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EVOLUTION IN THE PRODUCTION AND CONTROL OF EQUINE TETANUS ANTITOXIN DURING THE LAST 3 DECADES IN IRAN

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Summary

Evolution in the production and control of Equine teanus antitoxin during the last 3 decades in Iran.

During the second World War, since importation of human biologics to IRAN was very difficult, Razi Institute was asked to produce tetanus and diphtheria antitoxins and a few prophy lactics among them diphtheria, tetanus toxoids and whooping cough vaccine. Until 1949 the crude antitetanus serum, partly purified by Sodium Sulfate treatment was used. From 1949 the enzyme digestion of serums followed by ammonium sulfate fractionation was studied and since then locally made purified and concentrated tetanus antitoxins was available in our market.

A brief account about the activities of this unit, during the last 3 decades, is presented in this report.

INTRODUCTION:

The state Razi Institute has been involved with production of various heterogenous sera such as Diphtheria antitoxin, Tetanus antitoxin, Rabies antiserum, and snake & Scorpion antivenins, over a period of more than 50 years. Such products have been used throughout the country extensively, while the demand for Diphtheria antitoxin, because of effective vaccination, has dropped markedly in recent years. Production of tetanus antitoxin however, has been carried out on a large scale basis, because of the fact that the product has found application in Iran-Iraq War, mostly for serovaccination of the wounded army personnel.

In rather old day use were made at the Razi Institute of animals like cows and sheep, but for the following reasons, horses were found

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more suitable for serum preparation:

— Availability of larger plasma volumes.

— Easier management due to the domesticability of horses, easier bleeding and horse red cells being less fragile, leading to less hemolysis.

The present paper concerns a review of the modifications and improvements of such antisera preparation techniques, brought about, during the 30 years past.

CURRENT TECHNIQUE OF HETEROLOGUS TETANUS ANTITOXIN PRODUCTION

1- The animal used

— Selection of the animal

According to the government arrangement, the Razi Institute is entitled to make gratis use of horses, surplus to the requirements of the army of Iran. Additional horses are usually purchased from local breeders. Animals of 5-10 years of age are used irrespective of their sexes. They are vaccinated before undergoing hyperimmunization programme, while being kept in stables during both summer and winter time (fixed stabling).

Disadvantages:

— Money investment for stable construction
— More personnel needed as compare to those needed for free sabling (10 person/100 horse).
— Perparation of provender in large quantities.

Advantages:

— No need for green lands and farms for the maintenance of horses, as needed for free stabling.
— Easier food distribution.
— Better access to horses, in the time of bleeding, or immunization.
— Better health care of the horses.

Health Care

Before their arrival, the horses are locally checked from the standpoints of:

— A complete clinical examination.
— Testing for infectious diseases such as melioidosis and determination of serum antibody level, by searching for pseudomonas mallei.
— Stool examination for the internal parasites (...strongles, wound) and external parasites.
— Pest equine detection by gel diffusion.
3 months later, the animals are transferred to the Razi Institute.

Vaccination

Antidiphtheria, and antitetanus vaccins 3 times at 1 month interval.
Antirabies ..... one injection only.
Throughout their stays, the horses are observed for the following:
— Clinical observations.
— Antiparasite treatment.
prevention and detection of metabolic or infectious diseases.
— Yearly malleination, and the detection of glander antibody by agglutionation technique.

Immunization of the animals

— Antigen: Toxoids or toxins

Since the beginning of tetanus antitoxin production, horses have been immunized with toxoids (Anatoxine of Ramon) or with toxins produced in peptic or tryptic digest of meat.

The potency of such toxoids was low (5-15 Lf/ml), but because of non-specific antigens (adjuvants) the response of horses was rather satisfactory.

Since 1942 we have used a pancreatic digest of casein for tetanus toxin production.

At the present time, we are producing toxins in a semi-synthetic medium (Mueller and Miller), using Harward strain.

Adjuvants

A vast range of different materials has been found to improve the antibody response when mixed with antigens. To day there are many substances, such as yeast, alum, aluminum, Calcium phosphate, charcoal sodium alginate, saponin turpentine, bacterial toxins mineral oils etc...

Table 1:

Life style of the donors.

1- Upon the arrival of the new horses:
   — Quarantine.
   — Sampling for the detection of diseases, to make sure they are healthy.
   — Stool examination for parasites.
   — Treatment of the ailment.
   — Vaccination.
   — Rest period of 4-months duration.

2- Hyperimmunization: 2 months.

3- Bleeding: 15 days after the last inoculation.

Yearly Serum Production Cycle

Antigen inoculation, is performed 4 times with 5 days interval to reach a total of 500ml.

Pause, before first bleeding: 7 days.

First bleeding: 7 days after the first injection.

Second bleeding: 4 days after the first bleeding.

Blood sampling of horses for the determination of individual and pooled titres.

Third Bleeding: 4 days after the second bleeding.

Overall cycle duration: 35 days

Rest: One month

4- Repetition of hyperimmunization: After 4 weeks

Total yearly production cycle: 5 times.

Table 2:

Health care of antitetanus serum producing horses.

1- During the horse preparation course:
   — Clinical examination
   — Search for Melioidosis (Pseudomonase Mallei)
   — Search horse sikness
   — Search for glander

2- Quarantine at Razi Institute:
   — observation and treatment if necessary
   — 2 sample for detection of melioidosis
   — Refence samples

— Antiparasite treatment: external / internal

— Vaccination:

. Antidiphtheria and antitetanus

. Antirabies

3- During Production

— Clinical care

— Prevention of illness:

by systematic elimination of parasites

by systematic vaccination

by biological or biochemical test

by balanced nutrition

According to the results of many laboratories in charge of production of Diphtheria and tetanus antitoxins, $CaCl_2$ is a potent adjuvant for Diphtheria antitoxin production while alum is a suitable adjuvant for tetanus antitoxin preparation.

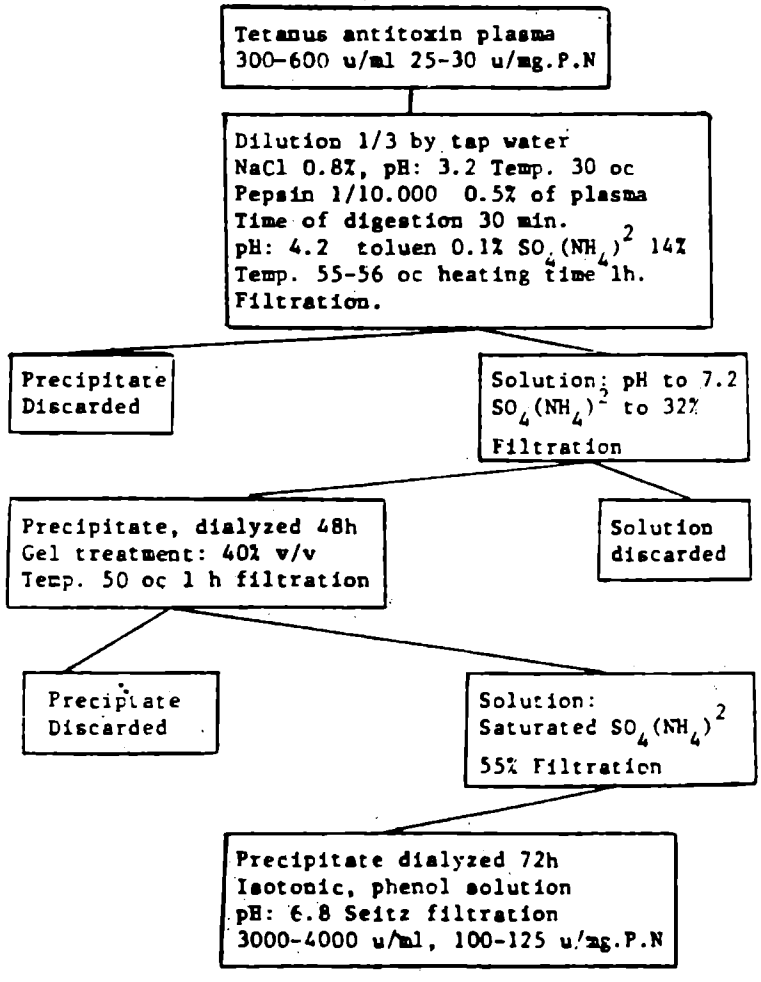
Collection of plasma

The night before beleeding, horses remain under diet and only water will be at their disposal. Sterile botttles of 9 literes having 200ml of 15 percent sodium-citrate are used. Blood 6 to 7 litres is directed from jugular vein to the bottles which remain in clean and cold room for 24 hours. The plasma is siphoned off in sterile bottle. Plasma of several horses may be pooled, providing that the horses have titers not very different.

Ether-phenol mixture is added slowly by mixing vigorously. The final concentration of phenol will be 0.5 percent. The bottles of plasma will be kept at cold room until several hundred litres of plasma are available for purification.

Table 3:

2- Diagram for Purification and concentration of tetanus antitoxin by enzyme amonim sulphate



General Testing Methods

1- Test for Flocculation
2- Test for Protein Nitrogen Content
3- Test for Plasma and Serum Protein Content
4- Test for Hydrogen ion Concentration
5- Electrophoretic Test
6- Test for Phenol Content
7- Test for Total Solid
8- Pyrogen Test
9- Potency Test
10- Test for Freedom from Abonormal Toxicity
11- Safety Test
12- Sterility Test

TABLE 4:

Refined & Concentrated Tetanus Antitoxin Production in 1957-1966

Years	No of horses	Antitoxin units
1957	90	425,810,000
1958	51	211,010,000
1959	90	427,310,000
1960	101	507,140,000
1961	96	488,950,000
1962	69	357,140,000
1963	112	603,120,000
1964	107	512,160,000
1965	115	538,264,000
1966	96	454,324,000

TABLE 5:

Refined & Concentrated Tetanus Antitoxin Production in 1967-1976

Years	No of horses	Antitoxin units
1967	118	433,544,000
1968	136	880,654,000
1969	152	957,416,000
1970	120	737,268,000
1971	107	631,212,000
1972	79	652,640,000
1973	90	885,144,000
1974	139	185,076,000
1975	132	1,010,426,000
1976	120	995,048,000

TABLE 6:

Refined & Concentrated Tetanus Antitoxin Production in 1977-1986

Years	No of horses	Antitoxin units
1977	89	792,500,000
1978	50	522,425,000
1979	61	689,325,000
1980	121	1,229,534,000
1981	116	1,088,786,000
1982	121	1,265,123,000
1983	102	979,385,000
1984	126	1,345,874,000
1985	79	876,968,000
1986	70	802,846,000

RESULTS:

With the improvement of both hyperimmunization and purification techniques, the yield of high titer antitoxins is increased thus, calling for less horses as compared to the older methods. In other words, less horses and more antitoxin recovery.

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