Production and control of tetanus vaccine

A training curriculum

MODULE II
Tetanus – microbiology and clinical aspects

World Health Organization
Geneva

in collaboration with
National Public Health Institute
Helsinki
PRODUCTION AND CONTROL OF TETANUS VACCINE
A TRAINING CURRICULUM

INTRODUCTION

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# MODULE II

**TETANUS - MICROBIOLOGY AND CLINICAL ASPECTS**

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Module II TETANUS - MICROBIOLOGY AND CLINICAL ASPECTS

1. Introduction

Tetanus is a life-threatening disease that is entirely preventable by vaccination. The widely used toxoid vaccine is very effective, very infrequently produces adverse events and is also cheap. Nevertheless, tetanus still remains a significant cause of mortality all over the world, especially in areas where the population is not extensively immunized.

Social and economic factors such as poverty, ignorance, religious beliefs, unhygienic customs and habits, together with lack of health services in regions with warm climate and fertile soil, are largely responsible for the high incidence of the disease in some places. Urbanization, industrialization and the mechanization of agriculture can interfere with the normal process of distribution of tetanus bacilli and reduce the morbidity rate, as has occurred in many industrialized countries during the last 70 years, even before active immunization against this disease was initiated (1).

Up to the 1960s, the overall case fatality rate for tetanus was 50-70%, but then started to fall with increasing availability of intensive care facilities (2). Nevertheless, tetanus mortality is still significant even in technically sophisticated settings. In 1977 in the United States 32 out of 75 cases had a fatal outcome (3). In Great Britain, the case fatality rate during the decade 1970-1979 was 10% (4).

The causative organism is very widely distributed and there are not many practical ways to avoid contact with it. Therefore active immunization remains the only feasible way of combatting this disease.

2. Etiological agent

Tetanus is caused by Clostridium tetani which is a gram-positive bacillus. It is motile, spore-forming and an obligate anaerobe. The bacterial cell measures typically 0.3 to 0.5 μm in width and 2 to 2.5 μm in length and the vegetative form often develops long filament-like cells in culture. Nonsporing bacteria carry numerous flagellae. The vegetative form is sensitive both to heating and detergents (5).

With sporulation, C. tetani takes on the more characteristic drumstick-like appearance. Spores usually form in a lateral position. The spores are extremely resistant, and are not affected either by boiling or by common disinfectants. Formaldehyde and glutaraldehyde will kill the spores but the
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best way to achieve this is autoclave sterilization, i.e. heating at $+120\, ^\circ \text{C}$ for 15 to 20 minutes (5).

C. tetani is an ubiquitous organism which is found very frequently in soil, particularly in cultivated soil. In addition to the natural environment the spores have dispersed widely in the man-made habitat. No city is free from tetanus spores, and they have been found even in operating theaters.

![Diagram of tetanus toxin structure]

The bacteria are also present in the normal intestinal flora of many animals and humans and are consequently detectable in faeces. In the intestines the bacteria are harmless as they lack invasive properties. An anaerobic environment is needed to transform the spores into vegetative organisms. An especially advantageous and nutritious environment for the bacteria is injured animal or human tissue. 

*C. tetani* produces two exotoxins, tetanolysin and tetanospasmin. Tetanolysin is an oxygen-sensitive hemolysin related to streptolysin and the θ toxin of *C. perfringens*. Tetanolysin may play a role in establishing local infection at the site of inoculation, but it is otherwise not involved in the pathogenesis of the disease. 

Tetanospasmin is a neurotoxin and the cause of the manifestations of tetanus. It is the second most potent toxin, known after botulinus toxin. As the lethal dose for humans is approximately 50 ng it can be estimated that 250 grams of tetanus toxin could annihilate the whole global population. As the more important of the two toxins tetanospasmin is usually referred to as tetanus toxin. It is this toxin that is used in the process of tetanus toxoid production. Its chemical structure is shown in Figure 1. 

3. Epidemiology 

3.1. Classical tetanus 

In 1966 Bytchenko reviewed the available data and estimated that during the decade 1951-60 there had been more than 1,000,000 cases of tetanus globally and of these approximately half had died, but these figures were considered underestimates (1). From those days the number of reported cases in many countries has risen sharply. This may be due to the development of more efficient epidemiologic surveillance rather than an actual change in tetanus incidence (6). In 1984 the annual incidence of (nonneonatal) tetanus was estimated to be at the level of 750,000 ± 250,000 with mean mortality rate of approximately 50% (7). 

In developed countries tetanus is a rare occurrence. For example in the United States approximately 100 cases have occurred annually during the last decade. Most European countries report fewer than 20 cases annually. However, even in technically developed medical centers the mortality remains significant, varying between 10 and 40% (4,8). 

3.2. Neonatal tetanus 

Neonatal tetanus is still one of the leading causes of infant mortality, accounting for approximately one-third of all neonatal deaths in some developing countries. In 1984 it was estimated that there are some 750,000 ± 250,000 cases of neonatal tetanus worldwide (7). In 1990 the EPI estimates were essentially at the same level. The reported mortality rates range from less than 5 to more than 60 per 1,000 live
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births which might account for 23 to 72% of all neonatal deaths (9). But even today the condition remains substantially underreported (10).

4. Pathogenesis

In an anaerobic milieu such as injured tissue, the spores give rise to the vegetative form of C. tetani. The transformation is enhanced for example by tissue necrosis, pyogenic infection or foreign bodies present in wounds or in the umbilical cord. The risk for tetanus is greatest in deep contaminated wounds, such as war or traffic injuries and piercing wounds related to agriculture, where massive contamination is typical. However, tetanus has also been reported to result from chronic otitis media, burns, animal bites wounds, chronic ulcerations and chronic dermatitis. Tetanus can complicate septic abortion, dental extraction or even elective surgical procedures (4).

In the tropics tetanus is often associated with childbirth. Some local customs, such as piercing of the ears or other parts of the skin, circumcision, or stopping of ear discharge with powdered soil, create conditions for contracting tetanus. Lately tetanus has been a growing complication of intravenous drug abuse. Sometimes the site of inoculation cannot be demonstrated (cryptogenic tetanus) (1).

The incubation period can vary from a few days to several months, but is usually between 3 to 21 days. The further the inoculation site is from the central nervous system, the longer the incubation period. A short incubation period is associated with fulminant disease and poor prognosis. Similarly, the shorter the period from the first symptom to the first generalized spasm (onset period), the worse the prognosis.

Transport of the toxin from the injured site has been a matter of much debate during the years. In fact most of the toxin is disseminated via the bloodstream. However, the toxin does not appear to cross the blood-brain barrier. Newer evidence has established neuronal transport as the predominant means by which the toxin enters central nervous system. The toxin can be demonstrated in motor end plates of muscle nerves (5).

After the toxin gains entry at neuromuscular junctions by binding to the cell membrane, it proceeds up the nerve to the ventral horns of the spinal cord or motor nuclei of the cranial nerves. The possible mechanisms of transport within the nerves include intra-axonal transport, transmission in the perineural space between nerve fibers or spread by lymphatics associated with the nerve (5).

5. Clinical presentation and complications

Few diseases present with such a classical clinical picture as tetanus. Modified cases are seen but most cases do present with the picture
which was already described in the writings of Hippocrates. Different clinical presentations are classified as follows:

1) Generalized tetanus: Usually the initial muscle spasms occur near to the initial wound. The most common presenting complaints are lockjaw (trismus) and dysphagia. This is followed by stiffness of back and abdominal muscles, progressing to persistent rigidity.

Tetanic muscle spasms result in marked extension of the back (opisthotonus), flexion of the arms, clenching of the fists, plantar flexion of the toes and the typical facial grimace (risus sardonicus). Spasms may be precipitated by almost any stimulus such as light touch, air current, noise or bright light. The spasms may be strong enough to produce fractures of the vertebral bodies.

2) Localized tetanus: A milder form of the disease. Symptoms include stiffness and pain in the muscle groups immediately adjacent to the wound. The condition may also progress to generalized tetanus, or it can be a mild, slowly developing disease. Should it remain localized, the prognosis is good, with a mortality of about 1%.

3) Cephalic tetanus: Presents as a weakness of muscle groups supplied by one or more of the cranial nerves, often the seventh, subsequent to injury of the face or head. Cephalic tetanus is rare and has a poor prognosis.

4) Neonatal tetanus: Is classified as a separate entity since it has a distinct epidemiology. The first signs are usually poor sucking and excessive crying followed by variable degrees of trismus, difficulty in swallowing, opisthotonos and other tetanic spasms. The symptoms occur 3 to 14 days after birth in approximately 90% of cases but they can occur from 1 up to 28 days (5).

5) Mild forms of tetanus: Occur in individuals with incomplete immunity from immunization. Includes subacute tetanus, seen in tropical areas.

6. Diagnosis

The clinical pattern of classical generalized tetanus is usually distinct enough to preclude other diagnoses. The diagnosis can be made quite reliably by taking careful history (wound, unimmunized or incompletely immunized status) and by observing the symptoms and signs of the patient. Localized forms of the disease may pose significant diagnostic difficulties. Frequently, laboratory investigations remain negative and services are not available where the disease poses the most common problem.

Characteristic gram-positive bacilli, some with terminal or subterminal spores, may occasionally be seen microscopically in aspirates from the affected area. Bacterial cultures have been reported positive only in about
30% of the clinically confirmed cases. With good sampling techniques and the employment of special transport systems for anaerobe specimens, significantly higher positive culture rates can be achieved.

Low or undetectable levels (< 0.01 IU/ml) of circulating anti-tetanus antibodies at the time of onset of the symptoms are compatible with the diagnosis; however, a few incidental cases with moderately high levels of antitoxin at the time of the diagnosis have been reported (5).

7. Treatment

The management of tetanus has three primary objectives: a) to overcome disturbances in the body due to the tetanus toxin already bound to the central nervous system; b) to neutralize toxin still circulating in the blood; and c) to eradicate the tetanus bacillus itself (11).

Good nursing care is critical in the management of a patient with tetanus. The patient should be kept in a quiet, dimly lit room. Sudden environmental stimuli, such as loud noises, should be avoided. Pharmacological treatment of hypertonicity and spasms depends on severity and frequency of the condition. The major purpose is to control spasms and muscle tone without impairing, if possible, voluntary movement, consciousness and, most importantly, respiration (12). If intensive care facilities are available, the patients are treated with general muscle relaxation, tracheotomized, in respirators.

Immediate attention needs to be given to neutralizing the effects of the toxin. While nothing can be done to discharge the toxin already bound to nerves, its further absorption can be prevented by the administration of antitetanus immunoglobulin.

The optimal dose of antitetanus immune globulin for treating acute tetanus is not known. Current practice, which is arbitrary, is to give doses ranging from 500 to 6,000 and up to 10,000 IU intramuscularly (13). Equine antitetanus serum could be given intravenously but is complicated by serious allergic reactions and its use cannot be advocated any longer where the human preparation is available.

The value of intrathecal administration of tetanus immunoglobulin still remains to be established, since several studies have given conflicting results (5).

Recently, it has been noted that gammaglobulins for intravenous use could be applied in treatment of tetanus. The anti-tetanus IgG antibody levels in these preparation has been found to be between 4 to 90 IU/ml (13). These preparations have the advantage that they could also be administered intrathecally.

Although definite proof of its usefulness is scanty, antibiotic prophylaxis is commonly recommended if the wound is soiled or if the object causing
the wound was contaminated (11). Penicillin and erythromycin have been recommended as drugs of choice. Tetracyclines may be used from the age of eight onwards.

8. Prevention

General measures in tetanus prevention must include proper wound management, cleaning, disinfection, careful inspection for foreign bodies in the wound, revision and suturing. Although not recommended routinely, prophylactic antibiotic treatment should be prescribed, if imminent risk for wound infection is present. Occupational health hazards should be minimized by offering protective clothing to workers (gloves and safety boots).

For neonatal tetanus, the important preventive approach is through improving the quality of prenatal, obstetric, and postnatal maternal and child health services. Unhygienic traditional birth practices should be changed (14,15,16).

However, the most important preventive measure for control of tetanus is immunization. Active immunization should be made available for all people. Passive immunization is necessary in unimmunized wounded patients in risk. The prevention of neonatal tetanus may be effected by active immunization of alternatives all women of childbearing age or pregnant women (17,18,19,20).

8.1. Passive immunization

To wounded patients in whom the risk for tetanus is considered high 250 IU tetanus immunoglobulin should be administered intramuscularly. If more than 10 years have passed from the last booster vaccination, 0.5 ml of toxoid should be given simultaneously. If the immunization status is incomplete, the vaccination series should be finished. If the patient has not been immunized at all or the immunization status is not known, the patient should receive a full course vaccination tetanus toxoid.

In many countries passive immunization is given also to children born in circumstances where exposure to tetanus spores is likely if they are seen with an unhealed umbilicus in the neonatal period.

8.2. Active immunization

8.2.1. Properties of the vaccine

Tetanus vaccine contains tetanus toxoid as an antigen (immunogen). The toxoid is prepared by treating tetanus toxin chemically (usually by formaldehyde, another possibility is glutaraldehyde) to render it nontoxic without losing its immunogenic potency. The toxoid is concentrated, purified and adsorbed onto a suitable adjuvant. It is supplied as a sterile solution in physiological buffer usually with preservative. The vaccines
currently produced are adsorbed on aluminum hydroxide or aluminum phosphate adjuvants (for more details see Module III).

The previously used unadsorbed fluid toxoid was not as effective as the adsorbed vaccine. More doses were required to achieve protection, and the levels also declined more rapidly. In combined active-passive immunization anti-tetanus immunoglobulin impaired the immune response if fluid toxoid was used. For these reasons WHO currently recommends only the use of adsorbed vaccine (21).

8.2.2. Timing of vaccinations

Today, basic immunization of infants against tetanus is usually given with the combined adsorbed Diphtheria-Tetanus-Pertussis (DTP) vaccine. Many countries have their own immunization schedules modified to local circumstances, reflecting established clinical practices, and different kinds of vaccines available, as shown in the example from Finland (Table 1). A schedule recommended by the WHO is to give the first dose at six weeks, the second at 10 weeks, and the third at 14 weeks. One completed basic immunization series during a lifetime is considered sufficient. After the completion of the primary immunization series, booster doses should be given at ten year intervals, or, in case of injury, after five years.

In some countries, however, the primary immunization series is currently boosterred with a fourth injection at 15 to 24 months of age, and a fifth dose at the age of four to six years. For the prevention of neonatal tetanus, EPI currently recommends a five dose schedule of TT to women of child-bearing age (Table 2). Two doses, properly spaced, are essential for the mother to pass protection to her infant.

8.2.3. Protection level

Immunization by tetanus vaccine results in formation of protective tetanus antitoxin (immunoglobulin) in serum. The immune response can be measured by several methods, both *in vivo* and *in vitro* (Module VII). The antitoxin levels are expressed in International Units (IU/ml).

Based largely on animal studies it has been estimated that circulating serum antitoxin levels of $\geq 0.01$ IU/ml are indicative of protection (5). This estimate has been confirmed in most clinical materials reported under all but the rarest situations (5).

It should be noted that the infection itself does not result in protective immune response, probably because the toxin is adsorbed into the neurons so quickly. Several cases of recurrent tetanus have been described. Therefore, the treatment schedule must always be supplemented with active immunization with tetanus vaccine.
## BASIC IMMUNIZATION SERIES AND BOOSTERS

<table>
<thead>
<tr>
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<th>VACCINE</th>
<th>SCHEDULE</th>
<th>NOTES</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTP vaccine</td>
<td>I</td>
<td>3 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>4 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>5 month</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>20-24 months of age</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>One complete basic immunization series during lifetime is considered sufficient.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. Booster dose</th>
<th>Td vaccine</th>
<th>at 11-13 years of age and to military conscripts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Td vaccine</td>
<td></td>
<td>Td vaccine includes a small dose of diphtheria toxoid, sufficient for a simultaneous diphtheria booster.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>3. Successive boosters</th>
<th>Tetanus toxoid or Td vaccine 0.5 ml i.m.</th>
<th>every 10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>The use of Td vaccine is currently recommended also for the adult age boosters to sustain immunity against diphtheria.</td>
<td></td>
</tr>
</tbody>
</table>

## TETANUS PROPHYLAXIS IN CONNECTION WITH PENETRATING WOUNDS

### A. Dirty wound, risk for tetanus assessed high

1. For a person with a full basic immunization series completed more than five years previously
   - tetanus toxoid booster 0.2-0.5 ml i.m.
   - or
   - Td booster 0.5 ml i.m.

2. For a person with a full basic immunization series completed more than ten years previously
   - tetanus toxoid booster 0.2-0.5 ml i.m.
   - and tetanus immunoglobulin 250 IU i.m.
   - or
   - Td booster 0.5 ml i.m.
   - and tetanus immunoglobulin 250 IU i.m.

3. Basic immunization series incomplete or immunization status not known
   - complete the basic immunization series (3 doses, first two at 4-8 week interval, the third 6-12 months from the second)
   - and tetanus immunoglobulin 250 IU i.m.

### B. Clean wound

1. Individual with full basic immunization series, last booster dose for more than 10 years previously
   - tetanus booster 0.2-0.5 ml i.m.

2. Unimmunized
   - basic immunization series as above

Table 1. Recommendation for immunization against tetanus in Finland (National Public Health Institute, 1980).
<table>
<thead>
<tr>
<th>DOSE</th>
<th>WHEN TO GIVE</th>
<th>EXPECTED DURATION OF PROTECTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT 1</td>
<td>at first contact or as early as possible in pregnancy</td>
<td>none</td>
</tr>
<tr>
<td>TT 2</td>
<td>at least 4 weeks after TT 1</td>
<td>1-3 years</td>
</tr>
<tr>
<td>TT 3</td>
<td>at least 6 months after TT 2</td>
<td>5 years</td>
</tr>
<tr>
<td>TT 4</td>
<td>at least one year after TT 3 or during subsequent pregnancy</td>
<td>10 years</td>
</tr>
<tr>
<td>TT 5</td>
<td>at least one year after TT 4 or during subsequent pregnancy</td>
<td>whole childbearing years</td>
</tr>
</tbody>
</table>

Table 2. Tetanus toxoid immunization schedule for women of childbearing age as recommended by the EPI.
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