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Asymptomatic Infections: The Hidden Epidemic

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Abstract

Asymptomatic infectious diseases (AI) are frequent, occasionally even more common than overt febrile presentations. Yet, the literature covering this topic is scarce. Acknowledging the role of AI is crucial both to public health practitioners, because AI silently perpetuate epidemics, such as influenza or malaria, and also crucial to newborns' health and their long-term sequelae.

The recent developments in metagenomics and rapid diagnostics open new horizons for the study of AI. The purpose of this review is to highlight key elements in the epidemiology, mechanisms and implications of well-studied infectious agents: *Plasmodium, T. gondii, C. burnetii, Cytomegalovirus, Rhinovirus* and Zika virus.

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Introduction

For centuries, physicians investigating infectious diseases have focused on overt infections, namely those presenting with fever, chills, aches, rash, cough, and diarrhea. However, as the tip of the iceberg, every symptomatic case may represent several asymptomatic cases. Asymptomatic infections span the entire microbiome: viruses [1], bacteria [2], parasites [3], and fungi [4]. The case of Mary Mallon, better known as Typhoid Mary, the first person in the United States identified as an asymptomatic carrier of *Salmonella*, is a formidable example of the importance of asymptomatic infections. Working as a cook, she was presumed to have infected 49 people, three of whom died.

Asymptomatic infections occur in both humans and animals. In fact, some of the notorious zoonoses arise from ostensibly asymptomatic animals (*Ebolavirus*-Bats [5], *Ehrlichia*-dogs [6], *Brucella*-sheep; *Bartonella*-cats). These animals may be completely asymptomatic, or just mildly ill, which may go unnoticed by the owner. While some microorganisms are transmitted to humans directly (airborne or food products), others are transmitted via an intermediary vector, such as ticks, or mosquitoes. Asymptomatic carriers of the pathogenic microbiota (animals or humans) constitute a reservoir maintaining their persistence in nature. The human gastrointestinal tract harbors prokaryotic cells at densities of 10 [11] cells/mL. These enteric cells have intimate functional and genetic relationship with both the local host and each other. These interactions probably keep the virulence of the organisms at bay, while maintaining the potential for infectivity (e.g. Poliovirus, Norovirus) [7]. Carriage of viruses is not rare; it has been estimated that each person may be concurrently infected with 10 different viruses, notably herpesviruses [8]. These viruses are transferable via airborne spread, body contact, blood transfusion, or vertically from mother to fetus.

How can asymptomatic infections be explained? Why do some people will not develop symptoms, yet clearly show serological conversion? Phylogenetically speaking, it can be argued that all creatures share the same archaic ancestor. As the phylogenetic tree has evolved over millions of years, some antigenic epitopes were lost, many were added, others have changed, but some remained conserved. Consequently, immunologically speaking, microorganisms belonging to one kingdom may share epitopes with microorganisms from another. This could partly explain why exposure to one microorganism may elicit an immune response which protects against a challenge with heterotypic microorganisms rendering the second infection asymptomatic [9]. Additional silencing mechanisms may be attributed to the less well-characterized cellular immune arm [10]. The recent developments in genetics have demonstrated the striking influence exerted by our genetic makeup on symptom development following an infectious challenge [11]. Speaking from a microbiological point of view, asymptomatic infections may be influenced either by a low inoculum size, or by low virulence microorganisms. Emerging data indicate that enteric viruses regulate, and are regulated by microbes in the gut through a process termed transkingdom interaction [12].

Why are asymptomatic infections important? Firstly, asymptomatic infections may provide immunity [13], Secondly, asymptomatic infections during pregnancy may produce devastating illness in the newborn (Zika virus, *T. gondii*, or cytomegalovirus) [14,15], or trigger changes leading to cancer (*H. pylori*, Hepatitis B virus, EBV, *Papillomavirus*). Thirdly, mice latently infected with gammaherpesvirus were resistant to infection with bacterial pathogens such as *Listeria and Yersinia* [7]. Fourth, with some microorganisms, failure to recognize their emergence in real-time may perpetuate epidemics, since a substantial proportion of transmissions occurs during the pre-symptomatic phase of infections (e.g. influenza, hepatitis A) [16]. Thus, timely diagnosis and perhaps treatment of asymptomatic infections is of prime importance.

This review will try to shed light on epidemiologic features of long-known and emerging pathogens known to cause AI, their importance in terms of public and personal health, and the immunologic mechanisms operating behind the scenes in a sample of parasites, bacteria, and viruses. Admitting these issues should foster efforts in diagnostics, public health, neonatology, vaccinology and perhaps therapeutics.

Definitions

An asymptomatic or subclinical infection may be defined, with a high degree of certainty, when an outbreak has been investigated in real time and certain exposed people, while not manifesting any symptom, demonstrate seroconversion [3]. However, when the timing

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of infection is remote, the term asymptomatic may not always apply due to recall bias. For example, a pregnant woman with IgG antibodies against *T. gondii* may have been infected in childhood, making it difficult if not impossible to ascertain the asymptomatic nature of infection. Thus, acute or recent asymptomatic cases more clearly fit the definition of true asymptomatic infections.

A rather difficult hurdle in the terminology is how to define carriage of a potential pathogen such as *H. pylori, Meningococcus* or MRSA. Quite often, the terms asymptomatic infection and carriage overlap. At times, carriage of a pathogen may advance to full blown disease. The agents included in this review represent a convenience sample: they were chosen either because they have a significant impact and/or enough data has accumulated on these infections.

Asymptomatic Protozoan Infections

Malaria

The World Health Organization's (WHO) fact sheet on malaria asserts that almost half of the world's population in 2015 were at risk of contracting the disease. Owing to massive efforts, the global malaria incidence between 2000 and 2015 fell by 37% and the death rate by 60%. According to the latest WHO estimates, there were 214 million cases of malaria in 2015 and 438,000 deaths [17]. Although older people in endemic areas may have asymptomatic parasitaemia, other patient-groups are prone to develop severe disease: infants, children under 5 years, pregnant women and patients with HIV/AIDS. Malaria is probably the best studied parasitic disease depicting asymptomatic infection. Despite the absence of a standard definition of asymptomatic parasitaemia, most researchers regard this state as detection of asexual or sexual parasites in the blood with absence of symptoms [18]. The method employed to diagnose malaria (microscopy vs. antigenemia vs. PCR), and the varying sensitivities of each method will ultimately determine the rate of asymptomatic infection. Lindblade and colleagues [19] rightly regarded asymptomatic parasitaemia in malaria as the silent threat. They argue that since treatment is usually provided to care-seeking patients, while a large proportion of malaria infections are asymptomatic, the global malaria epidemic is self-perpetuating.

The rate of asymptomatic infections varies by malaria subtype. Using microscopy for diagnosis, a larger proportion of *P. falciparum* infections (37.5%) compared with *P. vivax* infections (18.5%) in Brazil were asymptomatic [20]. Age, blood parasite density, concomitant illness, and immunity are cardinal factors determining whether malarial infection will be asymptomatic or not. In a survey of children in Mozambique, children <1 year of age had the highest rate of fever attributed to malaria (15.1%; as compared to 5.9% among 5-7 year olds). Children with a low-density *P. falciparum* parasitemia (<499 parasites/μL) had a lower rate of fever (7.2%) as compared with children whose parasite densities were ≥50,000/μL (42.1%) [21].

The importance of an intact immune system to combat malaria was demonstrated in a study of HIV infected patients. Those who had a lower count of CD4 had a higher likelihood of fever [22]. Others have found that IgG subclasses affected malaria symptoms, with children having elevated IgG1 antibodies showing better protection against severe malaria [23].

The role of the immune system in potentially keeping malaria under check for decades is best exemplified by the case of the 74-year-old Greek woman who had recrudescent P. malaria infection after decades of being symptomless [24]. Suffering from splenomegaly, she received a course of methotrexate (for suspected lymphoma). This was quickly followed by a quartan fever pattern. Her P. malaria infection was confirmed by antibody and RT-PCR assays.

Toxoplasmosis

T. gondii is a coccidian parasite of felids with humans and other warm-blooded animals as intermediate hosts [25] Historically, *T. gondii* infection has been considered asymptomatic in the vast majority of immunocompetent patients with only 10-20% of patients presenting with oligo-symptomatic cervical lymphadenopathy [25]. However, in the large outbreak associated with municipal drinking water in Victoria, BC, just 18% of seropositive patients were symptom-free [26]. Furthermore, a systematic review of 38 selected outbreaks found a fairly constant figure of symptom-free patients (~20%) [27].

What are the presumed mechanisms which silence acute toxoplasmosis? One way to answer this query requires understanding of the serological diagnosis of toxoplasmosis. In most tests (either agglutination, or the Sabin-Feldman dye test, or ELISA) the serum is diluted before testing. In fact, dilution is necessary to minimize the effect of the so-called cross-reacting antibodies. Western-blot (WB) study of these cross-reacting antibodies demonstrated that each seronegative individual has, in fact, antibodies against certain Toxoplasma epitopes [28]. The WB bands were both of IgG and IgM types and seemed to appear in early childhood. It was therefore hypothesized that these naturally occurring antibodies could be part of the mechanisms responsible for silencing the clinical picture of acute toxoplasmosis.

In parallel to the humoral arm, the cellular immune system (T cells, macrophages and type 1 cytokines) play a crucial role in the protection against toxoplasmosis and probably in transforming overt- into asymptomatic toxoplasmosis. Studies in mice revealed an expansion of both NK cells and Υδ T cells during early infection [29]. This expansion has been proposed to provide innate resistance. T-cells protect the host by secreting gamma INF, IL-2 and TNF-α [29]. Dendritic cells and macrophages also play a pivotal role in control of acute infection by early production of IL-12. A clinical correlate of the above can be found in ocular toxoplasmosis. Patients without ocular toxoplasmosis, as opposed to those with eye involvement had higher levels of IL-12 and INF-Υ [30].

Asymptomatic bacterial infections

Q fever

Coxiella burnetii is an obligate intracellular organism that causes both acute and chronic Q fever (QF). QF is a zoonotic illness easily transmitted to man by aerosol, and thus is considered an agent of bioterrorism. As of this review, more than 550 articles alluding to outbreaks of QF have been reported in PubMed. Large outbreaks, which constitute a formidable opportunity to study asymptomatic infections have occurred in European countries: The Netherlands, Switzerland, UK, Germany, and France. In the largest outbreak in the Netherlands, 80-86% of those tested had clinical illness, with 15-20% defined as asymptomatic [2]. During the large outbreak in Germany (2003) 21% (6/29) of vulnerable pregnant or cardiac patients tested positive, but were asymptomatic [31]. A more recent study from the Netherlands have found that 23/122 patients with vascular QF (grafts, aneurysms) had asymptomatic infection [32]. These figures certainly raise the question, what are the determinants that drive QF to be an asymptomatic one?

Age is probably one of these determinants. Children seem to be less symptomatic than adults [33]. During the outbreak in Switzerland in 1983, children <15 years old accounted for 19% of the 415 seropositive cases, but only 5% of the 191 symptomatic cases. Overall, 70/80 children were asymptomatic, vs. 121/335 of adults [33].

Upon inhalation, *C. burnetii* targets alveolar macrophages wherein it replicates in a lysosome-like parasitiphorous vacuole. Macrophages are unable to kill the organism which thus multiplies. *C. burnetii* seems to secrete effector proteins that control macrophage function [34]. Surprisingly, avirulent strains trigger a robust, early proinflammatory response characterized by secretion of TNFα, IL-6 and mature IL-1β. TNF production is specifically enhanced in patients who develop QF endocarditis.

Genetic traits surely exert an effect on disease presentation as studied by cytokine polymorphism [35]. Interferon-ϒ+874T/A, and the IL-10 -592C/A polymorphisms significantly affected both disease severity, cytokine protein levels and the duration of illness in three infections: EBV, QF, and Ross River Virus. This stood in sharp contrast to those with INF-ϒ+874AA genotype and IL-10 -592CC genotype who suffered a milder disease.

Asymptomatic Viral diseases

Cytomegalovirus

Cytomegalovirus (CMV) has a worldwide distribution. The seroprevalence rates of healthy adults in the US reach probably 70%, and may reach 100% in Africa [36]. Among Israeli army recruits it was found to differ by parental origin but was 60-84% [37]. The clinical features of acute CMV in the immunocompetent host may include fever, night sweats, nausea, cough and fatigue. Yet, asymptomatic infections are probably more common and may bear devastating consequences. For example, a recent study of 238 pregnant women from France has found that 79% were asymptomatic [38]. This statement holds true both for adults and neonates who acquire the virus perinatally [1].

Cytomegalovirus infection in neonates may cause sensorineural hearing loss, cognitive defects, and motor defects. The overall infection rate of CMV in neonates in the USA is 0.6% [39] but among mothers who seroconvert during pregnancy it is much higher. In a study which included 123 participants and tested the efficacy of hyperimmune globulin the rate of asymptomatic infection was 41.4% [40] this underscores the importance of serological follow-up.

Asymptomatic CMV has rarely been studied at the time of acute infection. Yet, Zanghellini and colleagues have followed 45 adolescents for 7.5 months for the acquisition of CMV [41]. Six of these 45 (13.3%) seroconverted and all were asymptomatic. CMV DNA was detected by PCR in the plasma and WBCs in all positive patients. The virus was also isolated from urine in 59.2% over 80 weeks' follow up, from saliva in 3 subjects, and vaginal swabs in 2/5. These data prove that asymptomatic viral shedders constitute an important source of ongoing infection in the community.

Immunity against CMV is tri-factorial: humoral, innate and adaptive. Three lines of evidence support the role of humoral immunity. First, in animals, immunization against glycoprotein B induces neutralizing antibodies. Second, in pregnancy, the probability of transmission of infection to the fetus greatly increases if the antibody response is of low avidity and poor neutralizing activity [1]. Finally, in solid-organ transplant recipients, primary infection is often more frequent and severe in seronegative rather than seropositive. The share of cellular immunity in CMV infection is likewise important. Natural killer cells were shown to play a role in clearance of murine CMV infection. Ablation of NK cells renders mice susceptible to lethal CMV infection [42].

Rhinoviruses

The remarkable improvements achieved in diagnostic assays over the past decade have increased our capability of identifying viruses that cause respiratory infections. Rhinoviruses are the causative agents of most common colds and are partly responsible for exacerbations of chronic obstructive pulmonary disease. Infections may be symptomatic or asymptomatic in both immunocompetent and immunocompromised patients.

Children may experience an average of 8-12 colds per year. After inoculation onto the nasopharynx the virus may cause disease and be shed thereafter for up to 21 days. Transmission is most effective via the oral route during the first five days of infection.

Srinivasan et al. have detected human rhinovirus (HRV) in 5 of 33 asymptomatic children before undergoing allogeneic hematopoietic cell transplantation [43]. Four patients had continued to shed HRV for ≥ two weeks, and two of the four later developed upper respiratory infections. Symptomatic infections with HRV are more common among young children as opposed to older children and adults [44] Peltola and others have found that among shedders aged <7 years, 12/13 were symptomatic, while only 2/14 of those aged >16 years were symptomatic. They concluded that the mechanisms that determine the appearance of clinical symptoms are probably of both viral and host origin.

To further elucidate the mechanism, Cohen et al. [11] inoculated 152 healthy adults with rhinovirus 39. Sixty-nine percent of the volunteers developed infection, while only 22% developed clinical illness, which means that 47% suffered an asymptomatic infection. Additional investigation revealed that shorter telomeres (in either PBMC or T-cell subsets) were associated with greater odds of infection, independent of pre-challenge virus-specific antibody or demographics. Furthermore, CD8CD28 − was the only cell population in which shorter telomeres were associated with greater risk of clinical illness. The association between CD8CD28 − telomere length and infection increased with age. Telomere shortening in leukocytes, as occurs with aging, has implications for immunocompetence. Shortening in the cytolytic CD8 T-cells is especially important for cancer and viral infections. The rapid loss of telomere length in cytolytic CD8 T-cells causes cell senescence marked by loss of expression of CD28, a co-stimulatory molecule important for antiviral function.

Zika virus

Zika virus (ZIKV) infection that swept through South America is the most recent example of an emerging infection capable of causing devastating consequences [14]. The prevailing consensus is that acute Zika infection may cause microcephaly and Guillain-Barré syndrome [45, 46] Thus, it came as no surprise when the WHO declared a state of public health emergency of international concern. The most common symptoms of Zika infection include headache, mild fever, rash, conjunctivitis and arthralgia. However, a non-negligible

proportion of those affected produce no symptoms. In The YAP island outbreak the symptomatic: asymptomatic ratio was estimated at 1:4.4 [47], while a recent seroprevalence conducted in French Polynesia have found a ratio of 1:1 among adults and 2:1 among children [48].

Asymptomatic Zika infections may have grave outcomes: first-pregnant women may fail to seek obstetric attention in due time, second-the virus may be carried by asymptomatic patients across borders and continents, thus perpetuating epidemics, third-It may be transmitted sexually [49] by an apparent asymptomatic patient and four- be transmitted by blood donations [50].

Studies of the immune response, specifically in asymptomatic Zika patients are unavailable. However, a few studies in symptomatic patient and mice shed light on important elements of the immune system [51-53] Studies in mice showed that an inhibitory antibody, ZIKV-117 broadly neutralized infection of ZIKV strains corresponding to African and Asian-American lineages. Monoclonal antibody based on ZIKV-117 treatment markedly reduced tissue pathology, placental and fetal infection, and mortality. Another study found that the most potent neutralizing antibodies targeted E protein domain III or quaternary epitopes on infectious virus [52]. Studies in immunocompetent mice infected with ZIKV have demonstrated a relatively mild disease which elicited both CD4+ and CD8+ effector cytokines and cytolytic molecules [53] It is still unclear which of these mechanisms is responsible for driving ZIKV infection into an asymptomatic one.

Summary and Future Directions

Asymptomatic infections are common. Moreover, for some of the infections like ZIKV, asymptomatic presentations are probably more common than overt febrile presentations. These asymptomatic infections pose a serious threat to the public either by perpetuating epidemics, or by damaging fetuses.

The developments achieved over the recent few decades in molecular biology, metagenomics, host-microbe immunology and rapid diagnostics have unmasked some of the secrets of asymptomatic infections. Generally speaking, the interplay between the pathogen's virulence and host's immunology is what drives the clinical presentation. Vaccines constitute a formidable example how manipulation of the immune system turns an overt, and at times lethal infection into a subclinical one. However, a few cardinal queries remain: At the current time of individualized medicine, can we distinguish people with specific vulnerability to infections? Can we study AI with the aid of attenuated vaccines? Should some of the asymptomatic infections be treated at all? Can the knowledge gained from metagenomics be translated into vaccinology?

The study of asymptomatic infections, although problematic to investigate in real-time, is an exciting field that may teach us important lessons on how to combat infections more effectively. Future studies should provide additional data on the genetic and immunologic determinants that drive a robust, yet discreet response against an invading pathogen.

Competing Interests

The author declare that no competing interests exist.

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