MMSL
Military Medical
Science Letters

Mil. Med. Sci. Lett. (Voj. Zdrav. Listy) 2015, vol. 84(2), p. 67-75
ISSN 0372-7025

REVIEW ARTICLE

BUBONIC PLAGUE: HISTORICAL ASPECTS AND THERAPY

Wilian A. Cortopassi [1,2], Teodorico C. Ramalho [3,4], Carlos A. M. Fraga [5], Iris Mangas [1], Kamil Kuca [4,6] and Tanos C. C. França [1,4] ✉

[1] Laboratory of Molecular Modeling Applied to the Chemical and Biological Defense (LMCBD), Military Institute of Engineering, Rio de Janeiro/RJ, 22290-270, Brazil.
[2] Department of Chemistry, Chemistry Research Laboratory, University of Oxford, Mansfield Road, Oxford, OX1 3TA (UK).
[3] Chemistry department, Federal University of Lavras, CP 3037, Lavras, MG, 37200-000, Brazil.
[4] Center for Basic and Applied Research, Faculty of Informatics and Management, University of Hradec Kralove, Hradec Kralove, Czech Republic.
[5] Laboratório de Avaliação de Substâncias Bioativas (LASSBio), Faculdade de Farmácia, Universidade Federal do Rio de Janeiro, Caixa-Postal 68023, Ilha do Fundão, 21941-902, Rio de Janeiro (RJ), Brazil.
[6] Biomedical Research Center, University Hospital Hradec Kralove, Czech Republic.

Received 18th February 2015.
Revised 15th May 2015.
Published 5th June 2015.

Summary

The bubonic plague or black plague is a zoonosis, caused by the bacterium *Yersinia pestis*, which quickly infects a great number of people, being able to decimate entire populations. This characteristic has turned plague into a dangerous biological warfare agent since the 16th century. Nowadays, the cure for plague is available; however the possibility of genetic engineering of *Y. pestis* strains could lead to the resurgence of this disease as a worldwide health problem of extreme gravity. In this work we have made a short resume and discussion on plague to provide readers with some information on its historical and clinical aspects, the currently used therapy and the potential of plague being used as a biological warfare agent nowadays.

Key words: Plague; Historical of plague; Plague therapy; Biological warfare.

INTRODUCTION

The Plague is a zoonotic disease that can be transmitted either via the bite of an infected flea or orally. This disease affects humans and other animals such as cattle, dogs and rats and is caused by the infection of *Y. pestis*, a gram negative bacillus belonging to the family of *Enterobacteriaceae*. Since its emergence around 1500 - 20000 years ago [1] *Y. pestis* has been responsible for three major pandemics of devastating magnitude [2]. The first pandemic occurred during the Binzantine empire under Justinian I (between 541 and 542 A.D.) causing the death of 100 million Europeans and almost 40% of the population of Constantinople. The second pandemic, called the black death, claimed the lives of about 25 million people between the years 1346 and 1352 and perhaps another 20 million at the end of the 14th century. This pandemic continued until 1720 when around 30 - 60% of the urban population in Europe succumbed to the plague. The third pandemic emerged in China in 1894 and spread out throughout the world, through the modern ways of transportation, until 1925, when

✉ Military Institute of Engineering, Laboratory of Molecular Modeling Applied to the Chemical and Biological Defense (LMCBD), Rio de Janeiro/RJ, 22290-270, Brazil
tanos@ime.eb.br

a more efficient sanitary control methodology eliminated the large urban populations of rats, thereby reducing the transmission to human. However, nowadays *Y. pestis* still remains endemic in some countries of the world and causes local epidemics [3].

Moreover, *Y. pestis* has been and continues being used as a biological warfare agent since the middle ages, when it was a common practice to throw pieces of infected corpses inside castles under siege and into fountains. More recently, the Japanese army was accused of launching bombs made of clay over the cities of Manchuria, in China, containing plague infected fleas, causing a large number of deaths among Chinese citizens during World War II. Allied troops were also accused of having bombed North Korea with insects capable of transmitting plague and other infectious agents during the Korean War. Also, the Soviet Union was suspected of having developed a mutant of *Y. pestis* resistant to antibiotics [4].

Y. pestis exists in urban and sylvatic reservoirs and can be transmitted by fleas of the species *Xenopsyllacheopis, X. brasiliensis, X. astia, Nosopsyllus fasciatus* and *Leptosyllasegnis*. *Ctenocephalides canis* and *C. felis* can also transmit plague from domestic animals to humans; *Polygenisbolhsi jordani* and *P. tripus* are fleas of wild rodents and, also, have had a great importance in the epizootization of plague [5]. Furthermore, lice and ticks can also be infected by *Y .pestis*, but there is no evidence so far pointing these insects as plague vectors to human [6 - 8]. Human can also be infected by direct contact with infected animals or, rarely, by secretions of other infected people. In addition, some mammals, such as squirrels, woodchuck, mice and rabbits can, eventually, be intermediate hosts and are, therefore, considered to be responsible for increasing the survival time of *Y. pestis* [6 - 8].

The most common infection of *Y. pestis* is the bubonic form, whose name derives from bubo, the name of the swelling lymph nodes that appear after the primary infection by the bacterium.They are similar in appearance to huge blisters and usually appear under the armpit, in the groin or on the neck and are also found in other infections such as gonorrhea, tuberculosis, chancroid or syphilis. Usually, the inguinal lymph nodes are the most commonly affected and, more rarely, cervical and auxiliary lymphs. However, there may also be no apparent forms of the disease. The bubonic form may have a mortality rate of around 50% if not properly treated. In 20% of the cases *Y. pestis* can spread out from lymph nodes into the blood, causing septicemia, and still reach the meninges and lungs in 10% of the cases. The pulmonary form is of particular importance not only because of its lethality, but mainly because the infection in this form can be efficiently transmitted from one person to another through droplets, initiating devastating epidemics, particularly in over populated regions. This form can be used in bioterrorism, to disseminate *Y. pestis* in aerosol sprays by aircraft or other ways of fast spreading.

HISTORICAL ASPECTS

Considering that plague is directly related to the lack of hygiene and rat infestations, individual cases may have occurred since pre-historic times, after the first contacts between men and the vector fleas. However, the first records of an epidemic of bubonic plague were found in the Bible. The old testament mentions a disease that wiped out much of the population of the Philistines in 1320 B.C. Although at that time the disease has not been defined yet as bubonic plague, there are some references that related the disease to rats, and the description of the symptoms with the presence of bubbles suggests that it was bubonic plague [9].

In the 6th century A.D., the called Justinian plague ravaged the Byzantine empire, or Roman empire of the east, whose capital was Constantinople. Many historians consider this episode as the first outbreak of bubonic plague. There is no doubt that it was plague due to the symptoms described by Procopius, an archivist of the Empire, who believed and documented that the disease was transmitted by the Egyptians to the Romans. This epidemic had a devastating effect, having wiped out about a third of the population of the Roman empire until the end of the 6th century and is considered one of the major factors that contributed to the fall of the Byzantine empire [10].

The second major outbreak of bubonic plague arose in Sicily in 1347 and became known as the black death. In the following years, the pandemic spread across Europe, coming to Norway, England and Sweden, and only disappeared completely three centuries later, in 1670. This epidemic was responsible for the higher mortality rate that has ever occurred in Europe. As an example, the population of England was so damaged that it took more than 150 years to full recovery. In the 14th century, the epidemic was less aggressive, but during the 15th, 16th and 17th century,

the disease has become more intense, peaking in 1630 in France and 1665 in England. After the outbreak, Jews and lepers were persecuted because they were considered the cause of the disease [10].

In 1855, new cases of plague were reported in the Chinese province of Yunnan. The disease has spread to Guizhou, Guangxi, Guangdong and Cantor. Due to the proximity to Cantor, Hong Kong was also affected and declared a site of proliferation of the disease in 1894. The epidemic of Hong Kong is considered the third and last great epidemic of bubonic plague. In 20 years, the disease has spread across southern China and from there around the world, causing over 10 million deaths. Only in 1959 the epidemic was controlled, when the World Health Organization (WHO) stated that the remaining infections were isolated cases [11]. Some cases of this epidemic were registered in Brazil five years after its beginning [12].

Currently, plague still disturbs world population, especially in areas with poor hygiene and over population, like the slums of the third world countries. However these numbers are small and do not characterize an epidemic. Also, most victims survive with a proper use of antibiotics therapy [13]. More recently, in 1994, an epidemic of the pneumonic form of plague has reached hundreds of people in India. This fact led many neighboring countries to close its borders with India in order to contain the spreading of the illness [13]. Another case occurred in 1997 when a man developed the pneumonic form of plague during a trip to Madagascar. The healer who performed the treatment of this man became also infected with the bacterium and transmitted it to his relatives, infecting a total of 16 people [14].

The plague in Brazil

The Bubonic Plague was first introduced in Brazil during the third great pandemic in 1900 through the port of Santos in the State of São Paulo and spread along the coast, reaching Fortaleza, capital of the state of Ceará. To contain the disease the Federal Government implemented several programs of vectors and hosts control. However, the disease spread through railways and roads to the States of Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Bahia, Minas Gerais and Rio de Janeiro.

A major control of the illness was achieved in 1941 with the creation of the National Plague Service (SNP). Afterwards, in 1970, a network of specialized laboratories investigating and fighting outbreaks of the disease was established through the creation of the Superintendence of Public Health Campaigns (SUCAM). In 1990, the National Health Foundation (FUNASA) replaced SUCAM and redistributed the pest control unit in regional health cells. Until 2003, the Secretariat of Health Surveillance, part of the Ministry of Health (SVS/MS), became responsible for the control of the disease [15].

Despite the(WHO) considers the bubonic plague as a reemerging disease in Brazil, few cases have been reported in recent years. Along the decade of 1980, 669 cases were registered with only seven deaths. In 2000, there was a case of bubonic plague in Bahia and five years later, another case of infection was reported in Ceará [16].

THE PLAGUE AS A BIOLOGICAL WEAPON

The plague has had great impact also on the military campaigns throughout history since old times. Its debilitating effects caused many times more damages than the enemy itself and were decisive in battles. However their use as a biological weapon frequently caused also self-contamination, since many individuals who tried to use infected bodies against the enemy, ended up contracting the illness.

The “Plague of Athens” was the episode that defined the defeat of the Athenians by Spartans in the war of the Peloponnesus, between 431 and 404 B.C. Athens was powerful and had the naval domain of the Greek seas. However an epidemic mowed down a quarter of the Athenian population being decisive for the Spartan victory. Emperor Pericles, of Athens, was one of the victims of the illness. Some historians believe that the plague of Athens could have been the bubonic plague, even though recent studies point to typhoid fever [17, 18].

The plague of Justinian was another example of the historical importance that the bubonic plague had on the military. It was one of the main causes of the decline of the Byzantine empire, mowing down a third of its population. The few that survived were weak and vulnerable to other illnesses and, consequently, incapable of defending the empire against their many enemies.

Although the plague was almost always present in the military campaigns throughout the centuries,

there were two moments in history when its use as an agent of biological war was remarkable. The conflict between the Tartars and Genovese in 1346 and during World War II. The Genovese worked on the trade of horses, furs and Russian slaves. They occupied the fortress of Caffa on the waterfront of the Black sea, in the eastern coast of Crimea (today Feodosia in Ukraine), where they watched the land routes for Turkestan and China. From the fortress, they viewed the tartar population of the near regions being devastated by the plague. The tartar governor Kipchak Khan Jani blamed the Genovese for the illness of his people, since he believed that the inhabitants of Caffa were spies under the service of Christianity. For this reason he started a conflict using a street fight between Genovese and Tartars as an excuse. Janibeg besieged Caffa and ordered to catapult the bodies of his own soldiers, killed by the plague, inside the fortress. It is believed that this contributed for the dissemination of plague among the traders. Trying to escape from that situation, the inhabitants of Caffa moved to the capital of the empire, Constantinople, taking the plague with them and spreading it out to the rest of the Roman empire, starting the first great epidemic of plague in Europe [19].

However, the hypothesis of transmission through infected bodies is questionable, since *Y. pestis* depends on the flea as a vector to be transmitted and fleas, since they like hot blood, don't feed from the corpse's blood. It is most likely that the plague has spread among the Genovese due to the big rats population existing around the black sea at the times of the siege of Caffa. These rats could have worked as reservoirs of *Y. pestis* [19].

During World War II the Japanese army launched ceramic pots containing fleas infected with *Y. pestis* over Manchuria and other regions of China. The bombs also contained grains to attract rats that were bitten by the fleas and got infected with the bacterium, transmitting it, later, to the population. There are records that around 700 people were killed in this biological attack. This number of deaths was not greater only because most of the fleas died with the impact of the pots against the floor [19].

Research on bubonic plague as a biological agent had been part of the biological warfare programs of the United States and Russia until the 1970s. These studies led to the development of new methods for the transmission of the illness, independent of a vector, and forms to transform *Y. pestis* in a war weapon (weaponization) [19].

According to the WHO, if 50 kg of *Y.pestis* were launched by aerosol over a city with 5 million inhabitants, 150,000 people will be infected and 36,000 will be fatal victims. The affected area would be of 10 km^2 and the lifetime of this agent would be 1 hour.

Americans stopped their research program on *Y. pestis* as a biological agent in 1970. But Russians kept developing research in this area in the following years and were able to dominate the production techniques of *Y. pestis* on a large scale [19, 20].

There are a few stories of anonymous groups who tried to use *Y. pestis* with terrorist purpose. However, in 1995, a microbiologist was imprisoned in Ohio, USA, under a suspicious activity, after illegally acquiring a sample of *Y. pestis* by mail [20].

CLINICAL ASPECTS

The human infection by *Y. pestis* is highly invasive due to its high proteolytic activity which promotes a massive destruction of the host's tissue. This tissue degradation provoked by the proteinases of *Y. pestis* normally facilitates the penetration and bacterial dissipation through the tissue barriers present in the extracellular matrix until reaching the blood circulation [21, 22].

The main symptoms of the bubonic plague are: local lymphadenitis (tumefaction), high fever, shiver, headache and, in some cases, gastrointestinal problems like nauseas, vomits and diarrhea. If the disease is diagnosed at its beginning there will be treatment for, at least, half of the cases [23]. Otherwise, it can cause death between 2 and 4 days after the first symptoms [24].

For the clinical diagnosis, besides the analysis of the symptoms, an analysis of the patient's history is made. For diagnostics purposes, both techniques can be used: the isolation and identification of the bacteria or serology focused on detection of antiplague antibodies [25].

The infection cycle of plague is initiated when the flea feeds from the blood of a rodent infected with *Y. pestis*. The organisms multiply inside the flea and, two days after the ingestion of the infected blood, accumulate in brown spots that appear in the stomach of the fleas. After that, a mass containing the bacteria is spread out in the flea's body in such a way that it

hinders any ingested food (in this case, blood) of reaching the stomach. Desperate with the necessity of feeding herself the flea tries, repeated times, to extract the blood from its victims, infecting them in the process. Once in the human host the bacteria spread in the lymphatic nodes responsible for the draining from the bitten place [26 - 28]. The inflammation of these nodes characterizes the main symptom of plague: the tumefaction, which can reach the size of a chicken egg [29, 30]. This tumefaction is an immunological response of the organism in the attempt to stop the proliferation of the microorganisms. The place where the bacteria settle and grow is still uncertain, however, it is believed that *Y.pestis* spreads by the organism through the blood current and is located mainly in the liver and spleen, also being able to develop in the lungs [26 - 28].

More severe forms of plague can occur when the bacteria have a protein capsule that facilitates its entry into the cells and prevents the protective action of white blood cells. *Y. pestis* is able to release both endotoxin and exotoxins, causing a circulatory collapse. The bacteria reach the lungs in around 5-15% of the cases. In these cases, the infected person can become a vector of plague transmission since the encapsulated bacteria can be easily exhaled by an infected person and inhaled by a health person nearby [31].

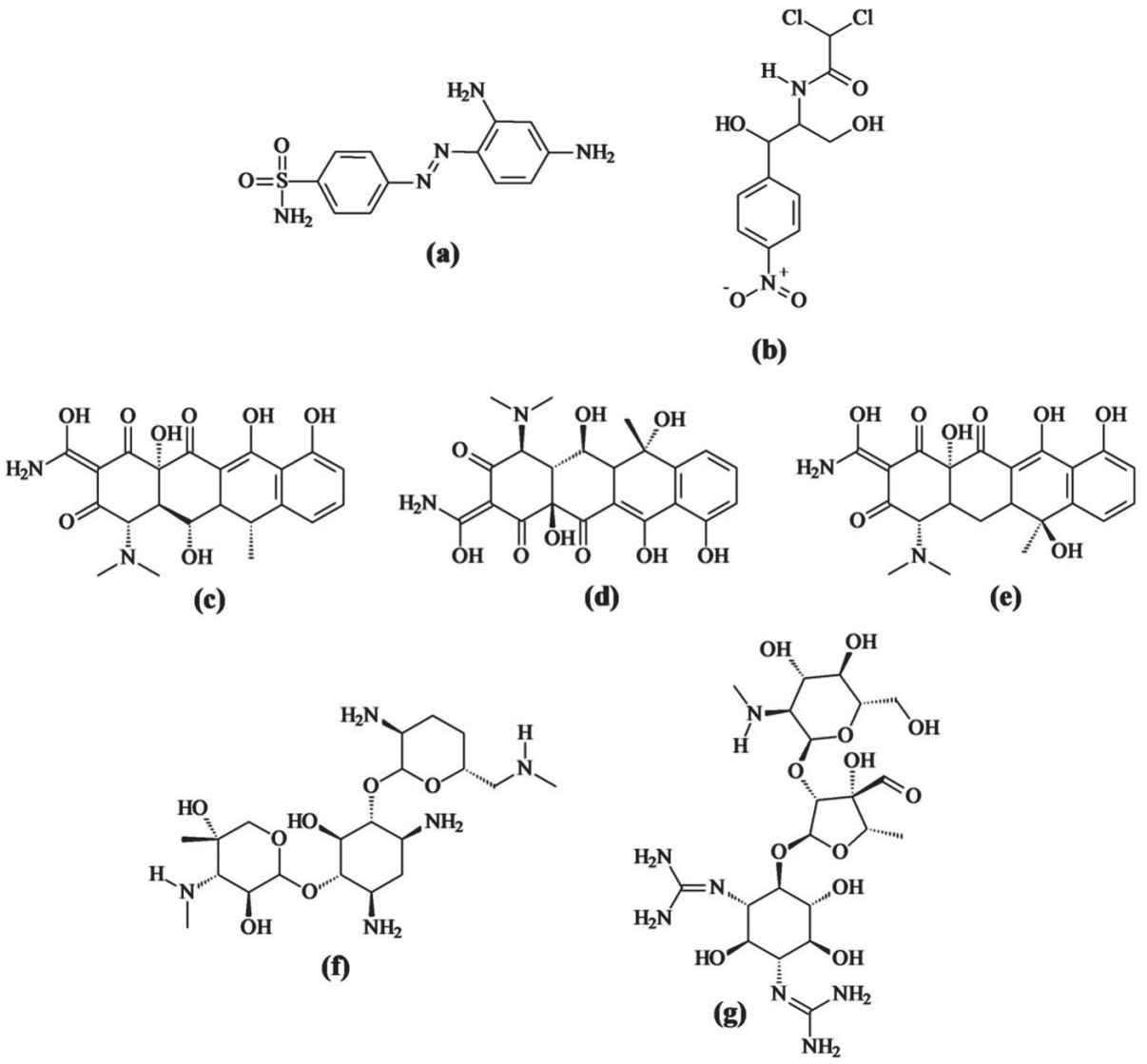

Figure 1. Chemical structures of antiplague drugs. **(a)** Prontosil; **(b)** Chloramphenicol; **(c)** Doxycycline; **(d)** Oxytetracycline; **(e)** Tetracycline; **(f)** Gentamicin and **(g)** Streptomycin.

CHEMOTHERAPY OF PLAGUE

Y. pestis was first discovered by a French-born Swiss bacteriologist named Alexander Émile John Yersin in 1894. In 1895, Yersin and co-workers published one of the first studies on a strategy of therapeutic intervention to cure the bubonic plague using serum from vaccinated rabbits [32]. Later, in 1896, Yersin was able to cure various infected patients using horse serum. The name *Y. pestis* was assigned in honor of Yersin because he was the first to identify this gram-negative bacterium as the causative agent of plague [19].

Figure 1 shows the chemotherapy in today use against *Y. pestis*. The first drug appeared in 1938 when the sulphonamide prontosil was successfully used by John A. Carman. This drug reduced the mortality of plague from about 100% to 50% [33, 34]. In 1943, J. W. Hornibrook successfully used streptomycin for the treatment of plague in rats and, in 1946, this drug was first used in humans [35]. Streptomycin is still the current drug of choice for plague. Other drugs also in use today are gentamicin, oxytetracycline, tetracycline, doxycycline and chloramphenicol (Figure 1).

Most of the antiplague drugs have its mechanisms of action associated to the inhibition of protein synthesis. This strategy is based on the fact that the inhibition of the bacterial protein synthesis does not affect the human protein synthesis due to the ribosomal differences between prokaryotic and eukaryotic cells. In bacterial ribosomes there are two subunits called 30S and 50S which contain RNA and several proteins [36]. Chloramphenicol (Figure 1), for example, is able of binding to one of the proteins in the 50S subunit which is responsible for associating the tRNA to the enzyme peptidyl transferase [36]. The main mechanism of resistance to chloramphenicol developed by *Y. pestis* relates to plasmids or modified permeability to drugs but the most common involves the production of an enzyme, acetyltransferase or nitroreductase, which inactivates the drug. The most serious adverse effect associated with chloramphenicol treatment is bone marrow toxicity, which may occur in two distinct forms: bone marrow suppression, which is a direct toxic effect of the drug and is usually reversible, and aplastic anemia, which is idiosyncratic and generally fatal. Other side effects caused by this drug are nausea, vomiting, change in taste, diarrhea, anal irritation and hematologic toxicity. Another serious side effect from the use of chloramphenicol is the "gray baby syndrome", that happens due to the inability of the newborn to eliminate the drug. This drug should not be taken by pregnant women because chloramphenicol crosses the placenta and is often found in milk. Today, the use of chloramphenicol is prescribed only in severe or specific cases [36].

Tetracycline, doxycycline and oxytetracycline are natural or semisynthetic molecules with a hydro naphthacene nucleus composed of 4 rings and different substituents. Tetracycline enters the cell by diffusion, a process dependent on energy expenditure, and is generally used for prophylactic purposes. This drug enters the bacterial cytoplasm and binds to the 30S ribosomal subunit, reversibly blocking the access of aminoacyl RNA carriers and preventing the protein synthesis [36]. The main mechanism of resistance developed by the bacteria consists of reducing the accumulation of these drugs within the cell. This mechanism can be either chromosomal or, more often, mediated by plasmids or transposons. Some studies point to the uncontrolled veterinary use of these drugs as the main cause of spreading resistance. The most common side effects of these drugs are allergic reactions such as hives, rash, periorbital edema and anaphylactic reactions; changes in the color of the teeth in children, enamel hypoplasia and abnormal bone growth, especially if used during pregnancy; nausea, vomiting and diarrhea; headache, inability to concentrate and, in rare cases, intracranial hypertension [36].

Gentamicin and streptomycin are aminoglycosides obtained from natural soil actinomycetes or semisynthetic products. These drugs exhibit complex structures consisting of sugars and amino groups and are able to bind irreversibly to the 30S ribosomal subunit, inactivating its initiation complex (30S-mRNA-tRNA). Thus, gentamicin and streptomycin prevent the onset of protein synthesis, as well as stimulate a misreading of the RNA [2, 36, 37, 38]. These aminoglycoside antibiotics bind to the bacterial cell surface and are subsequently transported through the wall by a process dependent on oxidative energy. Although rare, there are three recognized mechanisms of bacterial resistance to aminoglycosides: (1) alteration of the binding sites on the ribosome; (2) change in the permeability and (3) an enzymatic modification of the drug. The more common side effects observed due to the use of aminoglycosides are nephrotoxicity, ototoxicity and neuromuscular paralysis [36].

VACCINES AND PLAGUE CONTROL

The only plague vaccines known today were developed in the EUA, between the decades of 1940 and 1960, for military purposes. Currently, these vaccines are prepared by the Greer Laboratories (Lenoir, NC) and are administered in intramuscular doses, only to people with high exposure to virulent strains of *Y. pestis* and military who will work in endemic areas. The vaccine effectiveness was proven during the Vietnam war, where there were only eight cases of plague among soldiers of American troops throughout the conflict and none of these cases occurred among vaccinated individuals [39, 40]. Although being very effective, the vaccines available today cause secondary effects that can be harmful to health and, for this reason, are not released for the population. The elimination of these unwanted side effects motivates the current search for new vaccines against plague [41].

FINAL REMARKS

In this work we reviewed the more relevant historical aspects of plague as well as the main therapeutic strategies in use today to combat this disease. The possible emergence of genetically modified (GM) strains of *Y. pestis* and the vaccines development are also highlighted.

The bubonic plague is a disease that played a key role in Europe historically. The almost constant presence of the disease during the middle and modern ages helped to bring down great empires and was often a determining factor in the economic development of many European countries. The reports of the first manifestations of this disease have contributed to the advancement of medicine once the study of cases of infection became necessary to contain the spread of the plague.

The causative agent of plague has a complex life cycle partitioned between rodents, fleas and human. Eventually, other hosts can be part of this cycle, making the control of the disease difficult. Although currently there are no signs of epidemic and the reported cases are isolated ones, it is important to understand the need of sanitary control of fleas and rodents susceptible to plague, in order to prevent the spread of the disease. Also the search for new vaccines is needed, since the only existing vaccine for plague has harmful side effects to human. Cases of resistance to current antibiotics used to treat plague are rare, however the development of new and more effective antibiotics is necessary; not only to take care of individual cases, but also to replace those drugs with high toxicity problems, such as chloramphenicol. Besides, the actual risk of using GM strains of *Y. pestis* for terrorism purposes today makes the development of new drugs, the search for new molecular targets and the development of new strategies to combat plague a global emergency.

We hope that this paper will help to stimulate those starting a career in science to embrace the challenge of the development of tools which help enhance the discovery of appropriate treatments for many diseases with a major global impact and potential biological warfare agents such as plague.

ACKNOWLEDGMENTS

The authors thank to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior / Ministério da Defesa (CAPES/MD) (Edital PRODEFESA 2008) for financial support and to the Military Institute of Engineering (IME) and the Federal University of Lavras for the infrastructure and physical space. This work was also supported by Excellence project FIM. This work was also supported by the Project of Ministry of Health (Czech Republic) - 15-31847A.

REFERENCES

1. Achtman, M.; Zurth K.; Morelli, G.; Torrea, G.; Guiyoule, A.; Carniel, E. *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. *Proceedings of the National Acadademy of Sciences USA* **1999**, 96, 14043-14048.
2. Perry, R. D.; Fetherston, J. D. *Yersinia pestis*-etiologic agent of plague. *Clinical Microbiology Reviews.* **1997**, 10, 35-66.
3. Lahteenmaki, K.; Kukkonen, M.; Korhonen, T. K. The Pla surface protease/adhesin of *Yersinia pestis* mediates bacterial invasion into human. *FEBS Letters*. **2001**, 504, 69-72.

4. Huang, X.; Luther, L. The pH 6 Antigen Is an Antiphagocytic Factor Produced by *Yersinia pestis* Independent of Yersinia Outer Proteins and Capsule Antigen. *Infection and Immunity.* **2004**, 72 (12), 7212-7219.
5. Brazilian Ministry of Health/National Foundation of Health. Guide of vigilance and control of plague. Available in: http://portalsaude.saude.gov.br/images/pdf/2014/julho/24/manual-vigilancia-controle-peste.pdf acessed in: December 2014.
6. Traub, R.; Starcke, H. Seasonal changes in the flea fauna of nests of the southern flying squirrel. P*roceedings, International Congress on Fleas.* **1980**, 245-256.
7. Girard, G. Immunity in Plague. *Annual Review of Microbiology.* **1955**, 9, 253-276.
8. Traub, R.; Starcke, H. The roles of fleas and ticks in the epidemiology of human diseases. *Proceedings, International Congress on Fleas.* **1980**, 241-244.
9. Shrewsbury, J. F. D. The Plague of the Philistines and other medical-historical essays. Victor Gollancy Ltd, London, 1964.
10. Scott, S.; Duncan, C. J. Biology of Plagues: Evidence from Historical Populations, Cambridge University Press, Cambridge, 2001.
11. Ligon, B. L. Plague: A review of its history and potential as a biological weapon. *Seminars in Pediatric Infectious Diseases.* **2006**, 17, 161-170.
12. Silva, L. J. Biological warfare, bioterrorism and public health. *Revista Cadernosde Saúde Pública.* **2003**, 37 (3), 273-274.
13. Clem, A.; Galwankar, S. Plague: a decade since the 1994 outbreaks in India. *Journal of the Association of Physicians of India.* **2005**, 53, 457-464.
14. Kool, J. L. Risk of person-to-person transmission of pneumonic plague. *Clinical Infectious Diseases.* **2005**, 40 (8),1166-1172.
15. Aragão, A. I.; Pontes, R. J. S.; Seoane, A. C. M.; Nascimento, O. J.; Tavares, C.; Almeida, A. M. P. Tendência secular da peste no. Estado do *Ceará. Revista Cadernos de Saúde Pública.* **2007**, 23 (3), 715-724.
16. Brazilian Ministry of Health. Síntese epidemiológica da peste no Brasil em 2005. Available in: http://bvsms.saude.gov.br/bvs/publicacoes/peste.pdf acessed in: December 2014.
17. Papagrigorakis, M.; Yapijakis, C.; Synodinos, P.; Baziotopoulou-Valavani, E. DNA examination of ancient dental pulp incriminates typhoid fever as a probable cause of the Plague of Athens. *Journal of Infectious Diseases.* **2006**, 10, 206-214.
18. Cunha, C.; Cunha, B. Impact of plague on human history. *Infectious Disease Clinics of North America.* **2006**, 20, 253-272.
19. Crooddy, E.; Perez-Armendariz, C.; Hart, J. Chemical and Biological Warfare. A Comprehensive Survey for the Concerned Citizen. Springer-Verlag, New York, 2002.
20. Inglesby, T. V.; Dennis, D. T.; Henderson, D. A.; Bartlett, J. G.; Ascher, M. S.; Eitzen, E.; et al. Plague as a biological weapon: medical and public health management. *JAMA.* **2000**, 283, 2281-2290.
21. Ruback, E.; Lobo, L. A.; França, T. C. C..; Pascutti, P. G. Structural Analysis of Pla Protein from the biological warfare agent *Yersinia pestis*: Docking and Molecular Dynamics of Interactions with the Mammalian Plasminogen System. *Journal of Biomolecular Structure & Dynamics.* **2013**, 31, 477-484.
22. Lähteenmäki, K.; Kuusela, P.; Korhonen, T. K. Bacterial plasminogen activators and receptors *FEMS Microbiology Reviews.* **2001**, 25, 531-532.
23. Buttler, T. Plague and other Yersinia infections. Current Topics in Infectious Diseases. Plenum Medical Book Company, Nova York, 1983.
24. Laguna-Torres, V. A.; Benavides, J. G. La peste en el perú. *Revista Peruana de Medicina Tropical.* **2004**, 9 (1), 89-98.
25. Dong, X. Q.; Lindler, L. E.; Chu, M. C. Complete DNA sequence and analysis of an emerging cryptic plasmid isolated from *Yersinia pestis*. *Plasmid*. **2000**, 43, 144-148.
26. Bacot, A. W. Further notes on the mechanism of the transmission of plague by fleas. *Journal of Hygiene.* **1915**, 4, 774-776.
27. Bacot, A. W.; Martin, C. J. Observations on the mechanism of the transmission of plague by fleas. *Journal of Hygiene.* **1914**, 13 (3), 423-439.
28. Bahmanyar, M.; Cavanaugh, D. C. Plague Manual. WHO Publications, 1976.
29. Buttler, T. Yersinia infections: Centennial of the discovery of the plague bacillus. *Clinical Infectious Diseases.* **1994**, 19, 655-663.
30. Buttler, T.; Dennis, D. T. Yersinia species, including plague. Principles and Practice of Infectious Diseases, 6th Edition, Mandell, G. L.; Bennett, J. E. and Dolin. R. Eds., Elsevier, Philadelphia, 2005.
31. Kiple, K. F. The Cambridge World History of Human Disease. Cambridge University Press, Cambridge, 1993.
32. Yersin, A.; Calmette, A.; Borrel, A. La pestebubonique. *Annales de L'Institute Pasteur.* **1895**, 9, 589-592.

33. Carman, J.A. Prontosil in the treatment of oriental plague. *East African Medical Journal.* **1938**, 14, 362-366.
34. Kupferschmidt, H. Die Epidemiologie der Pest. *Gesnerus-Suppl.* **1993**, 43, 1-222.
35. Hornibrook, J. W. Streptomycin in experimental plague. *Public Health Rep*. **1946**, 61, 535-538.
36. Calvo, J.; Martínez-Martínez, L. Mecanismos de acción de los antimicrobianos. *Enfermedades Infecciosas y Microbiología Clínica.* **2009**, 27, 44-52.
37. Anand, D.; Davis, B. D. Damage by streptomycin to the cell membrane of *Escherichia coli. Nature.* **1960**, 185, 22-23.
38. Davies, J.; Gilbert, W.; Gorini, L. Streptomycin, suppression, and the code. *Proceeding of the National Academy of Sciences USA.* **1964**, 51, 883-890.
39. Meyer, K. F. Effectiveness of live or killed plague vaccines in man. *Bulletin of the World Health Organization.* **1970**, 42, 653-666.
40. Meyer, K. F.; Cavanaugh, D. C.; Bartelloni, P. J.; Marshall, J. D. Plague immunization. *Journal of Infectious Diseases.* **1974**, 129 (Suppl.), S13-S18.
41. Marshall, J. D.; Bartelloni, P. J.; Cavanaugh, D. C.; Kadull, P. J.; Meyer, K. F. Plague immunization. II. Relation of adverse clinical reactions to multiple immunizations with killed vaccine. *Journal of Infectious Diseases*. **1974**, 129 (Suppl.), S19-S25.