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Serological analysis and biological weapons

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Abstract— **In this paper, within the framework of forensic technical support, the importance of serological viral and bacterial tests during blood sample analysis in the recognition of biological agents is demonstrated. Due to a bioterrorist attack, the first indications in the attacked population range from individual diseases due to unusual, non-indigenous agents in patients with a similar syndrome with unusual characteristics, to an inexplicably increased incidence of a common syndrome above the seasonally expected level, or to unusual high morbidity. Application of serological tests is one of the key methods in uncovering the presence of biologically active substances in patients who reported with such symptoms. Statistical analysis and cadence monitoring is used in the analysis of the epidemiological situation and status in a certain territory.**

Index Terms— **bioterrorism, serology, biological agents, bacteria, viruses.**

I. Introduction

The development of information technologies and technologies for the isolation and identification of biological agents includes multimodal analyzes [1]. Along with the application of digital forensics [2], [3], these analyzes contribute to determining the control of epidemiological conditions in the observed area. Of particular importance to forensics is the detection of unpredictable biological agents that lead to bioterrorism.

Bioterrorism refers to the application of biological and chemical agents in the human environment and its food chain with the aim of causing mass panic, social disturbances, various psychological effects, epidemics and increasing the mortality of the population. Chemical agents are primarily used for the purpose of killing or incapacitating the enemy with chemical means - chemical poisons, smoke substances and incendiary means with permanent or time-limited contamination of a certain area. Chemical weapons are classified as weapons of mass destruction (WMD). Biological agents affect the fighting ability of the enemy due to the high degree of contagiousness and ease of the body absorption. Biological agents include: prions, viruses, bacteria, fungi and parasites [4,5]..

Biological agents are also used as non-conventional weapon of mass destruction and, due to their specific action, are difficult to detect during the incubation period. They are classified into three groups: A, B and C, according to the degree of risk for the national security and type of transmission. Biological agents can be transmitted through contaminated water, food, objects, aerosols, vectors, animals, and person-to-person.

Identification of biological agents is carried out by: microscopy (viruses are determined to the family level), cultivation, chain reaction of amplification (PCR) and antigen-antibody reaction [6], [7]. Serological methods are quick and easy to perform, available to most of the types of laboratories. The tests are highly specific and sensitive, and are therefore suitable for diagnostics and research. Most often, serum is used as a sample [8].

Bearing in mind the character and specifics of a bioterrorist attack, protection will be carried out post-exposure. The measures to be taken in that case are:

- recognition of biological attack,
- detection and identification of biological agents,
- taking care of the exposed persons,
- biological decontamination [9].

If the presence of certain causative agents from the mentioned category (or unregistered ones, which can be recognized based on the atypical clinical picture of patients and its frequency in a certain area) is determined in time, it is possible to undertake appropriate measures for epidemiological protection.

II. Biological agents

Some pathogenic microorganisms have more evolutionary morphological features that make them more contaguious and we distinguish:

Prions, which are proteins in structure, the mutation of which gives rise to pathogenic forms. They are transmitted through infected animals. Interhuman transmission is unknown. They have an affinity for the nervous system, where they accumulate. They cause severe clinical symptoms that are rapidly progressive and always fatal [10].

Viruses, which are two-component obligate intracellular infectious agents made of nucleic acid (DNA or RNA) and a protein coat. They are transmitted through vectors, direct contact, or animals [6], [7], [10].

Bacteria are unicellular organisms of prokaryotic structure. They do not have an organized nucleus, but the nucleic acid is located in the cytoplasm. Morphologically significant bacterial

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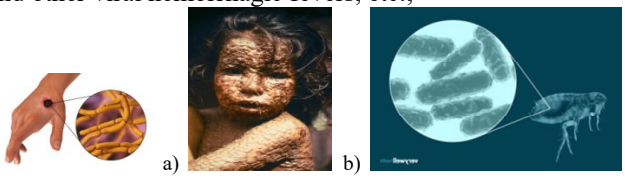
structures or metabolic products intended to cause disease are called virulence factors. Due to these factors, bacteria resist the mechanisms of the host's immune system to recognize and eliminate them in different ways. These factors include the cell wall, capsule, pili, fimbriae, ability to form spores, production of enzymes and toxins, etc. They are transmitted through vectors, direct contact, animals, aerosols, contaminated water or food [6], [7], [10].

Fungi are eukaryotic organisms. We can distinguish macromycetes (some species of which we use in the food) from micromycetes (they have a greater potential to be bioterrorist weapons), which can be grouped as yeasts, molds and biphasic fungi. Depending on the species, mushrooms can reproduce sexually or asexually. The final product of reproduction - spores (characteristic for each species) are used for dissemination. They can infect most tissues and organs [6], [7], [10].

Parasites are unicellular and multicellular eukaryotic organisms that feed at the expense of the host. Medical parasites can be classified into: endoparasites, which include protozoa (unicellular eukaryotic microorganisms) and helminths, and ectoparasites, which include arthropods. According to the degree of pathogenicity, we can group them into strictly pathogenic, facultatively pathogenic and apathogenic parasites. They are transmitted by feco-oral, direct contact, contaminated food and water [6], [7], [10].

In this paper, we analyze the biological agents of importance for the bioterrorism. According to the Centers for Disease Control and Prevention (CDC) Atlanta, we can classify them into three categories:

A. Ones which consist of agents that spread quickly, are highly contagious, have a high mortality rate, and for the adequate preparation of health institutions, special preparations are necessary. This group includes the causative agents of: variola (smallpox), anthrax, plague, tularemia, botulism, Ebola and other viral hemorrhagic fevers, etc.;



a) b)

Fig. 1. a) Cutaneous Anthrax [11] b) Variola vera [12] c) Yersinia pestis [13]

B. The others which consist of agents whose dissemination is moderate and have a moderately high mortality. This group characterize the causative agents of Q fever, brucellosis, eastern and western equine encephalitis viruses, and others.

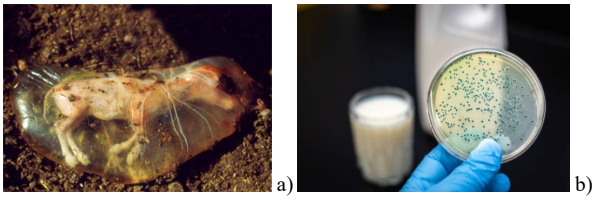


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Fig. 2. a) Q fever [14] b) Brucellosis [15]

C. It includes new, as well as already known pathogens that have potential as effective biological weapons due to availability, simple production, rapid dissemination, high morbidity, insufficiently prepared health institutions. Examples of such agents are the causative agents of tick-borne encephalitis, tick-borne hemorrhagic fevers, yellow fever, etc. [4], [5], [9].

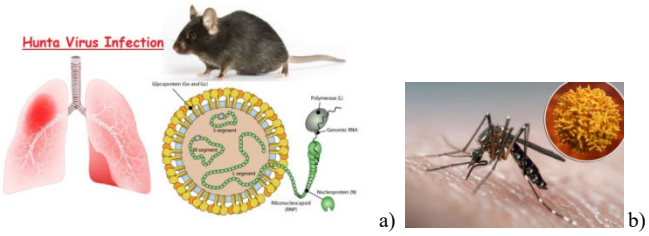

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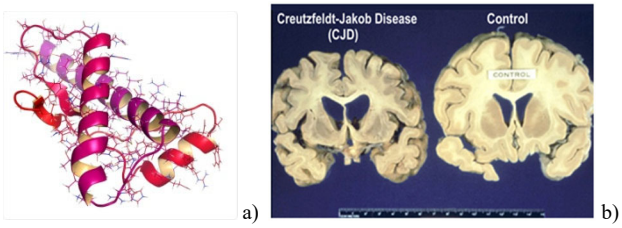
Fig. 3. a) Hantavirus [16] b) Yellow fever [17]

III. Diagnosis of Biological Agents

Recognition of a biological attack is based on observing: the sudden appearance of a large number of sick people and animals, high frequency of the disease and/or mortality in a short period of time, simultaneous infection of the patients with two or more pathogens, unusual geographical occurrence of infection, the appearance of seasonal diseases at non characteristic period of the year. Hence, the diagnosis of pathogenic agents is of great importance, as it enables timely detection of the disease and definition of adequate therapy.

The gold standard for ***prion diagnosis*** is autopsy. The methods of early diagnosis have not been sufficiently researched so far.



a) b)

Fig. 4. a) Prion [18] b) Prion disease [19]

Virus diagnostics appear as direct and indirect (serological). *Direct* diagnostics includes isolation and identification of viruses, examination and analysis of morphological characteristics of viruses, proof of viral antigens and viral nucleic acid. *Indirect* diagnostics proves the existence of specific antiviral antibodies in the sample. Immuno-fluorescence, agglutination and ELISA techniques are used.

There are several ways to ***identify bacteria*** in a sample. Microscopically, depending on the type of specimen (stained/native or direct/from culture), we can observe the shape, size, characteristic arrangement, presence or absence of a capsule or spore, and motility. Molecular techniques (PCR) amplify the sequence of interest and results can be obtained in a few hours. Antigen-antibody reactions reveal bacterial antigens, usually parts of the bacterial cell (capsule parts),

which cause the immune system to produce antibodies.

Fungi have the ability to colonize various tissues and organs, and depending on the localization of the changes, an appropriate sample is taken (skin scrap, nail, blood...). There are several methods for laboratory ***diagnosis of fungi***. Conventional methods, such as microscopy, cultivation, isolation, identification and antimycogram prove fungal infection. These methods are low sensitive and slow, with an average time period of 4 to 7 days (dermatophytes up to 21 days). Molecular (PCR) and serological methods are fast (up to 24 h), highly sensitive and show probable fungal infection.

Parasites include a large number of morphologically very distant genera and species, from protozoa, which are single-celled organisms, to helminths, arthropods and insects visible with bare eyes. During the parasite's life cycle, it is important to know their infectious stage, which represents the developmental form of the parasite capable of causing infection, and the diagnostic stage that we discover during the analysis. Parasites can cause immunopathological reactions, mechanical destruction of cells and tissues - by migration, or chemical destruction - by producing enzymes or toxins. ***Parasitological diagnosis*** mainly depends on the type of parasite and the localization of the change on the patient. Microscopy and detection of specific antigens, etc. are commonly used in diagnostics.

IV. Serology analyzes

Serology is a branch of science in human and veterinary medicine that studies serums and other body fluids. Serological analyzes diagnostically identify antibodies in the serum, which arise as an immune response to the presence of foreign molecules or to one's own proteins (in autoimmune diseases). We distinguish virological, bacteriological, mycological and parasitological serology.

Forensic serology is an important component of modern forensics. Based on the evidence of the presence of body fluids and their identification, violent acts can be proven. In the case of a bioterrorist attack, this method is used to quickly and efficiently sample the population, determine the presence of various pathogenic biological agents, while epidemiological procedures determine their progression.

Several different serological techniques are used: immunoagglutination, immunoprecipitation, enzyme-linked immunosorbent assay (ELISA), immunofluorescence and immunochromatographic tests.

Immunological tests are based on antigen-antibody reactions that take place in the laboratory conditions (in vitro). Since in most cases serum is used as a sample, these reactions are often called serological. Based on these tests, the presence of various antigens or antibodies in a sample is determined. They represent the interaction between specific antibodies produced by B lymphocytes and an antigen, a foreign molecule that triggers an immune response.

Antigens are specifically and with high avidity bound to the antibody, which makes them one of the most sensitive tests for detecting biomolecules.

As a product of a positive reaction, immune complexes are formed, which can be visible with the naked eye or marked microscopically with a marker. The marker is usually attached to antibodies and it can be an enzyme, a radioactive isotope or a fluorescent dye.

The most commonly used serological method is the enzyme immunological test, which, using a microtiter plate, enables simultaneous analysis of a large number of liquid samples.

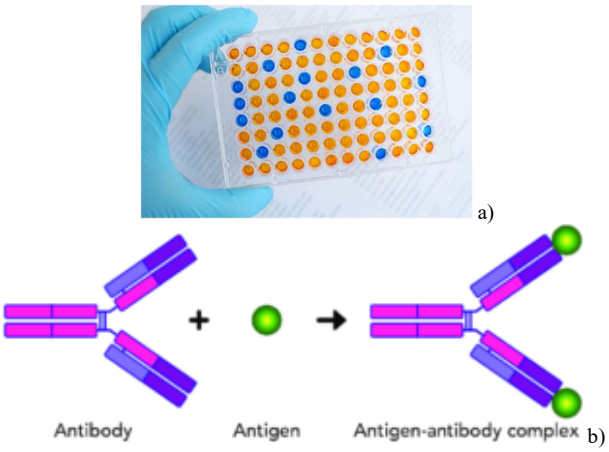


Fig. 5. a) ELISA test [20] b) Antigen - antibody complex [21]

After the reaction between the antigen and the antibody from the sample, a suitable substrate is added, which creates a colored product under the catalytic action of the enzyme. Depending on the color intensity, the concentration is determined colorimetrically. [8].

V. Discussion

Although the path of transmission of infectious diseases, caused by biological agents in natural conditions is wide, the most effective way of transmission is through air, i.e. aerosols. The method of dispersal of pathogens, suitable for biological weapons is different and ranges from aerosols formed by sprays or aerosols inserted into ventilation systems, to dispersal from aircraft platforms, long-range rockets or explosive bombs with infectious material packed in porcelain shells. It can also be successfully used to disperse biological agents (intestinal infectious diseases) and water systems in cities. Food dispersal is feasible after the introduction of genetically modified foods (GMOs). Dissemination of pathogenic agents through biological vectors (fleas, lice, mosquitoes, ticks) is the least suitable in a limited space due to unreliable effectiveness and the impossibility of controlling the effects [6,7,9].

Bearing in mind that pathogenic biological agents spread quickly, it is of great importance to urgently identify their presence in a certain territory. Emerging or undiscovered pathogens are major public health problems. In such situations, a well-organized healthcare system and discipline in the implementation of isolation measures play a key role.

In order to solve such a situation, in addition to regular staff training, mobile laboratories and field hospitals with higher biological safety levels are necessary.

VI. Conclusion

As a prevention for crisis situations caused by a biological accident, or an act of bioterror, it is necessary to strengthen the national strategy of protection and handling in emergency situations. It is also necessary to work on vigilance and raising public awareness of the possible catastrophic consequences of the action of infectious agents.

Therefore, continuous improvement of detection and identification measures of dangerous pathogens and potential biological agents is of particular importance. The application of serological tests, to emphasize once again, is of particular importance because it enables cheap, simple and fast application with high sensitivity, along with constant epidemiological and epizootological surveillance.

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