Chapter 2
Vaccines, Sera and "New" Viruses: Ebola, Zika and Other Infectious Challenges for Human Health

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Abstract Following the basic description of the immunological science, we may now go in more detail into the practical applications involving immunological prevention against infectious diseases (vaccine use, requiring availability of suitable antigenic preparations, safe and effective immunization schedules and time to allow for immunity to develop) and immunotherapy of dangerous infective conditions (serotherapy, involving availability of immune sera and their use for injection in subjects needing protection against infectious agents).

All this is discussed with special regard of the many new micro-organisms described in human pathology and also in situations in which the deliberate use of them for biological warfare or related menace (bioterrorism) is realized.

Keywords Immunology • Virus • New infectious diseases • Bioterrorism

2.1 Vaccines and Sera

What is a vaccine? In a dangerous disease caused by micro-organisms and "foreign" not-self substances (an "antigen"), it may be possible to raise a vaccine, that is an innocuous preparation of that "antigen" which can induce the production of an immune response and create immune memory in the subjects we want to protect. A vaccine can be developed in many ways: killed (denatured) or non-replicating pathogens (viruses), recombinant protein antigens, live, attenuated (less harmful) strains of pathogens (for instance cowpox virus to protect against smallpox).

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Vaccines can be very effective: cases of smallpox (extinction of the disease: no more cases on earth, for the success of global vaccine campaigns) and of polio (near extinction of disease, except in some nomadic populations and in critical areas of today's world, like Syria and also, surprisingly, remote Chinese provinces) are very instructive on the subject.

What is a serum? If there is no time to confer protection by means of a vaccine (which, when available, requires at least 7–10 days before sufficient amounts of specific antibodies are synthesized by the vaccinated individual) we may protect the subject exposed to the dangerous contagion by means of the serum of individuals (humans or animals) already immunized against the micro-organism or lethal toxicant in action (examples: anti-tetanus serum, anti-snake venom serum, anti-diphtheria serum, etc.).

The theme of parasitism has been treated briefly in the previous chapter by the same authors: so now we will briefly review the old and new micro-organisms posing challenges for human health in relation to Bioterrorism.

2.2 Main Diseases of Interest in the Field

In light of recent concern and interest about the potential for biological terrorism (biowarfare) there are several diseases and bacterial toxins that must be considered in particular, like anthrax [1, 2], smallpox [3, 4], plague [5], botulinum toxin [6], and tularemia [7]. A very detailed discussion of such diseases and other infectious diseases with similar risks in terms of bioterrorism goes beyond the scopes of this concise chapter, but some features of these and other infectious diseases representing important threats in the biowarfare field will be mentioned.

In this respect, we may distinguish in time diseases which are:

- Old diseases which are disappearing and sometimes returning, like smallpox and polio virus infections (which are either extinct or close to be eradicated, thanks to planetary vaccination programs);
- Diseases still active at present times, like carbuncle (anthrax), plague, tularemia, tetanus, botulinum, TBC, etc.;
- New diseases, which are appearing/spreading, like SARS (Severe Acute Respiratory Syndrome) and its more recent variety of MERS (Middle-East Respiratory Syndrome), infections by Ebola/Marburg viruses, Hantavirus, filovirus, novel Flu virus strains, Zika virus, etc.

Now we will summarize the essential facts about some of these diseases.

For a more complete medical reference to all of them, see for instance the Merck Manual of diagnosis and Therapy [8] and recent reviews which appeared on the most recent epidemics and that are listed in the references (9–25).

2.3 Definitions and History of Bioterrorism

Practice of bioterrorism goes back to very remote times of human existence and indeed to pre-historical conflicts between humans, when groups of people devised to deliberately use biological agents for conflict sustainment (damaging weapons) and for propagation of fear in the enemy populations.

Since the early times of combat between humans, some biological means have been used: for instance through the contamination of water wells in conflict areas, tainted with rotting animal remains, or through hunting-fighting arrowheads dipped in toxic plant extracts or venomous substances, before throwing them at the living target.

Usable biological agents for harm are in fact all the pathogens of biological nature, like micro-organisms (bacteria, viruses, fungi, prions), toxins, animal and plant venoms, together with the related carriers (fomites, instruments) or vectors (insects, etc.).

Terrorism is the use of violence to condition societies or governments in their political choices.

Bioterrorism is the use (or menace of use) of biological agents to enact terrorist events and induce generalized *fear* concerning negative consequences in target populations.

Here follow some examples of bioterrorism enacted in various ages:

(a) Use of poison darts/arrows (primitive populations): mostly for hunting, but also for battles against enemies.

From here derived many of the advances of toxicology, the science of toxic substances (the word "toxicology" derives indeed from the Greek words "toxon" = arch, while "farmakon" = poison; toxicon farmakon = poison for arch hunting): from such practice derives the knowledge we have of stricnin, curare, ouabain, aconite, other plant/animal poisons primarily devised for hunting and fighting.

(b) Impingement of darts in decomposing cadavers or putrefaction soil (or manure > > tetanus) before throwing at enemies (New Guinea, tribal combats; Sciites 400 B.C.)
(c) Last but not least, the use of fear that human have toward beasts. Here comes the example of Hannibal (from Carthago), leading the ships of Prussia I, king of Bithynia (West Turkey) in a battle of year 184 B.C. against Eumene II (Attalides, Pergamon); he won that naval battle because he managed to throw canisters full of reptiles at the enemy ships, causing terror and uncoordinated reactions leading to his victory. He therefore used fear as a weapon: snakes were not even harmful (not poisonous), but big was the surprise and reactions were out of control!
(d) In recent history, we can see that First World War (also named the war of Chemistry) contributed to the development and use of Nervine gases, chemical weapons banned everywhere but still existing in some countries. The Second World War (also named the war of Physics) led to the development and use of the atomic bomb.

Peace Treaties: *Geneva Protocol* ruled against chemical weapons (1925) and was followed later by additions concerning bacteriologic war. Not all the states however subscribed it. Though, most world states had banned chemical and bacteriological weapons by 1975.

Today, how scared should we be of biological and chemical terrorism? Well, since these are lethal and cheap weapons, they are of considerable concern. They may be seen as the cheap atomic bomb and represent remarkable threats to peace in local conflicts and in terroristic attacks worldwide. We should all know more on the subject and do extensive prevention.

Albert Einstein once said "I do not know by what weapons the Third World War will be fought, but for sure the Fourth will be fought with stones". Well, we do not want any more World Wars, for sure.

2.4 Main Infectious Agents in Bioterrorism and New Infections

In the following paragraphs, the main infectious agents usable for biological warfare are concisely described, together with some of the most recent biological agents appearing as world epidemics (Ebola and Zika viruses).

2.4.1 Smallpox (Variola)

Smallpox is a highly contagious disease (incubation 10–12 days) caused by the smallpox virus, an orthopoxvirus. It causes death in up to 30% of infected subjects. Indigenous infection has been eradicated (last case, Ethiopia, 1990 – WHO) [3, 4]. The main concern for outbreaks of smallpox today is from bioterrorism.

Smallpox is characterized by severe constitutional symptoms (fever, headache, extreme malaise) and a characteristic pustular rash. Treatment is supportive; prevention involves vaccination which, because of its risks (eczema, encephalitis, etc.), is done selectively [3, 4].

Pathogenesis of smallpox demonstrates that the virus is transmitted from person to person by direct contact or inhalation of droplet nuclei. Clothing and bed linens can also transmit infection. Most contagions are in the first 7–10 days after the skin rash appears. Once the crusts appear, the infectivity declines. The virus invades the oropharyngeal and respiratory mucosa, multiplies in regional lymph nodes, causing viremia and localization in small blood vessels of the skin (rash) and rarely in CNS (encephalitis) [3, 4].

Officially, smallpox is eradicated on Earth. There have no longer cases detected in the world population since 1990, but the question that often arose was: can we destroy the samples of smallpox virus existing in some virology laboratories around

the world? [3]. The logical answer is certainly not, because we would lose the capability to prepare vaccine doses without the live virus samples to start from. Needless to say that without a vaccine, a small amount of wild virus could ignite a wide epidemic killing a large portion of the human population, since the vaccination is no longer mandatory in any country and a large percentage of young populations have no longer been vaccinated after the early 1990s.

2.4.2 Poliomyelitis (Infantile Paralysis)

Poliomyelitis is an acute infection caused by a poliovirus. Manifestations include a non-specific minor illness (abortive poliomyelitis), sometimes aseptic meningitis without paralysis (nonparalytic poliomyelitis) and, less often, flaccid weakness of various muscle groups (paralytic poliomyelitis) [8–10]. Diagnosis is clinical, although laboratory diagnosis is possible. Treatment is supportive. Vaccination is available, still mandatory in many countries, although soon legislations may change this. Childhood vaccination produces immunity in 95% of recipients. Declared cases worldwide have diminished remarkably, but recently some areas with particularly poor sanitary services or with conflicts preventing health services to operate are recording increased numbers of cases (Syria, 2013; China 2013).

Polioviruses have three serotypes. The virus enters the mouth via the fecal-oral route, and then enters the lymphoid tissues of the GI tract. If not contained, infection may enter the CNS with significant damage in the spinal cord and brain, specifically to nerves controlling motor and autonomic function (breathing). Spreading is through the enteric route [9–11]. The vaccine is live, attenuated virus, able to immunize many contacts in respect to the vaccinated subjects (community vaccination strategies; problems in nomad populations) [9–11].

2.4.3 Anthrax (Carbuncle)

Anthrax is caused by *Bacillus anthracis,* toxin producing, encapsulated, aerobic or facultative anaerobic organisms. Anthrax, an often fatal disease of animals, is transmitted to humans by contact with infected animals or their products (woolsorter's disease) [12].

In humans, infection typically occurs through the skin. Inhalation infection is less common; oropharyngeal, meningeal and GI infections are rare. For inhalation and GI infections, nonspecific local symptoms are typically followed in several days by severe systemic illness, shock, and often death. Empyric treatment is with ciprofloxacin or doxycycline. A vaccine is also available (antitoxin) [13].

Pathogenesis of anthrax takes place since *Bacillus anthracis* readily forms spores when germs encounter dry environment -a condition unfavorable for growth-. Spores resist destruction and can remain viable in soil, wool, and animal hair for

decades. Spores germinate and multiply in favorable conditions (wet skin, tissue, blood) and can give human disease by contact (papules, black eschars, contagious also via fomites) ingestion (raw meat > fever, nausea, vomiting, diarrhea), and inhalation (flu-like illness, respiratory distress, cyanosis, shock, coma) [12, 13].

Of note is the anthrax bioterrorist attack through mail (using spores in powder form) that took place in the USA in 2001 (US Postal Service, Washington DC). An event that highly sensitized the public to the global theme of bioterroristic attacks.

2.4.4 Plague (Pestis, Black Death)

The Plague is caused by *Yersinia pestis* (formerly named *Pasteurella pestis*). Short bacillus with hairpin shape, infects wild rodents and can infect humans via tick bites [5]. Symptoms are either severe pneumonia or massive lymphadenopathy with high fever, often progressing to septicaemia. Diagnosis is epidemiological and clinical, confirmed by culture and serologic testing. Treatment is with streptomycin or doxycycline. Unfortunately, a vaccine is not available for the Plague [5].

2.4.5 Tularemia

Tularemia is a febrile disease caused by *Francisella tularensis,* it may resemble typhoid fever as symptoms are a primary local ulcerative lesion, regional lymphadenopathy, profound systemic symptoms, and, occasionally, atypical pneumonia [14]. Diagnosis is primarily epidemiological and clinical and supported by serologic tests. Treatment is with streptomycin, gentamycin and other antibiotics.

2.4.6 Tetanus

Tetanus is an acute poisoning from a neurotoxin produced by *Clostridium tetani* [15]. Symptoms are intermittent tonic spasms of voluntary muscles [15]. Spasm of the masseters accounts for the name “lockjaw” (trismus). Incubation requires 2–10 days. Diagnosis is clinical. Treatment with immune globulin and intensive support. Only unbound toxin can be neutralized. A vaccine is available, with a good extent of preventive protection.

2.4.7 Botulism

Botulism is a neuromuscular poisoning due to *Clostridium botulinum* toxin. Botulism may occur without infection if toxin is ingested [6]. Symptoms are symmetric cranial nerve palsies accompanied by a symmetric descending weakness and flaccid paralysis without sensory deficits. Diagnosis is clinical and by laboratory identification of toxin. Treatment is with antitoxin and support therapies [6].

2.4.8 Tuberculosis (TBC)

TBC is a chronic, progressive infection by *Mycobacterium tuberculosis*, often with a long period of latency following initial infection. It occurs most commonly in the lungs, with productive cough, chest pain, and dyspnea [16]. Diagnosis is most often by sputum culture and smear. TBC can involve any tissue (organ disease). Treatment is with multiple antimicrobial drugs. Forms of multiresistant TB bacteria are becoming more and more frequent [25].

2.4.9 Severe Acute Respiratory Syndrome (SARS)

Coronavirus infections in humans most frequently cause common cold symptoms; however in 2002, a relatively new coronavirus caused an outbreak of Severe Acute Respiratory Syndrome (SARS), which was much more severe than other coronavirus infections [17]. SARS is an influenza-like disease leading to progressive respiratory insufficiency with significant mortality rate. First detected in China, the SARS epidemic spread to more than 30 countries. In mid-July 2003, there were >8000 cases with >800 deaths (10% mortality) [17].

Then the outbreak subsided and no new cases have been identified from 2004 to 2012. In 2012 a new similar epidemic (sustained by the virus nCoV, novel coronavirus) started in Middle East (Arabia), with an estimated mortality above 40% (WHO, 2013). Later the nCoV epidemic has been named MERS (Middle East Respiratory Syndrome) and is being studied as a new zoonosis transmitted to humans from Dromedary camels. Studies are currently in progress, with great attention by the international sanitary authorities [17].

WHO in 2013 alarmed many countries against the new SARS-like coronavirus responsible of MERS, that has infected at the moment of this writing (April 2017; www.who.int/en) more than 1900 persons (Arabia, Great Britain, France, Germany, Tunisia, Italy, Abu Dhabi, United Arab Emirates, etc.) with reduced infective capacity as compared to SARS, but still highly lethal and communicable via close contact (family members). The latest available numbers call for 1938 ascertained diagnoses in humans, with 691 deaths (mortality, 35.7%).

Updates can be found at the following web sites: www.who.int/en; www.cdc.gov and (recommendations for clinicians) emergency.cdc.gov, emphasizing the need to consider the novel (nCoV) coronavirus when treating patients with a severe respiratory illness who have recently traveled to the Arabian Peninsula (or been in close contact with such travelers).

2.4.10 Ebola/Marburg Diseases

Marburg and Ebola are filoviruses that cause hemorrhage, multiple organ failure and high mortality rates. Diagnosis is with enzyme-linked immunosorbent assay, PCR or electron microscopy. Treatment is supportive. Strict isolation and quarantine measures are necessary to contain outbreaks. Incubation 5–10 days [18]. Marburg virus has been identified in bats and in primates. Human to human transmission occurs via skin and mucous membrane contact (humans/primates).

Filoviruses can affect intestines (nausea, vomiting, diarrhea), respiratory tract (cough, pharingitis), liver (jaudice), CNS (delirium, stupor, coma), and cause hemorrhagic phenomena (petechiae, frank bleeding) with high mortality rates (up to 90% with Ebola virus). Survivors recover very slowly and may develop long lasting complications (hepatitis, uveitis, orchitis) with only supportive care available: no specific antivirals nor vaccines are available for filovirus infections. A recent outbreak in West Africa was extremely severe in 2013–2014 [19]

2.4.11 Hantavirus, Lassa Fever, etc

Bunyaviridae contain the genus Hantavirus (4 serogroups, 9 viruses) causing hemorrhagic fevers that start with flu-like symptoms and evolve with severe renal and pulmonary consequences. Hantaviruses are single-stranded, enveloped, negative sense RNA viruses which can kill humans. Lethal in 10–15% of cases [20].

Lassa fever is an often fatal arenavirus infection occurring mostly in Africa. It may involve multiple organs, except CNS. Treated with ribavirin. No vaccinations are available so far for hantavirus infections [21].

Outbreaks of such infections have been recorded in Nigeria, Liberia, central Africa, with some rare imported cases in the USA and the United Kingdom. The animal reservoir of such viruses is in wild African rats (*Mastomys natalensis*), frequently found in African homes [19, 20]. Direct human to human transmission is documented via urine, feces, saliva or blood. Mortality (up to 45%) can be reduced by prompt ribavirin treatment. Universal hygiene precautions, airborne isolation, and surveillance of contacts are essential.

2.4.12 *Zika Virus Disease*

The original isolation of Zika virus goes back to 1947 during studies of yellow fever in macaques of the Zika forest in Uganda [22], where this new virus was first identified (a member of the flavivirus family). Then, after about 70 years, the infection spread in Africa and also diffused to South America (mainly in Brazil) where it spread substantially and showed to be highly neuropathogenic in embryos (newborns with microcephaly) and in infants (linked with Guillain-Barré syndrome) [23].

2.4.13 *Influenza Virus, with New Strains Continuously Appearing*

Last but not least, we must mention now influenza! Flu viruses are in nature among the most rapidly changing (mutating) organisms through their ability to infect a variety of hosts: birds (migrating waterfowl -ducks-, stantial poultry -chickens-), mammals (pigs, felines) and humans. In South East Asia (mostly in China, but also in Vietnam, Laos, Thailand, etc.) it is very common to have mixed farms of pigs, poultry, and ducks attended by humans [24, 25].

Every year, new strains appear in SE-Asia, favored by the reciprocal passage between migrating birds (mostly fowl), pigs and chickens, with exposure to many humans in farms, markets, rooster fighting sports, and food preparation places. A common saying in China tells that "Anything with four legs (except chairs) and anything that flies (except airplanes), can be eaten". With this philosophy, there is a lot to be desired in food safety and in general hygienic prevention in such geographical areas.

After the avian flu H5N1 of 2005–2006, highly lethal but unable to give human to human contagion, new combinations of flu strains are expected and feared, with high lethality and high human to human transmissibility.

On this widely interesting theme, of the world diffusion of new virus strains with pandemic potential, readers are encouraged to peruse the book entitled "Pandemics - virology, pathology and prevention of influenza" (Bollati Boringhieri publisher, Turin, Italy, 2010) [25].

2.5 Conclusions

In summary, we can see that a continuous surveillance is being devoted world-wide to the appearance of new strains of influenza viruses, in order to isolate as soon as possible potentially pandemic new strains and to prepare biological stocks suitable for massive vaccine preparations in due time to prevent the global spreading of potentially lethal new variants of the influenza viruses. Examples in time recall the

cases of the highly lethal pandemics known as “Spanish flu” in 1917–18 (in excess of 40 million deaths worldwide), “Asian flu” in 1956 (in excess of one hundred thousand deaths worldwide) and “Hong Kong flu” in 1978 (in excess of seven hundred thousand deaths worldwide). The basic question is: when will the new pandemic strike? Sometimes soon, as international experts say. The so called “Avian flu” came close to that, but sometime in the future new mutations may emerge with the potential of being much worse.

To conclude this wide, although rapid, overview of the most frequent or alarming causes of micro-organism related human diseases with potential interest for bioterrorism, we hope to have provided sufficient matter for discussion and for further future diffusion of medical and microbiological culture that may be useful for prevention and the betterment of human social relationships and the promotion of peace.

References

1. Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, Hauer J, McDade J, Osterholm MT, O'Toole T, Parker G, Perl TM, Russell PK, Tonat K (1999) Anthrax as a biological weapon: medical and public health management. Working group on civilian biodefense. JAMA 281(18):1735–1745
2. Inglesby TV, O’Toole T, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Friedlander AM, Gerberding Hauer JJ, Hughes J, McDade J, Osterholm MT, Parker G, Perl TM, Russell PK, Tonat K (2002) Anthrax as a biological weapon. Updated recommendations for management. JAMA 287(7):2236–2252
3. Henderson DA, Inglesby TV, Bartlett JG, Ascher MS, Eitzen E, Jahrling PB, Hauer J, Layton M, McDade J, Osterholm MT, O'Toole T, Parker G, Perl TM, Russell PK, Tonat K (1999) Smallpox as a biological weapon: medical and public health management. Working group on civilian biodefense. JAMA 281(2):2127–2137
4. Lovinger S (2002) Addressing the unthinkable: preparing to face smallpox. JAMA 288(20):2530
5. Inglesby TV, Dennis DT, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Fine AD, Friedlander AM, Hauer J, Koerner JF, Layton M, McDade J, Osterholm MT, O'Toole T, Parker G, Perl TM, Russell PK, Schoch-Span M, Tonat K (2000) Plague as a biological weapon: medical and public health management. Working group on civilian biodefense. JAMA 283(17):2281–2290
6. Arnon SS, Schechter R, Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Fine AD, Hauer J, Layton M, Lillibridge S, Osterholm MT, O'Toole T, Parker G, Perl TM, Russell PK, Swerdlow DL, Tonat K (2001) Botulinum toxin as a biological weapon: medical and public health management. JAMA 285(8):1059–1070
7. Dennis DT, Inglesby TV, Henderson DA, Bartlett JG, Ascher MS, Eitzen E, Fine AD, Friedlander AM, Hauer J, Layton M, Lillibridge SR, McDade JE, Osterholm MT, O'Toole T, Parker G, Perl MT, Russell PK, Tonat K (2001) Tularemia as a biological weapon: medical and public health management. JAMA 285(21):2763–2273
8. Porter RK (ed) (2011) The merck manual of diagnosis and therapy, 19th edn. Merck Sharp & Dohme Corp, Whitehouse Sation
9. Poliomyelitis (2012) Center for disease control and prevention
10. Wallace GS, Alexander JP, Wassilak SGF (2013) Traveler’s health: poliomyelitis. Center for Disease Control and Prevention

11. Herremans T, Reimerink JHJ, Koopmans MPG (2000) Antibody responses to antigenic sites 1 and 3 of serotype 3 poliovirus after vaccination with oral live attenuated or inactivated poliovirus vaccine and after natural exposure. Clin Vaccine Immunol 7(1):40–44
12. Hendricks KA, Wright ME, Shadomy SV, Bradley JS, Morrow MG, Pavia AT, Rubinstein E, Holty JE, Messonnier NE, Smith TL, Pesik N, Treadwell TA, Bower WA (2014) Workgroup on anthrax clinical, guidelines. Centers for disease control and prevention expert panel meetings on prevention and treatment of anthrax in adults. Emerg Infect Dis 20(2). doi:10.3201/eid2002.130687. PMC 3901462.PMID 24447897
13. Turnbull P (2008) Anthrax in humans and animals, 4th edn. World Health Organization, Geneva, pp 20–36
14. Ryan KJ, Ray CG (2004) Sherris medical microbiology, 4th edn. McGraw Hill, New York, pp 488–490
15. William A (2012 May) Tetanus epidemiology and prevention of vaccine-preventable diseases, 12 edn, Public Health Foundation, pp 291–300
16. Cudahy P, Sheela VS (2016) Diagnostics for pulmonary tuberculosis. Postgrad Med J 92(1086):187–193
17. Smith RD (2006) Responding to global infectious disease outbreaks, Lessons from SARS on the role of risk perception, communication and management. Soc Sci Med 63(12):3113–3123
18. World Health Organization (2014) Ebola and Marburg virus disease epidemics: preparedness, alert, control, and evaluation, pp 123, WHO/HSE/PED/CED/2014.05
19. WHO Ebola Response Team (2014) Ebola virus disease in West Africa — the first 9 months of the epidemic and forward projections. N Engl J Med 371:1481–1495
20. Johnson KM (2001) Hantaviruses: history and overview. Curr Top Microbiol Immunol 256(256):1–14
21. Yun NE, Walker DH (2012) Pathogenesis of lassa fever. Virus 4(12):2031–2048
22. Sikka V, Chattu VK, Popli RK et al (2016) The emergence of zika virus as a global health security threat: a review and a consensus statement of the INDUSEM Joint working Group (JWG). J Global Infect Dis 8(1):3–15
23. Lyle R, Petersen LR, Jamieson D, Powers A, Honein M (2016) Zika virus. N Engl J Med 374:1552–1563
24. Mitka M (2013) Deadly mers coronavirus not yet a global concern. JAMA 310(6):569
25. Bologna M, Lepidi A (2010) Pandemie, Virologia, patologia e prevenzione dell'influenza. Bollati Boringhieri Publisher, Turin, Italy, ISBN 978–88–339-2037-5