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The transmission, virulence and etiology of an epidemic ailment "Plague" - A crucial review

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Abstract

Plague is often fatal without prompt and appropriate treatment. It affects mainly poor and remote populations. Late diagnosis is one of the major causes of human death and spread of the disease, since it limits the effectiveness of control measures. There are currently no approved vaccines for protection against this organism. The current U.S. Department of Defense candidate plague vaccine is a fusion between two *Yersinia pestis.* Also the protein recombinant models by which the proteins significantly augmented the IgG antibody response to the plague vaccine increased the probability of survival in murine models of plague. However, the attenuated plague vaccine still caused a high rate of severe local and systemic reactions in humans. Now, Rapid diagnostic tests (RDTs) for infectious diseases are of much value in facilitation of major improvements in disease management, especially in developing countries. The integration of the recombinant DNA into the nuclear genome of the plant cell is the most widely used strategy for production of plant-made Vaccine. Plant-made vaccine antigens can be delivered to a mucosal surface. Mucosal surfaces lining the digestive tract, respiratory tract and genitourinary tract are the most important portals of entry for mammalian pathogens. Thus, focus on plant-made vaccine against *Y.pestis* would lead us for approved vaccine development and plague management.

Keywords: Plague, *Yersinia pestis,* epidemiology, bio-terrorism, vaccine.

Introduction

Plague is an acute, severe, zoonotic infectious disease caused by the Gram-negative bacillus *Yersinia pestis*, which is a member of the Enterobacteriaceae family. As it lies as a flea-borne rodent disease that is occasionally transmitted to man, which is still prevalent in more than 20 countries**.** Eleven species belong to the genus *Yersinia*, including the three human pathogenic species, i.e. *Y. pestis*, *Y. pseudotuberculosis*, and *Y. enterocolitica*. The most notorious species of the three, *Y. pestis*, causes bubonic, septicemic and pneumonic plague *Y. pestis* is considered as a clone that is evolved from *Y.pseudotuberculosis* .It has been recorded, since biblical times and has generally affected humans in explosive epidemics, causing large reductions in the global population. It was not until the late 19th century that a vaccine against plague was first developed.

Some RDTs are based on techniques such as immunochromatography, with conjugated gold or latex particles used to detect specific antigens from bacterial, viral, or parasitic agents. In Humans, it develops varying degrees of illness, from abdominal pain to many symptoms. In most instances, the infection is self-limiting and can be effectively treated with antibiotic therapy. *Y. pestis* cannot live freely. Maintenance of plague in nature is dependent upon cyclic transmission between fleas and mammals. The comparative genomics and microevolution of *Y. pestis* have recently been discussed in detail. Still, it has been many years since the last compilation of knowledge about

Y. pestis infection was published. *Yersinia pestis*, the causative agent of plague, is an extremely virulent bacterium. However, there are no approved vaccines for protection against it.

A vaccine that would address ease of delivery, mucosal efficacy, safety, rapid scalability, and cost would serve a better choice to the lead novel production and delivery system for a plague vaccine. In addition, many classy models are being evolved such as Plants represent an economical and safer alternative to fermentation-based expression systems for the production of therapeutic proteins.

The recombinant plague vaccine candidates produced in plants are based on the most immunogenic antigens, antigen fusion protein expressed in tomato.etc., serve a better choice. There are currently no approved vaccines for protection against this organism. Many strategies is being evolved such as, nuclear transformation, chloroplast transformation and plant-virus-based expression vectors.

Extent from historical, epidemiological, political, economical and social viewpoints *Yersinia pestis* is the etiological agent of bubonic and pneumonic plague, diseases, which have caused over 200 milllion human deaths in the past. *Plague still occurs throughout the world today, though for reasons that are not fully understood pandemics of disease.* Antibiotic treatment of bubonic plague is usually effective, but pneumonic plague is difficult to treat and even with antibiotic therapy.

About the Causative agent

Plague-*"The syndrome that sustain decades"*

Yersinia pestis

The etiological agent of plague is *Yersinia pestis*, a "Gram-negative bacterium", which is a member of the enterobacteriacae family. *Y. pestis* is closely related to the other human pathogenic *Yersiniae*. However, unlike *Y. enterocolitica* and *Y. pseudotuberculosis*, *Y. pestis* does not infect the host by the enteric route.

The major difference is that *Y. pestis* is unable to survive outside of an animal host, whereas *Y. enterocolitica* and *Y. pseudotuberculosis* can survive in the environment. These findings suggest that *Y. pestis* might have evolved from the other human pathogenic *Yersiniae*. These suggestions are supported by a comparison of the genetic diversity of several housekeeping genes in Y. pestis and Y. pseudotuberculosis and suggest that *Y. pestis* evolved from *Y. pseudotuberculosis*. The life cycle of *Y. pestis* differs from the other human pathogenic *Yersiniae* because the bacterium is transmitted from one animal host to another either directly or via a flea as a vector. In areas of the world where plague is endemic, the bacterium appears to survive by causing chronic disease in animals.

Bubonic, septicaemic and pneumonic plague

The occasional transfer of the bacteria to other mammalian hosts can result in acute disease, which is recognised as plague. There are three recognized forms of plague in man.1. bubonic plague, 2. septicaemic plague,3. pneumonic plague.

Bubonic plague

Bubonic plague is the most common form of disease and arises following a bite from a flea, which has fed, previously on an infected animal. The bacteria are disseminated from the initial site of infection to the draining lymph nodes, which become swollen and tender forming a bubo. The bubo can reach the size of a fowl's egg and is the classical feature of bubonic plague.

A bacteraemia may develop with blood culture counts. Almost all of the plague, which now occurs in the world, is the bubonic form of the disease.

Septicaemic plague

Septicaemic plague occurs when there is a bacteremia without the development of buboes and is characterised by an elevated temperature, chills, headache, malaise and gastrointestinal disturbances. Because of the generalised nature of these symptoms, a diagnosis of plague is often delayed, and even

with medical intervention. About half of the patients die, probably because of the induction of the systemic inflammatory response syndrome. The most feared form of plague arises when there is colonisation of the alveolar spaces leading to pneumonia. Pneumonic plague results in the production of a highly infectious bloody sputum. Coughing results in the production of airborne droplets containing bacteria, which can be inhaled by susceptible individuals? The pneumonic form of the disease is feared because of the rapidity with which the disease develops (1-3 days), the high mortality rate in infected individuals and the rapid spread of disease from man to man . In the context of the illegitimate use of *Y.pestis* as a weapon, pneumonic plague is the likely outcome.

Pneumonic plague

The pneumonic form of plague that is epidemic in an industrial cities is the deadliest and most easily communicable form of the bacterial disease that was known as the Black Death in the Middle Ages. Pneumonic plague had not been heard of anywhere for decades. Experts says that they could not recall an outbreak of pneumonic plague in the world since early in this century, and they had no recollection of where the last outbreak occurred. Although confirmation is lacking in many cases, the disease appears have struck, where unofficial accounts suggest that at least 100 people have died from plague in recent days."*It is certainly an epidemic, and it is the most serious outbreak of the disease in many years,"* .Occasional cases of plague occur in the United States each year, but those are the less deadly bubonic form that leads to the swelling of lymph nodes in the body. Studies have shown that the swollen lymph nodes known as buboes occur most often in the upper leg and groin areas.

Prevalence of disease

Yersinia pestis is generally recognised to have caused three major pandemics of disease in the, 14th–17th and 19th centuries. Credible estimates indicate that together these resulted in 200 million deaths. It is likely that both bubonic and pneumonic forms of plague occurred during the past pandemics.During the second pandemic of plague (the Black Death) it is estimated that over 30% of the population of Europe died from plague. Although *Y. pestis* no longer causes disease on this scale there is still a public health problem from plague, especially in Africa, Asia and South America.

During the period 1967–1993, the average worldwide incidence of plague worldwide was 1666 cases. Although the incidence trend was downwards until 1981, there has been an apparent increase in the incidence of disease over the last decade, possibly because of more diagnosis that is efficient and reporting of cases. However, many cases of plague are not diagnosed and it is likely that the true worldwide incidence of disease is several times the WHO figures out.

The Surat outbreak of plague in 1994 reminded the world that plague was still a potential problem. Although the extent of the disease was probably overstated, there were at least 876 presumptive cases of plague and 54 fatalities. The potential for the rapid spread of the disease throughout the world by air transport systems was of particular concern during the Indian outbreak of plague. This concern was related especially to the pneumonic form of the disease, were being referred.

Mechanism of *Yersinia pestis*

The innate immune system (nonspecific immunity) is able to discriminate between self and a variety of pathogens by recognizing the highly conserved sets of molecular structures specific to microbes (pathogen-associated molecular patterns [PAMPs]) via a limited number of germ line-encoded pattern recognition receptors (PRRs). Different PRRs react to specific pathogen-associated molecular patterns, exhibit distinct expression patterns, and activate immune cells directly to induce the expression of a variety of genes involved in the innate and adaptive immunity.

PRRs activate the complement pathway of innate immunity and induce production of cytokines such as interleukins, Tumor necrosis

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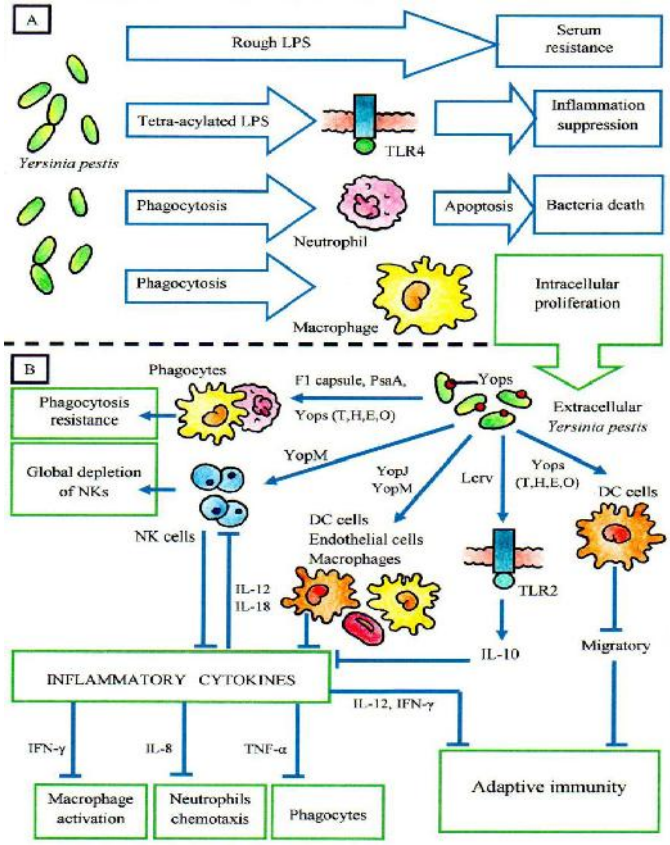
Fig.1. Y. pestis resistance mechanisms in opposition to host innate immunity (Amedei et al.,2011).

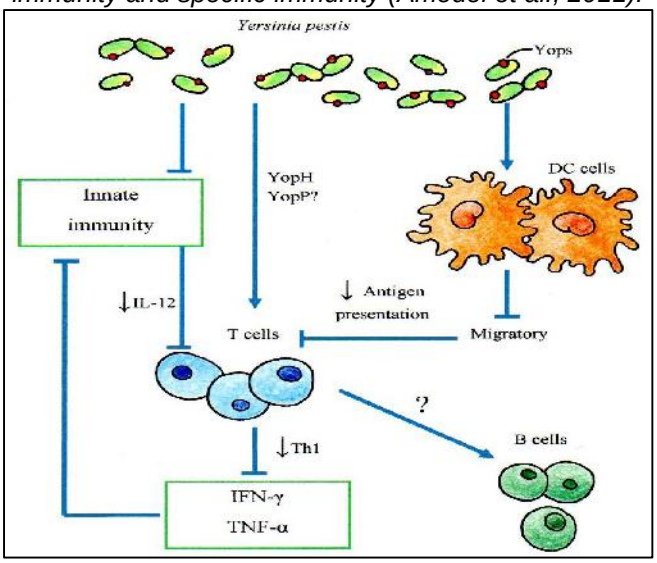


factor (TNF), and chemokines to collectively induce inflammatory responses to pathogens, recruit neutrophils to the infection site, and activate macrophages to kill the microbes.

By the bite of an infected rodent flea, *Y. pestis* may invade directly into the host through the barrier structure of the host skin and encounter phagocytes such as polymorphonuclear leukocytes (PMNs) (predominantly neutrophils) and macrophages at the site of invasion.

Most of them might be killed by neutrophils. However, the facultative intracellular *Y. pestis* preferentially infects host macrophages, possibly via recognition of specific surface-associated CCR5 molecules, and survives inside of macrophages at the early stage of infection. After proliferation and expression of various virulence determinants in macrophages, *Y. pestis* can be released into the extracellular compartment and spread systemically with acquisition of phagocytosis resistance. During this process, *Y. pestis* may

Fig.2. Defense apparatus of Y. pestis versus the specific immunity and the link between innate immunity and specific immunity (Amedei et al., 2011).



circumvent destruction by the components of the host innate immune system.Fig.1. *Y. pestis* resistance mechanisms in opposition to host innate immunity.

a) Resistance mechanisms at the early stage of infection. The LPS structure varieties of ***Y.pestis*** during transition between flea and host temperatures make the bacteria resistant to the serum mediated lysis and repress the proinflammatory response. In the meantime, the bacteria phagocytosed by macrophages can grow and express different virulence determinants to act on host immune responses.

b) Resistance mechanisms after the release of ***Y.pestis*** from macrophages. The bacteria released from macrophages attain the capacity to resist phagocytosis and can inhibit the production of proinflammatory cytokines, which also attenuate the host's adaptive immunity.

Y. pestis defense mechanisms against host innate immunity.

(a) Defense mechanisms at the early stage of infection. The LPS structure diversities of *Y. pestis* during transition between flea and host temperatures make the bacteria resistant to the serum-mediated lysis and suppress the proinflammatory response.

Meanwhile, the bacteria phagocytosed by macrophages can proliferate and express various virulence determinants to act on host immune responses.

(b) Defense mechanisms after the release of *Y. pestis* from macrophages.

The bacteria released from macrophages acquire the ability to resist phagocytosis and can inhibit the production of proinflammatory cytokines, which also attenuate the host's adaptive immunity.

The adaptive immunity versus ***Y.pestis*** was influenced not only by inhibition of cytokines induction provided by the innate immunity but also by the direct yops action on the immune cells of specific immune responses. In contrast, the inactivation of T cells reducing the IFN and TNF secreatiion linfluences the aqctivity of the innate immunity.

Vaccines and antibiotics

Both vaccines and antibiotics are used to prevent or treat the disease. However, the killed whole cells plague vaccine requires a course of injections over a period of 6 months. Therefore, this vaccine is used mainly in those individuals who might be exposed to the pathogen there are currently no approved vaccines for protection against this organism. Plants represent an economical and safer alternative to fermentation-based expression systems for the production of therapeutic proteins. The recombinant plague vaccine candidates produced in plants are based on the two most immunogenic antigens of *Y. pestis*: 1. The fraction-1 capsular antigen (F1) , 2. The low calcium response and 3. Virulent antigen (V) either in combination or as a fusion protein (F1-V).

These antigens have been expressed in plants using all three known possible strategies:

- Nuclear transformation.
- chloroplast transformation and
- Plant-virus-based expression vectors.

Both live attenuated and killed whole cells vaccines have been used in man. Killed whole cells vaccines are used throughout the Western World, whilst live attenuated vaccines have been used especially in the former USSR and in the former French colonies.

Although there is circumstantial evidence for the efficacy of these vaccines, none have been subjected to a controlled and randomised clinical trials In view of the continuing worldwide incidence of plague and the increased likelihood of illegitimate use of *Y. pestis*, there is a requirement for a vaccine which protects against both bubonic and pneumonic plague. Ideally this should be a reduced dose vaccine (two doses or ideally a single dose) which is free of any adverse side effects. Essentially there have been two approaches to the development of such a vaccine; the identification of a rationally attenuated mutant strain of *Y. pestis* and the identification of sub-units of the bacterium, which could be formulated for single dose delivery.

Generally, Vaccines discovered in later Generations, vaccines where in various forms in various strured dosages, Whole cell killed vaccines ,live attnueated vaccines, e. coli based vaccines, DNA vaccines etc.

These are classified and detailed under generation-based discovery: 1. First generation plague vaccines, 2. Second-generation plague vaccines and 3. Third generation plague vaccines.

First generation plague vaccines

Commercially available human plague vaccines are based on either the live, attenuated strain EV76 or a killed, whole-cell preparation. There is no currently available vaccine for plague in the US but some plague vaccines are still being commercialized in other parts of the world. The advantages and disadvantages of these vaccines are summarized and compared.

Formaldehyde killed whole-cell vaccine (KWCV): Formaldehyde killed whole-cell vaccine (KWCV) was originally devised by Waldemar Haffkine in 1897 and produced in 1946 by the US Army for human use. The KWCV was commercialized first by Cutter Laboratories and later by Greer Laboratories.

The efficacy of killed plague vaccines have never been evaluated in controlled clinical trials. The evidence for protection has been based on animal trials, immunogenicity studies in humans, and observations on its use in US military personnel during the Vietnam War. The KWCP vaccine is effective against bubonic plague but it does not protect against pneumonic plague.

Immunization against plague with any of the killed whole-cell vaccines requires a course of subcutaneous injections given over a 2- to 6-month period. Unfortunately, multiple reactions to the vaccine, including malaise, headaches, elevated temperature and lymphadenopathy, are relatively common. The killed whole-cell plague vaccine is no longer available. Because of its multiple side effects and poor protection against pneumonic plague.

Live attenuated vaccines: Live attenuated vaccines, based on pigmentation mutants of fully virulent Y. pestis strains, have been used in humans mainly in the former Soviet Union (FSU) and in the former French colonies and are still in use in the FSU and Mongolia. *Y. pestis* EV76 is the most widely used and best-characterized pigmentation mutant that has been used as a vaccine. The characteristic of this Y. pestis mutant is that it appears pigmented when grown on certain solid media.

This is a consequence of the deletion of a chromosome region, including the so-called haemin storage locus and high pathogenicity island. Vaccination with this strain induces protection against subcutaneous and inhalation challenges in mice. However, these vaccines are not licensed or commercially available in Europe or the US because of their severe side effects that sometimes require hospitalization and the evidence that the EV76 strain can regain virulence and cause disease in primates.

Plague vaccines based on live attenuated Y. pestis provide the theoretical advantage of priming immunity against many antigens at the same time. It is believed that combining multiple defined mutations should lead to safer, more attenuated, live vaccines. Researchers at the USAMRIID (US Army Medical Research Institute of Infectious Diseases) reported that a Y. pestis strain with mutations in both the pigmentation and plasminogen activator loci safely induces humoral responses in African green monkeys. Higher immunogenicity and protection than the EV76 strain in mice. More recently, intra-nasal vaccination with a highly attenuated *Y.pestis* YopH mutant has been reported to confer protection againstbubonic and pneumonic plague (Table.1).

Second-generation plague vaccines

The limitations of commercial live attenuated and killed whole cells plague vaccines along with the concern about the illegitimate use of Y. pestis have greatly increased the need for an improved, safer and effective plague vaccine. Different approaches have been investigated and, at present, the most promising candidates are recombinant subunit vaccines that express both F1 and V antigens of Y. pestis which are individually immunogenic and have additive protective effect in combination. The fraction I (F1) antigen is the major capsular protein. It forms a polymer composed of a protein subunit and plays an important role in inhibiting phagocytosis by macrophages.

The use of F1 antigen subunit may allow oligomerization, which appears to enhance the immunogenicity of the protein. However, F1 antigen alone in a subunit vaccine is not desirable because virulent F1-negative strains have been reported. The V antigen is a secreted protein that regulates the translocation of the cytotoxic effector proteins. From the bacterium into the cytosol of mammalian cells promoting the death of phagocytic host cells and inhibiting the normal inflammatory response. It has been recently demonstrated that F1 and V antigens activate dendritic cells to induce primary T cells response essential to raise a protective immunity against plague. Different strategies have been used in the development of subunit vaccines for plague including the expression of F1 and V antigens in the bacterium Escherichia coli, DNA vaccines and

Table 1.Comparison of the different system used to produce plant derived plague vaccines (Alvarez & Cardineau, 2010)

System (product)	Advantages	Disadvantages
Nuclear stable transformation (transgenic plants)	High (10-16%tsp[A]) F1-V antigen expression in tomato fruit. Stable at room temperature (freeze dried plant material). Antigen purification is not necessary if accumulates in edible tissues. Inheritable transgene: a stable transgenic line can be used as permanent resource Very high scale capacity. Applicable to a broad range of plant hosts. Potential for crossing transgenic lines to obtains a line expressing multiple vaccine antigens.	Relatively long time of development (from 6 weeks to 18 months depending on the plant species). Positional effect and gene silencing. In general, low to modest recombinant Protein expression level (0.01-16% TSP). Potential for out crossing (it is prevented if plants grow in a green house).
Chloroplast stable transformation (transplastomic plants)	High (14.8% TSP) F1-V antigen expression in tobacco leaves Inheritable transgene: a stable transgenic line can be used as permanent resource. No positional effect and gene silencing. Allow expression of multiple proteins from a single transcript (similar to bacterial operon based system). Maternal inheritance (no gene transfer to other plants by pollen). Very high scale capacity.	Applicable to a limited range of plant hosts. Long time of development: approx 6 months in tobacco leaves. No protein glycosylation. Laborious: three rounds of transgenic plants selection are required compared to only one for nuclear transformation.
Plant-virus-based transient expression ("Magnifection " and "launch vector" systems)	High (1-2 mg/g FW) F1, V and F1-V transient expression in tobacco leaves . short production timescale. In general modest to high recombinant protein expression level (up to 80% for GFP).	Foreign genes are not inheritable. Products need to be purified (increase cost). Purified proteins need refrigeration for storage and transport (increase costs). Only parental vaccination (lower mucosal immune response). Limited to *N.benthamiana* leaves

TSP: Total soluble protein; FW: fresh weight;GFP: green fluorescent protein.
*High F-V fusion protein expression (up to 16% in freeze dried fruits) was achieved in tomato after reversion of gene silencing using P19

Table 2. Plant derived candidate plague vaccines developed using nuclear or chloroplast transformation (Alvarez & Cardineau, 2010)

Expression system	Antigen	Administration via and days	Treatment groups	Immunogenicity in mice	Protect in mice	Reference
Stable nuclear tomato Transformation (constitutive 35S promoter)	Bacterially produced F1-V (purified)+F1-V tomato fruit	**Vaccination:** Prime:s.c.10µg bact. F1-V (day 0) Boost: oral vaccination by feeding of 2 g F1-V tomato or non transgenic tomato . **Dose:** 300 µg/dose F1-V on days 21.28 and 35; 1200 µg/dose on day 42. **Challenge:** s.c.*Y.pestis* 20XLD$_{50}$ 18 months after last boost and observed for 25 days.	G1(n=4); PBS+adj. (non vaccinated control) G2(n=5);s.c.10 µg bact F1-V+ oral transgenic tomatoes G3(n=6);s.c.10 µg bact. F1-V+ oral F1-V transgenic tomato	Undetectrable fecal anti-V or anti-F1 IgA or serum anti- V or anti -F1 IgG1 or IgG2 Similar serum anti-F1 and anti-VIgG1 and IgG2 pre and post boost with non-transgenic tomatoes. Undetectable fecal anti-F1 and anti-V IgA. Serum anti-F1 and anti-V IgG1 increase 3X and 5X respectively, after boosting with F1-V tomato. Fecal IgA increase after boosting with F1-V tomato in 2 out of 6 mice IgG1>IgG2	0%(0/4) 20%(1/5) 50%(3/6)	Alvarez et al., 2006 and this paper.
Stable chloroplast tobacco transformation (Light inducible promotor)	F1-V crude extract enriched from transgenic tobacco chloroplasts (enF1-V) or F1-V tobacco cells suspension	**Vaccination:** Prime: s.c.25 µg enF1-V (day 0), boost:s.c.10 µg/dose enF1-V on days 14,28,126 and 164; or oral non transgenic tobacco cells suspensioin on days 8,15,22,29,119,126,164, and 171. **Challenge:** Aerosolized *Y.pestis* CO92 15XLD$_{50}$	G1(n=10);s.c.enF1-V G2(n=10):s.c.enF1-V+oral (gavage) F1-V tobacco cells suspension G3(n=10):s.c.enF1-V+oral (gavage) non transgenic plant cells suspension. G4(n=5):s.c.AIH G5(n=5):untreated	Lower serum anti-F1and anti-V IgG1 than in G2 High serum anti-F1-V, anti-F1 and anti-V IgG1 (peak ast day 140). Serum anti F1-V IgG1>IgG2a. Low serum anti F1-V IgA and undetectable fecal anti-F1-V IgA. Lower serum anti-F1-V IgA than in G2. Lower serum anti-F1-V IgA than in G2 Undetectable serum anti-F1 and anti-V or anti-F1-V IgG1, IgG2 or IgA. Undetectable serum anti-F1, anti-V or anti-F1-V IgG1, IgG2 or IgA.	33%(3/9) 88%(7/8) 0%(0/10) 0%(0/5) 0%(0/5)	Arlen et al (2008)

Particulate vaccines. Below we have summarized the advantages and disadvantages of these different approaches for the development of a subunit vaccine for prevention of bubonic and pneumonic plague.

E. coli-based vaccines: The production of F1 and V antigens has been greatly facilitated by the development of recombinant systems for the expression of the encoding genes in E. coli. Different formulations incorporating the individual subunits or an F1–V antigen fusion protein have been reported. The protein may be advantageous to combining individual F1 and V antigens as purifying and characterizing one protein, rather than two, should lead to lower manufacturing cost . Also, an injected subunit vaccine based on F1 and V antigens, using alhydrogel as an adjuvant, provides good protection against an airborne challenge with Y. pestis in mice reported with promising results with a two-dose intra-muscular F1 and V subunit vaccine in humans.

DNA vaccines: DNA vaccines consist of purified recombinant nucleic acids (‘‘naked DNA’’) that can be delivered in vivo, allowing protein expression in mammalian cells. Immunization with naked DNA vaccines encoding either the F1 antigen or V antigen is able to provide protection against bubonic plague in mice. DNA vaccine co-expressing *Y. pestis* antigens F1 and V confers protection against pneumonic plague in mice. In some studies, a prime–boost strategy, where naked DNA immunized mice were boosted with purified protein, was necessary to provide good protection. A theoretical limitation of DNA vaccines is that the cytotoxic T cells evoked by the vaccine may kill the cell that produces the immunizing antigen. There are some concerns about the safety of DNA vaccine in humans since there is a low possibility that the DNA integrates in the host genome, induces tumors, or generates pathogenic antibodies against DNA.

Particulate vaccines: It has been established that microencapsulation of peptidic vaccines confers an adjuvant effect by improving uptake into antigen presenting cells (APCS) and by sustaining the release of antigenic material over time. Encapsulation protects labile material from enzymatic and chemical destruction and improves uptake into M-cells. Reported that intra-tracheal, intra-nasal and intra-muscular poly-L-lactide microsphere co-encapsulated with F1 and V antigens was immunogenic in mice. More recently, microspheres containing F1 or V antigens were used to immunize mice on a single occasion, by either the intra-nasal or intra-muscular route. Both routes of immunization induce systemic and local immune responses in mice, with high levels of serum IgG being developed in response to both vaccine antigens. This particulate vaccine confers protection against virulent plague challenge by the parenteral and aerosol routes (Table.2).

Salmonella-based vaccines: Mucosal (oral or intra-nasal) immunization with a live attenuated Salmonella enterica serovar Typhimurium expressing F1 antigen , V antigen or F1–V fusion has been reported to provide protection against bubonic and/or pneumonic plague. However, there is a concern about plasmid instability that causes loss of antigen expression and reliance on antibiotic resistance

genes, along with the risk involved in using live vaccines particularly in immuno-compromised patients, children, elderly people and pregnant women.

Third generation plague vaccines: plant-derived vaccines

Plants are emerging as an economical alternative to fermentation- based expression systems for producing vaccine antigens, especially for the manufacturing of high-volume reserves of subunit

vaccines. Since plant-derived vaccines were first described by, a range of different plant systems have been used for the production of a long list of antigens that includes viral, parasite, bacterial, enteric, and non-enteric pathogen antigens as well as autoimmune antigens, for humans and animals. The seven Phase I human clinical trials accomplished with plant-made vaccines have shown the potential of using the plant-made vaccine

technology. The first licensed plant-derived vaccine is Dow Agroscience's tobacco cell derived vaccine against the Newcastle diseases.

Plants share the advantages of other systems used to produce recombinant subunit vaccines compared to traditional vaccines made with live, killed or attenuated/modified pathogens, including increased safety, less antigenic competition and targeting to specific sites plants have some advantages over subunit vaccine production carried out in the bacterium E. coli since plants, being eukaryotics, can produce more complex proteins that fold correctly and undergo post-translational protein modifications (e.g., N-glycosylation) that do not occur in bacteria.

Furthermore, plants do not require the construction and operation of expensive fermentors and culture media like the vaccines based on E. coli or mammalian cells. Plants can also be produced virtually indefinitely from seeds, allowing a cost effective production of recombinant proteins. Antigens can be expressed and accumulated in edible parts of the plants and oral vaccines can be effectively administered directly in the food product in which they are grown after minimal processing like freeze-drying, eliminating purification costs. Plant-derived vaccines are safer than DNA vaccines because they cannot integrate in the genome or induce tumors. Compared to particulate vaccines, plant-derived vaccines are more cost-effective particularly because they may not require antigen purification

Stable expression of plague antigens in tomato fruit using nuclear transformation

The integration of the recombinant DNA into the nuclear genome of the plant cell is the most widely used strategy for production of plant-made Vaccines. DNA can be transferred into the nuclear genome by either direct or indirect method depending on the target plant. In the direct method, named particle bombardment or "biolistics", high velocity micro-projectiles are used to carry DNA past cell walls and membranes. The term "biolistics" stands for "biological ballistics" since in this process DNA is being 'shot' into cells.

The indirect method to transfer DNA into the plant cells involves a natural plant pathogen, *Agrobacterium tumefaciens*, which can efficiently transport DNA into plant cells and cause nuclear DNA integration at random sites. This is the preferred method for nuclear transformation of most dicot and a few monocot species. However, there are still serious handicaps with Agrobacterium mediated transformation of some major cereals including wheat, barley and sorghum, but they still can be transformed using the biolistic method. From the cells transformed using the direct or indirect method, a fertile transgenic plant can be regenerated. Several independently transformed lines are analyzed and the ones that perform best (the ones with the highest recombinant protein accumulation) are selected (Fig.3.) Production systems for recombinant vaccines.

Plant-made vaccine antigens can be delivered to a mucosal surface. Mucosal surfaces lining the digestive tract, respiratory tract and genitourinary tract are the most important portals of entry for mammalian pathogens. An effective vaccine should be able to stimulate a mucosal immune response to block both initial stages of disease and disease development mucosal immune responses are characterized by the production of secretory immunoglobulin type A (sIgA). This immunoglobin prevents the binding of the pathogens with receptors on the mucosal cell surface. Mucosal vaccination is more effective than traditional parental vaccination at providing protection against enteric and respiratory diseases since systemic vaccination is a poor inducer of mucosal immunity.

Mucosal vaccines have several advantages over traditional systemic vaccines. They can be administered orally or nasally rather than via injection, thereby decreasing cost of vaccination. Mucosal vaccines are more widely accepted by the public, and they are simpler to administer and distribute.

Epidemology and its clinical features

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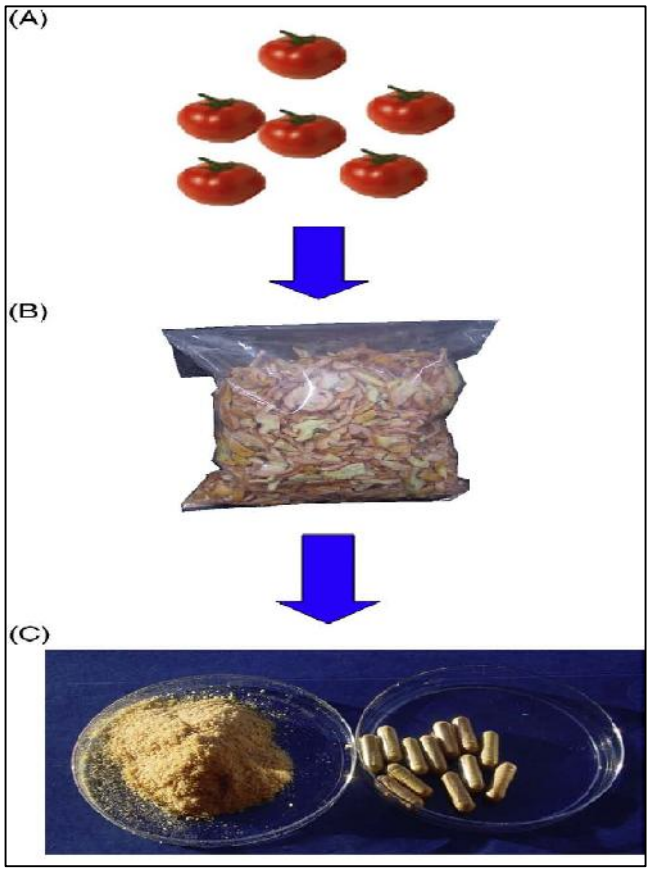
Fig.3.Production systems for recombinant vaccines (Alvarez & Cardineau, 2010)



Epidemiology

Wild rodents are the natural reservoirs of plague. In many areas, human infection occurs sporadically when humans are exposed to wild rodents and the fleas (Xenopsylla cheopis) they harbour, which are highly effective vectors. In endemic zones, infection is most common in the summer and spring in hikers and outdoor campers.

In its epidemic form, plague occurs in areas affected by earthquakes or war, when forest-dwelling (sylvan) rodents are forced to move to urban areas and thereby come into contact with domestic rats and humans. The first sign of plague in an area is often the death of large numbers of rats (which may fall from the rafters of houses – so-called 'rat-fall').

Plague may also be transmitted from rodents to domestic animals (e.g. cats, dogs) and thence to humans. In severe cases, plague is transmitted between humans, when large quantities of bacteria are expectorated in sputum. This form is highly infectious and has a high mortality. In recent years, concerns have been expressed about the potential use of plague bacilli as a source of bioterrorism involving aerosolization and infection of humans and the local rodent population. Awareness of plague as a potential cause of an outbreak of sepsis and necrotizing pneumonitis is necessary.

Pathogenesis

Plague is usually transmitted to humans by a flea bite. Fleas regurgitate Y. pestis into the skin, and these travel rapidly via the lymphatic system to the local lymph nodes, where they elicit a severe inflammatory response and marked lymph node enlargement (bubo).

Within hours, the infection and the inflammatory process spread to other lymph nodes and the bloodstream, with subsequent bacteraemia and sepsis. Haematogenous spread to the lungs leads to pneumonic patches in 10–20% of patients.

These are often multilobar and are associated with hypoxaemia and respiratory failure. Severe inflammation and necrosis with occasional abscess formation is seen in secondarily infected organs (e.g. lungs, liver, spleen).

Disseminated intravascular coagulation (DIC) results from uncontrolled sepsis, and patients ultimately die from the effects of septicaemia, shock and multi-organ failure. In untreated plague, mortality is more than 50%.

Clinical features

The fleabite is often unnoticed, though an eschar may develop. The first presenting symptom is high fever (often 39–40°C) in association with painful buboes. The femoral and inguinal nodes are most commonly involved, but the axillary and cervical groups may also be affected.

The lymphadenitis is extremely tender, and patients often immobilize their extremities in an attempt to reduce the tenderness elicited by contact and movement. In most cases, the lymph nodes are discrete with considerable surrounding oedema, but may later coalesce to form abscesses, and local cellulitis may occur. Most lymph nodes are 2–5 cm in diameter, but

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may enlarge to 8–10 cm. Rupture of the bubo with sinus formation is uncommon.

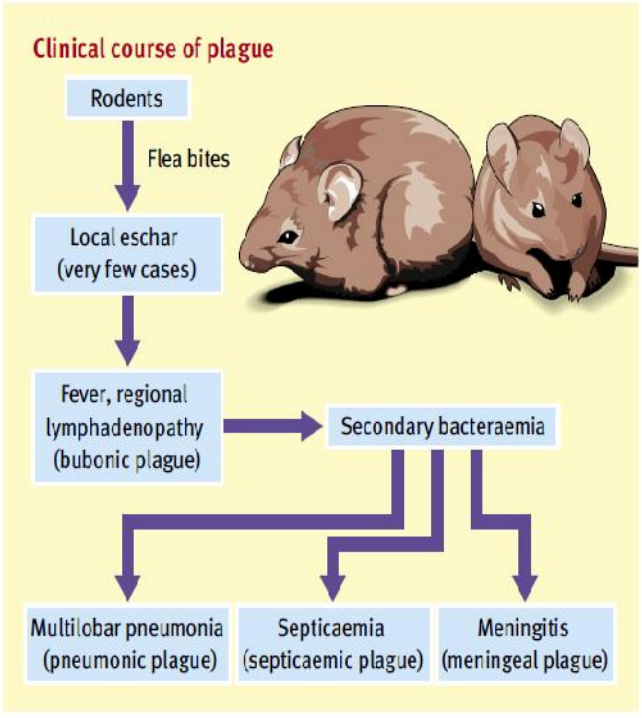
Patients are usually toxic and moribund in appearance. Cough, sputum and widespread crackles with or without signs of frank lobar consolidation often herald the onset of pneumonic plague, which is highly infectious (health-care personnel are at particular risk) and has a high mortality from hypoxaemic respiratory failure (Fig.4).

Primary plague pneumonia acquired by inhalation directly from humans is uncommon and occurs without bubo formation. Purpura and petechiae are manifestations of septic coagulopathy and signal end-stage disease. (This discoloration of the skin led to the term 'black death'.) Septicaemic plague mimics typhoid; the buboes are too deep-seated to be palpated and there is extensive bacteraemia.

Diagnosis

Attempts should be made to identify the organism. Y. pestis is slow growing, but is easily recovered from bubo aspirates, blood or sputum on standard culture media (e.g. blood agar, MacConkey's agar), where it grows aerobically.

Fig.4.Bacterial tropical infections



Differentiation in the laboratory is not difficult. Y. pestis is a Gram-negative bacillus with bipolar beading in a 'safety-pin' appearance. A rising serological titre is useful evidence of infection, but in endemic areas baseline titres in controls need careful evaluation.

Rapid dipstick tests may be useful for detecting Y. pestis antigen in patients in field studies. Neutrophilic leucocytosis occurs in almost all patients and in some cases may reach leukaemoid levels. Regular laboratory tests and chest radiography are needed to monitor complications such as adult respiratory distress syndrome, renal failure and DIC.

Management

Prompt administration of antibiotics is the mainstay of treatment. Despite occasional reports of in vitro resistance, there are almost no reports of clinical failure of streptomycin or tetracycline. However, isolation of a multi-drug-resistant strain of Y. pestis in Madagascar in 1995 has caused concern. Streptomycin is given intramuscularly in two divided doses of 15 mg/kg. Gentamicin, 3–5 mg/kg 8-hourly, appears equally effective and may be the safest drug in pregnant women. Tetracycline is given orally in a dose of 10–20 mg/kg 6-hourly. Treatment with more than one antibiotic is unnecessary. Once the patient has been afebrile for 5 days, streptomycin may be stopped and treatment changed to tetracycline, 5–10 mg/kg p.o. 6-hourly. The total duration of treatment should not be less than 10 days. Chloramphenicol, 15 mg/kg i.v. 6-hourly, is useful in those with meningeal infection and shock.

Prevention

Vaccines are generally unavailable. A formalin-killed vaccine given in two doses 3 months apart confers some immunity in laboratory personnel and health-care workers, but its role in mass immunization during an outbreak remains unevaluated.

Conclusion

A comprehensive review towards pneumonia plague heads us towards the

empowering threatening disease to our knowledge and the upcoming endemic economic global issues that to be solved either by therapies and research oriented methods and a better knowledge towards its prevention and management. Identification stage lies a crucial factor of these aggressive disease we also state the research to this particular area to be accelerated and must cover a wide research to beat up the health issues and challenges towards this deadly disease which also illustrates the importance for a serious proposal towards a wide research. And vaccines should be developed which could heads us towards a particular site specific strategy. Rapid diagnostic test (RDT)could serve as a better option to these types of Diseases.

References

1. Achtman M, Zurth K, Morelli G, Torrea G, Guiyoule A and Carniel E (1999) *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia* pseudotuberculosis. *Proc. Natl. Acad. Sci.* USA. 96, 14043–14048.
2. Adamovicz JJ and Andrews GP (2005) Plague vaccines. In: Lindler LE, Lebeda FJ, Korch GW, editors. Biological weapons defense: infectious diseases and counterbioterrorism. Totowa, New Jersey: Humana Press. pp: 121–153.
3. Amedeo Amedei et al. (2011) Role of immune response in *Yersinia pestis* infection, Accepted 23 May 2011, *J. Infect .Dev.* Courtries. 5(9), 628-639.
4. Baker EE, Sommer H, Foster LE, Meyer E and Meyer KF (1952) Studies on immunization against plague. 1. The isolation and characterization of the soluble antigen of *Pasteurella pestis*. *J. Immunol.* 68,131-145.
5. Chanteau S, Ratsifasoamanana L, Rasoamanana B et al. (1998) Plague, a reemerging disease in Madagascar. *Emerg. Infect. Dis.* 4**,** 101–104.
6. Christakos et al (2007) Recent results on the spatiotemporal modeling and comparative analysis of black death and bubonic plague epidemics. *J. royal institute of public health.* Public Health. 121, 700 – 720.
7. Cooper CL, Davis HL, Morris ML, Efler SM, Al Adhami M, Krieg AM, et al. CpG 7909, an immunostimulatory TLR9 agonist oligodeoxynucleotide, as adjuvant to Engerix-B HBV vaccine in healthy adults: a double-blind phase I/II study. *J. Clin. Immunol.*24, 693–701.
8. Crook LD and Tempest B (1992) Plague - a clinical review of 27 cases. *Arch. Int. Med.* 152, 1253-1256. Kei Amemiya et al (2009) CpG oligodeoxynucleotides augment the murine immune response to the *Yersinia pestis* F1-V vaccine in bubonic and pneumonic models of plague. *Vac.* 27, 2220-2229.
9. Dongsheng Zhou et al (2006) Molecular and physiological insights into plague transmission, virulence and etiology. Microbes and Infection 8 (2006) 273–284.
10. Galimand M, Guiyoule A and Gerbaud G et al (1997) Multidrug resistance in Yersina pestis mediated by a transferable plasmid. *N. Engl. J. Med.* **37,**667-680.
11. Galimand M, Guiyoule A, Gerbaud G, Rasoamanana B, Chanteau S, Carniel E and Courvalin P (1997) Multidrug resistance in *Yersinia pestis* mediated by a transferable plasmid. *N. Engl. J. Med.* 337,677–680.
12. Harris S (1992) Japanese biological warfare research on humans: a case study of microbiology and ethics. *Ann. NY. Acad. Sci.* 555, 21–52.
13. Heath DG, Anderson GWJ, Mauro JM, Welkos SL, Andrews GP, Adamovicz J, et al (1998) Protection against experimental bubonic and pneumonic plague by a recombinant capsular F1–V antigen fusion protein vaccine. *Vac.* 16,1131–1137.
14. Hinnebusch BJ, Perry RD and Schwan TG (1996) Role of the *Yersinia pestis* hemin storage (hms) locus in the transmission of plague by fleas. *Sci.* 273, 367–370.
15. Jehangir S Sorabjee (2005)" Plague "is Consultant Physician at the Bombay Hospital Institute of Medical Sciences, Mumbai, India. Conflicts of interest: none declared. "Bacterial tropical infection". The Medicine Publishing Company Ltd. pp: 30 -31.
16. Kapusta J, Modelska A, Figlerowicz M, Pniewski T, Letellier M and Lisowa O (1999) A plant-derived edible vaccine against hepatitis B virus. *FASEB J.* 13, 1796–1799.
17. Lillard JW Jr, Fetherston JD, Pedersen L, Pendrak ML and Perry RD (1997) Sequence and genetic analysis of the hemin storage (hms) system of *Yersinia pestis. Gene.* 193, 13–21.
18. Lucrecia Alvarez M (2006) Plant-made subunit vaccine against pneumonic and bubonic plague is orally immunogenic in mice. *Vac.* 24 , 2477–2490.
19. Lucrecia Alvarez M et al (2010) Prevention of bubonic and pneumonic plague using plant-derived vaccines. *Biotechnology Adv.* 28, 184-196.
20. Naktin J and Beavis KG (1999) *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. *Clin. Lab. Med.* 19 , 523–536 (vi).
21. Perry RD and Fertherston JD (1997) *Yersinia pestis*, etiologic agent of plague. *Clin. Microbiol. Rev* .10, 35-66.
    The World Health Organization (2003) Outbreak news. *Wkly Epidemiol. Rec.* 75, 337–344.
22. Richard W and Titball et al (2001) Vaccination against bubonic and pneumonic plague.

www.elsevier.com/locate/vaccine. Vac. 19, 4175–4184.

23. Russell P, Eley SM, Hibbs SE, Manchee RJ, Stagg AJ and Titball RW (1995) A comparison of plague vaccine, USP and EV76 vaccine induced protection against *Yersinia pestis* in a murine model.*Vac.*13,1551–1556.
24. Smego RA, Frean J, Koornhof HJ and Yersiniosis I (1999) Microbiological and clinicoepidemiological aspects of plague and non-plague *Yersinia* infections. *Eur. J. Clin. Microbiol. Infect. Dis.* 18, 1–15.
25. Suzanne Chanteau *et al (*2003*)* Development and testing of a rapid diagnostic test for bubonic and pneumonic plague. 361, Pp: 211-216. www.thelancet.com
26. Tacket C, Mason H, Losonsky G, Clements J, Levine M and Arntzen Ch (1998) Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. *Nat .Med.* 4, 607–609.
27. Tacket C, Mason H, Losonsky G, Estes M, Levine M and Arntzen C (2000) Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. *J . Infect. Dis.* 182, 302–305.
28. Thanavala Y, Mahoney M, Pal S, Scott A, Richter L, Natarajan N, et al.(2005) Immunogenicity in humans of an edible vaccine for hepatitis B. *Proc. Natl. Acad. Sci. USA.* 102, 3378–3382.
29. Titball RW and Williamson ED (2004) *Yersinia pestis* (plague) vaccines. *Expert. Opin. Biol. Ther.* 4(6), 965-973.
30. Varthelyi D and Klinman DM (2003) Immunoregulatory activity of CpG oligonucleotides in humans and nonhuman primates. *Clin. Immunol.*109, 64–71.
31. Wren BW (2003) The yersiniae-a model genus to study the rapid evolution of bacterial pathogens. *Nat. Rev. Microbiol.* 1, 55–64.