi.org/10.4161/hv.8.1.17623>
- [14] Centers for Disease Control and Prevention (CDC). Pregnant women and flu vaccination, Internet Panel Survey, United States, November 2015. Available at: <http://www.cdc.gov/flu/fluview/pregnant-women-nov2015.htm> Accessed on: September 27, 2016.
- [15] European Centre for Disease Prevention and Control. Technical Report. Seasonal influenza vaccination in Europe: overview of vaccination recommendations and coverage rates in the EU Member States for the 2012–13 influenza season. Available at: <http://ecdc.europa.eu/en/publications/Publications/Seasonal-influenza-vaccination-Europe-2012-13.pdf> Accessed on: September 27, 2016.
- [16] Englund JA, Walter E, Black S, Blatter M, Nyberg J, Ruben FL, Decker MD, GRC28 Study Team. Safety and immunogenicity of trivalent inactivated influenza vaccine in infants: a randomized double-blind placebo controlled study. *Pediatr Infect Dis J* 2010; 29:105-10; PMID:19934787; <https://doi.org/10.1097/INF.0b013e3181b84c34>
- [17] Principi N, Senatore L, Esposito S. Protection of young children from influenza through universal vaccination. *Hum Vaccin Immunother* 2015; 11:2350-8; PMID:26090704; <https://doi.org/10.1080/21645515.2015.1055428>
- [18] Mbawuike IN, Six HR, Cate TR, Couch RB. Vaccination with inactivated influenza A virus during pregnancy protects neonatal mice against lethal challenge by influenza A viruses representing three subtypes. *J Virol* 1990; 64:1370-4; PMID:2304146
- [19] Sweet C, Jakeman KJ, Smith H. Role of milk-derived IgG in passive maternal protection of neonatal ferrets against influenza. *J Gen Virol* 1987; 68:2681-6; PMID:3668509; <https://doi.org/10.1099/0022-1317-68-10-2681>
- [20] Englund JA, Mbawuike IN, Hammill H, Holleman MC, Baxter BD, Glezen WP. Maternal immunization with influenza or tetanus toxoid vaccine for passive antibody protection in young infants. *J Infect Dis* 1993; 168:647-56; PMID:8354906; <https://doi.org/10.1093/infdis/168.3.647>
- [21] Benowitz I, Esposito DB, Gracey KD, Shapiro ED, Vazquez M. Influenza vaccine given to pregnant women reduces hospitalization due to influenza in their infants. *Clin Infect Dis* 2010; 51:1355-61; PMID:21058908; <https://doi.org/10.1086/657309>

- [22] Poehling KA, Szilagyi PG, Staat MA, Snively BM, Payne DC, Bridges CB, Chu SY, Light LS, Prill MM, Finelli L, et al. Impact of maternal immunization on influenza hospitalizations in infants. *Am J Obstet Gynecol* 2011; 204(6 Suppl. One): S141-8; PMID:21492825; <https://doi.org/10.1016/j.ajog.2011.02.042>
- [23] Eick AA, Uyeki TM, Klimov A. Maternal influenza vaccination and effect on influenza virus infection in young infants. *Arch Pediatr Adolesc Med* 2011; 165:104-11; PMID:20921345; <https://doi.org/10.1001/archpediatrics.2010.192>
- [24] Regan AK, Klerk Nd, Moore HC, Omer SB, Shellam G, Effler PV. Effectiveness of seasonal trivalent influenza vaccination against hospital-attended acute respiratory infections in pregnant women: a retrospective cohort study. *Vaccine* 2016; 34:3649-56; PMID:27216758; <https://doi.org/10.1016/j.vaccine.2016.05.032>
- [25] Dabrera G, Zhao H, Andrews N, Begum F, Green H, Ellis J, Elias K, Donati M, Zambon M, Pebody R. Effectiveness of seasonal influenza vaccination during pregnancy in preventing influenza infection in infants, England, 2013/14. *Euro Surveill* 2014; 19:20959; PMID:25411687; <https://doi.org/10.2807/1560-7917.ES2014.19.45.20959>
- [26] Omer SB, Goodman D, Steinhoff MC, Rochat R, Klugman KP, Stoll BJ. Maternal influenza immunization and reduced likelihood of prematurity and small gestational age births: a retrospective cohort study. *PLoS Med* 2011; 8:e1000441; PMID:21655318; <https://doi.org/10.1371/journal.pmed.1000441>
- [27] Sheffield J, Roberts S, Lorimer M, Casey B, McIntire D, Wendel G. The efficacy and safety of influenza vaccination in pregnancy. In *Obstetrics Ajog*, editor. Society for maternal-fetal medicine: 31st Annual Meeting: the pregnancy meeting, 2011.
- [28] Ludvigsson JF, Ström P, Lundholm C, Cnattingius S, Ekblom A, Örtqvist Å, Feltelius N, Granath F, Stephansson O. Maternal vaccination against H1N1 influenza and offspring mortality: population based cohort study and sibling design. *BMJ*; 351: h5585; PMID:26572546; <https://doi.org/10.1136/bmj.h5585>
- [29] Mbawuie IN, Six HR, Cate TR, Couch RB. Vaccination with inactivated influenza A virus during pregnancy protects neonatal mice against lethal challenge by influenza A viruses representing three subtypes. *J Virol* 1990; 64:1370-4; PMID:2304146
- [30] World Health Organization. Immunization, Vaccines and Biologicals. WHO recommends seasonal influenza vaccination to pregnant women as the highest priority. Available at: http://www.who.int/immunization/newsroom/newsstory_seasonal_influenza_vaccination_pregnancy/en/ Accessed on: September 28, 2016.
- [31] Centers for Disease Control and Prevention (CDC). Prevention and control of seasonal influenza with vaccines. Recommendations of the Advisory Committee on Immunization Practices-United States, 2013-2014. *MMWR Recomm Rep* 2013; 62(RR-07):1-43.
- [32] Public Health Agency of Canada. An advisory committee statement on seasonal influenza vaccine for 2015-2016. Modified 2016-03-08. Available at: <http://www.phac-aspc.gc.ca/naci-ccni/assets/pdf/flu-2015-grippe-eng.pdf>; Accessed on September 28, 2016
- [33] Public Health England. Annual flu programme. Available at: <https://www.gov.uk/government/collections/annual-flu-programme>. Accessed on: October 3, 2016.
- [34] Zaman K, Roy E, Arifeen SE. Effectiveness of maternal in fluenza immunization in mothers and infants. *N Engl J Med* 2008; 359:1555-64; PMID:18799552; <https://doi.org/10.1056/NEJMoa0708630>
- [35] Thompson MG, Li DK, Shifflett P, Sokolow LZ, Ferber JR, Kurosky S, Bozeman S, Reynolds SB, Odouli R, Henninger ML, et al. Effectiveness of seasonal trivalent influenza vaccine for preventing influenza virus illness among pregnant women: a population-based case-control study during the 2010-2011 and 2011-2012 influenza seasons. *Clin Infect Dis* 2014; 58:449-57; PMID:24280090; <https://doi.org/10.1093/cid/cit750>
- [36] Puleston RL, Bugg G, Hoschler K, Konje J, Thornton J, Stephenson I, Myles P, Enstone J, Augustine G, Davis Y, et al. Observational study to investigate vertically acquired passive immunity in babies of mothers vaccinated against H1N1v during pregnancy. *Health Technol Assess* 2010; 14:1-82; PMID:21208547; <https://doi.org/10.3310/hta14550-01>
- [37] Zuccotti G, Pogliani L, Pariani E, Amendola A, Zanetti A. Transplacental antibody transfer following maternal immunization with a pandemic 2009 influenza A (H1N1) MF59-adjuvanted vaccine. *JAMA* 2010; 304:2360-1; PMID:21119083; <https://doi.org/10.1001/jama.2010.1729>
- [38] Manske JM. Efficacy and effectiveness of maternal influenza vaccination during pregnancy: a review of the evidence. *Matern Child Health J* 2014; 18:1599-609; PMID:24272875; <https://doi.org/10.1007/s10995-013-1399-2>
- [39] Wilson RJ, Paterson P, Jarrett C, Larson HJ. Understanding factors influencing vaccination acceptance during pregnancy globally: a literature review. *Vaccine* 2015; 33:6420-9; PMID:26320417; <https://doi.org/10.1016/j.vaccine.2015.08.046>
- [40] McCarthy EA, Pollock WE, Nolan T, Hay S, McDonald S. Improving influenza vaccination coverage in pregnancy in Melbourne 2010-2011. *Aust N Z J Obstet Gynaecol* 2012; 52:334-41; PMID:22486173; <https://doi.org/10.1111/j.1479-828X.2012.01428.x>
- [41] Esposito S, Bosis S, Pelucchi C, Tremolati E, Sabatini C, Semino M, Marchisio P, della Croce F, Principi N. Influenza vaccination among healthcare workers in a multidisciplinary University hospital in Italy. *BMC Public Health* 2008; 8:422; PMID:19105838; <https://doi.org/10.1186/1471-2458-8-422>
- [42] Centers for Disease Control and Prevention (CDC). Influenza vaccination in pregnancy: practices among obstetrician-gynecologists - United States, 2003-04 influenza season. *MMWR Morb Mortal Wkly Rep* 2005; 54:1050-2; PMID:16237376
- [43] Green Book. Chapter 19. Influenza. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/456568/2904394_Green_Book_Chapter_19_v10_0.pdf Accessed on: September 27, 2016.
- [44] Nunes MC, Cutland CL, Jones S, Hugo A, Madimabe R, Simões EA, Weinberg A, Madhi SA, Maternal Flu Trial Team. Duration of infant protection against influenza illness conferred by maternal immunization: secondary analysis of a randomized clinical trial. *JAMA Pediatr* 2016; 170:840-7; PMID:27380464; <https://doi.org/10.1001/jamapediatrics.2016.0921>
- [45] Munoz FM. Infant protection against influenza through maternal immunization: a call for more immunogenic vaccines. *JAMA Pediatr* 2016; 170:832-3; PMID:27380323; <https://doi.org/10.1001/jamapediatrics.2016.1322>
- [46] Proveaux T, Lambach P, Ortiz JR, Hombach J, Halsey NA. Review of prescribing information for influenza vaccines for pregnant and lactating women. *Vaccine* 2016; 34:5406-09.
- [47] The American College of Obstetricians and Gynecologists. Immunization resources for obstetrician-gynecologists. A comprehensive tool kit. Available at: <https://www.acog.org/-/media/Departments/Immunization/ImmunizationToolkit.pdf> Accessed on: September 30, 2016.
- [48] Wong VW, Lok KY, Tarrant M. Interventions to increase the uptake of seasonal influenza vaccination among pregnant women: a systematic review. *Vaccine* 2016; 34:20-32; PMID:26602267; <https://doi.org/10.1016/j.vaccine.2015.11.020>
- [49] Fleming DM, Crovari P, Wahn U, Klemola T, Schlesinger Y, Langussis A, Øymar K, Garcia ML, Krygier A, Costa H, et al. Comparison of the efficacy and safety of live attenuated cold-adapted influenza vaccine, trivalent, with trivalent inactivated influenza virus vaccine in children and adolescents with asthma. *Pediatr Infect Dis J* 2006; 25:860-9; PMID:17006278; <https://doi.org/10.1097/01.inf.0000237797.14283.cf>
- [50] Ashkenazi S, Vertruyen A, Aristegui J, Esposito S, McKeith DD, Klemola T, Biolek J, Kühn J, Bujnowski T, Desgrandchamps D, et al. Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections. *Pediatr Infect Dis J* 2006; 25:870-9; PMID:17006279; <https://doi.org/10.1097/01.inf.0000237829.66310.85>
- [51] Belshe RB, Coelingh K, Ambrose CS, Woo JC, Wu X. Efficacy of live attenuated influenza vaccine in children against influenza B viruses by lineage and antigenic similarity. *Vaccine* 2010; 28:2149-56; PMID:20003926; <https://doi.org/10.1016/j.vaccine.2009.11.068>

- [52] Grohskopf LA, Olsen SJ, Sokolow LZ, Bresee JS, Cox NJ, Broder KR, Karron RA, Walter EB, Centers for Disease Control and Prevention. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP)—United States, 2014–15 influenza season. *MMWR Morb Mortal Wkly Rep* 2014; 63:691-97; PMID:25121712
- [53] Public Health Agency of Canada. Canadian immunization guide chapter on influenza and statement on seasonal influenza vaccine for 2016-2017. Available at: <http://www.phac-aspc.gc.ca/naci-ccni/flu-2016-grippe-addendum-children-enfants-eng.php#footnote28>. Accessed on: October 3, 2016.
- [54] European Medicines Agency. Fluenz Tetra. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002617/human_med_001713.jsp&mid=WC0b01ac058001d124. Accessed on: October 3, 2016.
- [55] Joint Committee on Vaccination and Immunisation (JCVI). JCVI statement on the annual influenza vaccination programme – extension of the programme to children. Available at: <https://www.gov.uk/government/publications/jcvi-statement-on-the-routine-annual-influenza-vaccination-programme>. Accessed on: October 3, 2016.
- [56] Chung JR, Flannery B, Thompson MG, Gaglani M, Jackson ML, Monto AS, Nowalk MP, Talbot HK, Treanor JJ, Belongia EA, et al. Seasonal effectiveness of live attenuated and inactivated influenza vaccine. *Pediatrics* 2016; 137:e20153279; <https://doi.org/10.1542/peds.2015-3279>
- [57] Flannery B. LAIV vs IIV effectiveness: Summary of evidence since 2009. Presented to Advisory Committee on Immunization Practices, Atlanta, 2016. Available at: <http://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2016-06/influenza-07-flannery.pdf>. Accessed on: October 3, 2016.
- [58] Caspard H, Gaglani M, Clipper L, Belongia EA, McLean HQ, Griffin MR, Talbot HK, Poehling KA, Peters TR, Veney N, et al. Effectiveness of live attenuated influenza vaccine and inactivated influenza vaccine in children 2-17 years of age in 2013-2014 in the United States. *Vaccine* 2016; 34:77-82; PMID:26589519; <https://doi.org/10.1016/j.vaccine.2015.11.010>
- [59] Kwong JC, Pereira J, Quach S, Pellizzari R, Dusome E, Russell M, Hamid JS, Feinberg Y, Winter AL, Gubbay JB, et al. Randomized evaluation of live attenuated vs. trivalent inactivated influenza vaccines in schools (RELATIVES) pilot study: preliminary results from the household surveillance sub-study. *Vaccine* 2015; 33:4910-5; PMID:26232348; <https://doi.org/10.1016/j.vaccine.2015.07.044>
- [60] Skowronski DM, Chambers C, Sabaiduc S, De Serres G, Winter AL, Dickinson JA, Gubbay J, Fonseca K, Charest H, Krajden M, et al. Integrated sentinel surveillance linking genetic, antigenic, and epidemiologic monitoring of influenza vaccine-virus relatedness and effectiveness during the 2013–2014 influenza season. *J Infect Dis* 2015; 212:726-39; PMID:25784728; <https://doi.org/10.1093/infdis/jiv177>
- [61] Pebody RG, Green HK, Andrews N, Zhao H, Boddington N, Bawa Z, Durnall H, Singh N, Sunderland A, Letley L, et al. Uptake and impact of a new live attenuated influenza vaccine programme in England: early results of a pilot in primary school-age children, 2013/14 influenza season. *Euro Surveill* 2014; 19: pii 20823
- [62] Pebody R, Warburton F, Andrews N, Ellis J, von Wissmann B, Robertson C, Yonova I, Cottrell S, Gallagher N, Green H, et al. Effectiveness of seasonal influenza vaccine in preventing laboratory-confirmed influenza in primary care in the United Kingdom: 2014/15 end of season results. *Euro Surveill* 2015; 20:36; PMID:26535911; <https://doi.org/10.2807/1560-7917.ES.2015.20.36.30013>
- [63] Flannery B, Chung J. Influenza vaccine effectiveness, including LAIV vs IIV in children and adolescents, US Flu VE Network, 2015-16. Presented to Advisory Committee on Immunization Practices, Atlanta, 2016. Available at: <http://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2016-06/influenza-05-flannery.pdf> Accessed on September 30, 2016.
- [64] Ambrose C. 2015-16 US influenza vaccine effectiveness: Influenza clinical investigation for children (ICICLE) study. Presented to Advisory Committee on Immunization Practices, Atlanta, 2016.
- [65] Public Health England. Influenza vaccine effectiveness in adults and children in primary care in the UK: provisional end-of-season results 2015-16. 2016. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/530756/Influenza_vaccine_effectiveness_in_primary_care_in_children.pdf Accessed on October 3, 2016.
- [66] Skowronski DM. Live attenuated influenza vaccine (LAIV) vs. inactivated influenza vaccine (IIV): summary of effectiveness evidence since 2009. Presented to NACI IWG, Ottawa, 2016.
- [67] Nohynek H. Seasonal childhood influenza vaccinations: experiences from Finland. Presented at the Nordic Vaccine Meeting, Iceland, 2016.
- [68] Flannery B, Chung J. Influenza vaccine effectiveness, including LAIV vs IIV in children and adolescents, US Flu VE Network, 2015-16. Presented to Advisory Committee on Immunization Practices, Atlanta, 2016. Accessed: July 21, 2016. Available at: <http://www.cdc.gov/vaccines/acip/meetings/downloads/slides-2016-06/influenza-05-flannery.pdf>. Accessed on: October 3, 2016.
- [69] Centers for Disease Control and Prevention. ACIP votes down use of LAIV for 2016-2017 flu season. 2016. Available at: <http://www.cdc.gov/media/releases/2016/s0622-laiv-flu.html> Accessed on: October 3, 2016.
- [70] US Food and Drug Administration. FDA information regarding FluMist quadrivalent vaccine. 2016. Accessed: June 30, 2016. Available at: <http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm508761.htm>. Accessed on: October 3, 2016.
- [71] Public Health England. Use of live attenuated influenza vaccine (LAIV) FluMist quadrivalent: frequently asked questions for health-care workers. Available at: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/469992/Q_A_for_FluMist_Quadrivalent.pdf. Accessed on: October 3, 2016.
- [72] Department of Health Protection. Seasonal childhood influenza vaccinations experiences from Finland. Available at: http://www.nvm2016.is/sites/default/files/Nohynek_NorVac_influenzaLessonsNohynek_short.pdf. Accessed on: October 3, 2016.
- [73] Ohmit SE, Thompson MG, Petrie JG, Thaker SN, Jackson ML, Belongia EA, Zimmerman RK, Gaglani M, Lamerato L, Spencer SM, et al. Influenza vaccine effectiveness in the 2011-2012 season: protection against each circulating virus and the effect of prior vaccination on estimates. *Clin Infect Dis* 2014; 58:319-27; PMID:24235265; <https://doi.org/10.1093/cid/cit736>
- [74] MedImmune LLC. A Study to evaluate the immunogenicity of quadrivalent live attenuated influenza vaccine (LAIV) in children. 2011. Available at: <https://clinicaltrials.gov/ct2/show/results/NCT01091246?sect=X5>. Accessed on: October 3, 2016.
- [75] Cotter CR, Jin H, Chen Z. A single amino acid in the stalk region of the H1N1pdm influenza virus HA protein affects viral fusion, stability and infectivity. *PLoS Pathog* 2014; 10:e1003831; PMID:24391498; <https://doi.org/10.1371/journal.ppat.1003831>
- [76] Centers for Disease Control and Prevention (CDC). Vaccine temperature best practices for refrigerated vaccines-Fahrenheit (F). Available at: <http://www.cdc.gov/vaccines/hcp/admin/storage/downloads/temp-fridge.pdf>. Accessed on: October 3, 2016.
- [77] Caspard H, Coelingh KL, Mallory RM, Ambrose CS. Association of vaccine handling conditions with effectiveness of live attenuated influenza vaccine against H1N1pdm09 viruses in the United States. *Vaccine* 2016; 34:5066-5072; PMID:27613072; <https://doi.org/10.1016/j.vaccine.2016.08.079>
- [78] Ambrose CS, Bright H, Mallory R. Potential causes of the decreased effectiveness of the influenza A(H1N1) pdm09 strain in live attenuated influenza vaccines. *Euro Surveill* 2016; 21:pii 30394; PMID:27918259