Seroprevalence of antibodies anti-pertussis toxins of *Bordetella pertussis* in blood donors in Casablanca, Morocco

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**SUMMARY**

Despite vaccination coverage, whooping cough is re-emerging around the world. Anti-pertussis toxins (PT) serology remains the technique of choice for the epidemiological surveillance of this disease. The objective of our study is to assess the seroprevalence of anti-PT IgG / IgA in blood donors in Casablanca.

This is a retrospective study of 91 blood donor samples collected at the regional blood transfusion center of Casablanca (CRTS) over 28 days carried out at the level of the Immunology Laboratory of the Faculty of Medicine and Pharmacy of Casablanca in collaboration with the Microbiology Laboratory of the same faculty. We measured the serum titers of anti-PT IgG / IgA by the ELISA method, and we compared the seroprevalences of these immunoglobulins according to age and sex.

The seroprevalence of anti-PT IgA was 41.76% (mean serum titer of 16.03 ± 20.8 IU / ml), and that of anti-PT IgG was 6.59% (mean serum titer of 24.49 ± 35.26 IU / ml). The seroprevalence in subjects over 50 years old was significantly higher than in subjects under 50 years old, for both IgG and IgA anti-PT (p=0.001 and 0.04; respectively).

Thus, our results demonstrate the existence of a resurgence of *B. pertussis* with the modification of its seroepidemiology in the vaccinated adult population;
however they need to be confirmed by more representative studies to establish a new preventive strategy against this pathology in our country.

1. Introduction

Pertussis is a highly contagious disease of the respiratory tract caused by *Bordetella pertussis*, which is a particularly serious disease and can be even fatal for infants. It remains a major cause of child mortality worldwide, even in countries with high immunization coverage (Tone *et al.*, 2020). The World Health Organization encourages careful epidemiological surveillance of this disease to monitor its burden and the impact of vaccination. In Morocco, pertussis vaccination was introduced and expanded by the national vaccination program in the early 1980s, reducing its incidence from around 15,000 cases per year in 1980 to less than 100 cases per year in 2011 (Katfy *et al.*, 2020). In recent years, however, there has been a resurgence of Pertussis around the world (Levy *et al.*, 2014). This current re-emergence of pertussis is of great interest to our study.

Serology and especially Enzyme-linked immunosorbent assay (ELISA) remains the technique of choice for epidemiological surveillance and may be necessary as a diagnostic tool for adults vaccinated during childhood and for whom the presence of anti-pertussis toxins (anti-PT) antibodies is a sign of infection or carriage of *B.pertussis* bacteria.

This diagnosis has always been retrospective since it requires two serologies taken 1 month apart (one serum collected in the acute stage and a second one collected in the convalescence stage). A case is confirmed if there is an increase or a decrease in antibody levels between the two. However, if the antibody level is sufficiently high in the first serum, for either an adolescent or an adult, the test is increasingly seen as confirmation of an infection, especially if immunoglobulins G and A are detected.

The objective of our study is to evaluate the seroprevalence of anti-PT IgG/IgA according to age and sex in blood donors in Casablanca Morocco.

2. Material and methods

This is a monocentric retrospective study of the prevalence on serums of 91 blood donors collected at the Casablanca Regional Blood Transfusion Centre (CRST) over 28 days. The samples were analyzed at the Immunology Laboratory of the Faculty of Medicine and Pharmacy of Casablanca. The study was anonymous and all the mandatory laboratory health and safety procedures have been complied.

Thus, serum samples from random anonymous blood donors were centrifuged at 4000 rpm in a refrigerated centrifuge and stored in the laboratory serum library at \(-20°C\) until needed. We used to measure IgG and IgA anti-PT toxin (anti-PT) serum titers by ELISA method with a kit "SeroPertussis TM Toxin IG, from Savyon ® Diagnostics Ltd".

We included in our study all serums from blood donors who met the requirements mentioned by the manufacturer in the Kit's technical notice. And by the criteria mentioned therein, we excluded all heat-inactivated, lipemic, cloudy, or contaminated serums, as well as those with a serum volume less than 10μL.

Qualitative variables are represented by numbers and percentages and quantitative variables by means and standard deviations. Interpretation of the results is only possible after validation of the technique and calculation of the IU/ml concentrations of IgG/IgA anti-PT. The statistical analysis was carried out using the following software: STATA and SPSS version 24.0. The significance
threshold used was \( p<0.05 \) and the thresholds used for the interpretation of the results are those recommended by the manufacturer of the Kit:

For anti-PT IgA:

- Threshold \(<12 \text{ IU/ml}\): Result considered negative.
- Threshold \( \geq 12 \text{ IU/ml}\): Result considered positive.

For anti-PT IgG (IU/ml):

- Threshold \(<40 \text{ IU/ml}\): Negative result (no indication of ongoing infection).
- Threshold \( \geq 40 \text{ to } <100 \text{ IU/ml}\): Intermediate result (possible infection, re-test IgG after 2 to 4 weeks or test for IgA).
- Threshold \( \geq 100 \text{ IU/ml}\): Positive result (indication of current infection or recent contact if no recent vaccination has been received).

Thus, the interpretation of anti-TP IgG/IgA profiles will be as follows:

- If IgG negative and IgA negative: no \textit{B.pertussis} infection.
- If intermediate IgG and IgA negative: no recent infection.
- If intermediate or negative IgG and IgA positive: indication of recent infection.
- If IgG positive and IgA negative or positive: indication of recent infection.

3. Results

- Description of the studied population:

The population studied was composed of 35.1\% of women and 64.84\% of men with a sex ratio of 1.84. The average age of blood donors was 37.41 ± 10.8 years (extremes: 20 and 61 years old). The distribution of blood donors by age group was 49.45\% (for 20-35 year old), 30.77\% (for 35-50 year olds), and 19.78\% (for 50-65 year old).

- Average serum titer of anti-PT IgA/IgG:

The anti-PT IgA/IgG serum titer mean were 16.03 ± 20.8 IU/ml (extremes: 1.17 and 143.25 IU/ml). Besides, the average anti-PT IgG titer was 24.49 ± 35.26 IU/ml (extremes: 0.81 to 229.24 IU/ml).

The average titer of the anti-PT IgG found was, statistically, significantly different between the two sexes (\( p=0.0393 \)), contrary to the average titer of the anti-PT IgA.

The results of the anti-PT IgG/IgA serum titers mean by gender are shown in Figure 1.

![Figure 1. Average serum IgA and IgG anti-PT titers by sex](image)

The mean of anti-PT IgA/IgG serum titers in blood donors by age group are shown in Figure 2.
Seroprevalence of anti-PT IgA/IgG in blood donors by sex and age group are shown in Tables I and II respectively. There was a statistically significant difference between these seroprevalences with a p=1.5327×10⁻⁸ for IgA and p=0.0001 for IgG.

Immune status of blood donors

Thanks to the correlation between the serological profiles of anti-PT IgA and IgG recommended by the manufacturer of the kit used, it has been possible to study the immune status of blood donors towards pertussis by dividing them into three groups: "non-immune", "immune" and "recently infected". Of the 91 blood donors, 54.95% were reported to be non-immune to pertussis, 3.3% were reported to be immune, while 41.75% had recent exposure to B. pertussis (Figure 3).
The distribution of blood donors’ immune status towards pertussis according to age and sex is summarized in Table III.

Table III. Breakdown of the studied population-based on pertussis immunity status by age and sex

<table>
<thead>
<tr>
<th>Age</th>
<th>Immunity status</th>
<th>Serological profiles</th>
<th>Female</th>
<th>Male</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Prevalence(%)</td>
<td>Number</td>
<td>Prevalence(%)</td>
</tr>
<tr>
<td>[20-35] yrs</td>
<td>Non-immune</td>
<td>IgG and Ig A (+)</td>
<td>11</td>
<td>61.11</td>
</tr>
<tr>
<td></td>
<td>Immune</td>
<td>IgG (I) and Ig A (+)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Recently infected</td>
<td>IgG (I) or (-) and Ig A (+)</td>
<td>7</td>
<td>38.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgG (+) and Ig A (-) or (+)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[35-50] yrs</td>
<td>Non-immune</td>
<td>IgG and Ig A (+)</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Immune</td>
<td>IgG (I) and Ig A (+)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Recently infected</td>
<td>IgG (I) or (-) and Ig A (+)</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgG (+) and Ig A (-) or (+)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>[50-65] yrs</td>
<td>Non-immune</td>
<td>IgG and Ig A (+)</td>
<td>3</td>
<td>33.33</td>
</tr>
<tr>
<td></td>
<td>Immune</td>
<td>IgG (I) and Ig A (+)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Recently infected</td>
<td>IgG (I) or (-) and Ig A (+)</td>
<td>2</td>
<td>22.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgG (+) and Ig A (-) or (+)</td>
<td>4</td>
<td>44.45</td>
</tr>
</tbody>
</table>

4. Discussion

Over the last few years, pertussis has re-emerged all over the world, even in vaccinated populations. The resurgence of this disease and the modifications in its epidemiology have raised the interest of several authors who have conducted numerous studies, most of which are essentially epidemiological (Di Mattia et al., 2019).

According to Domenech et al. (2018), vaccination is the powerful factor that directly changed the epidemiology of pertussis. Before the introduction of vaccination, the highest incidence was among children aged between five and seven years who were infected in the community (Gaelle et al., 2020). Thereafter, adolescents and adults who had regular contact with contaminated children received the so-called natural reminder doses (Guiso et al., 2010).

After generalized vaccination for children, the disease decreased significantly in this age group and as a result, adolescents and adults no longer had "natural booster shots" and had not had any natural vaccine recalls, resulting in a decrease in immunity over time (Hallander et al., 2005; Van Amersfoorth et al., 2005). They then became a source of contamination for the rest of the population according to Zhang et al., (2019). The transmission of the disease, which was therefore from child to child during the pre-vaccination era, is from adult to child (Guiso et al., 2010). Adolescents and adults are therefore now recognized as the main source of contamination of infants who are not or not fully vaccinated (Koufakis et al., 2017; Baxter et al., 2014; Macina et al., 2021).

On the one hand, it was the introduction of the vaccine and its widespread use that changed the transmission way and thus the epidemiology of the disease. On the other hand, the main reason for the current resurgence of pertussis may be linked to the change in vaccination protocol, which now uses a vaccine containing purified acellular bacterial components, which is less reactogenic and more tolerated than first-generation vaccines consisting of whole killed bacteria (Pósfay-Barbe et al., 2006). Several studies show that the protective immunity induced by the acellular vaccine is of shorter duration (Kurova et al., 2018; McGirr et al., 2015).

The increase in cases of pertussis may also be the result of improved diagnostic capabilities with the development of more efficient techniques such as polymerase chain reaction (PCR) and the improved availability of these
diagnostic tests. Also, other factors may contribute to this resurgence, including the decline in vaccine-induced immunity and the adaptation of the causal pathogen \textit{B.pertussis} (Mooi et al., 2014).

Given the increased incidence of pertussis, epidemiological surveillance is required. Now, serology allows us to obtain a more realistic estimation of the circulation of \textit{B. pertussis} in a population. Sero-epidemiology has thus become an essential component in surveillance programs, and pertussis toxins antibodies are used as the main markers. This surveillance provides the opportunity to study the decline in immunity in a given population. After excluding subjects who have been vaccinated within the last two years, it would therefore be possible to estimate the proportions of recent infections according to Huygen et al. (2014).

The study concerns the seroprevalence of IgG/IgA anti-PT antibodies in a population of 91 Moroccan blood donors. We observed higher mean serum titers in women than in men with a significant difference (p=0.0393) for IgG only. Furthermore, the titers tended to increase with age since donors aged 50 years and over had the highest serum titers, and only the 50 to 65 age group showed an increase in antibody titers in women compared to men with significant differences for both IgA (p=0.05) and IgG (p=0.0395).

IgG/IgA seropositive cases were found in all age groups and both sexes. The majority of cases were female and elderly subjects in whom the decline in immune response was likely to be more severe. Thus, we noted that the protective immunity to pertussis in blood donors decreased with age, in contrast to the prevalence of the infection, which increased with age. This may be due to several factors, including immune senescence and a decrease in the protective effect of the vaccination over time since vaccination lasts only a few years (Kurova et al., 2018; Aye et al., 2020); as a result, elderly adults would no longer be protected and would be exposed to infection again.

A study approved by the Institutional Review Board of Seoul carried out on a sample of 1192 volunteer to assess the seroprevalence of pertussis in Korea showed an average anti-PT IgG titer and seroprevalence of 35.53 ± 62.91 IE/ml and 41.4%, respectively, with no significant difference between age groups (Lee et al., 2014). However, the seroprevalence of people aged over 51 was significantly higher than that of people under 50 (Lee et al., 2014), confirming our results. On the other hand, the seroprevalence of IgG positive in men population was higher unlike in our study. This could be explained by the difference in the number of samples, which would automatically influence the sex ratio.

Therefore, our results suggested that the risk of exposure to pertussis would increase with age, especially among women. This was confirmed in a meta-analysis by Wiley and al, who concluded that most of the identified sources of \textit{B.pertussis} infection in young children were women, particularly the mothers (Wiley et al., 2013). Also, other studies have shown that mothers are a significant source of \textit{B.pertussis} infection of young children (Skoff et al., 2015; Gill et al., 2020).

Many experts agree that there is a clear trend towards an increase in pertussis in children aged 7-10 years and adults, whereas, in the past, the disease affected newborns. However, in our study, we reported a high seroprevalence among older adults, which could be due to a possible pertussis epidemic during the period when our serum samples were collected. In other words, it can be attributed to the cyclical patterns that are part of the natural history of the disease, as well as to the collection of country-specific
evidence, which in 2012 showed a resurgence of pertussis in many countries including Morocco (Elgarini et al., 2018).

Unfortunately, the majority of studies concerning pertussis focus on anti-PT immunoglobulins type IgG and sometimes IgM but never IgA, which is not the case in our study. In any way, the titration of IgA allowed us to evaluate the immune status of the studied population on the one hand, and on the other hand, it was useful for us to confirm the infection.

It would have been useful to know the vaccination status of the blood donors recruited in our study to be able to distinguish the vaccine response from the natural response to the infection.

Overall, our results would suggest that the decrease in immunity in elderly adults and the absence of pertussis vaccine recalls may be one of the reasons for the increase in the prevalence of this disease and highlight this trend of increasing prevalence in our country.

5. Conclusion

It is important to remember that pertussis is still a live issue and that it cannot be considered as a strictly pediatric disease. It can affect the adult population and is not a lifelong immunizing disease. By contracting pertussis, adults can become the main source of contamination for pregnant women or elderly people or infants who are not or incompletely vaccinated, in whom the disease can have dramatic consequences. This is why it is time to adopt new vaccination strategies such as pertussis vaccine booster shots while extending vaccination to the entourage of those at risk. In addition to regular revaccination, raising awareness among the general population seems to be essential to provide the best possible information on the risks and ways of contamination.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References


