IMMUNITY TO TETANUS IN PREGNANT WOMEN AND MALE BLOOD DONORS IN DAR ES SALAAM, TANZANIA

By

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A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Medicine (Microbiology) of the University of Dar es Salaam.

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CERTIFICATION

The undersigned supervisors certify that they have read and hereby recommend for acceptance by the University of Dar es Salaam a dissertation entitled: *Immunity to Tetanus in Pregnant Women and Male Blood Donors in Dar es Salaam, Tanzania,* in partial fulfilment of the requirements for the degree of Master of Medicine (Microbiology).

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DECLARATION AND COPYRIGHT

I, Said Aboud, declare that this dissertation is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

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DEDICATION

To my parents, Aboud Said Saleh and Masaad Salim Magram.

ABSTRACT

The aim of the present study was to investigate immunity to tetanus among pregnant women and male blood donors with tetanus toxoid (TT) vaccination in Dar es Salaam, Tanzania. In a cross-sectional study 176 pregnant women and 200 male blood donors from Muhimbili National Hospital antenatal clinic and blood bank, respectively, were examined. Using an antigen competition, indirect and avidity ELISA techniques, serum tetanus antitoxin levels, distribution of anti-TT IgG subclasses and their binding avidities, respectively, were determined. Our findings showed that 5.1% of pregnant women and 36% of male blood donors had tetanus antitoxin levels below 0.1 IU/ml. In addition to the most dominant anti-TT IgG_1 antibody, anti-TT IgG_2, anti-TT IgG_3 and anti-TT IgG_4 subclass antibodies were also detected. In pregnant women, the avidities of anti-TT IgG_1 and anti-TT IgG_3 antibodies were found to be high, whereas avidity of anti-TT IgG_4 antibody was found to be low. It is concluded that 5.1% of pregnant women and their newborns, and 36% of male blood donors were susceptible to tetanus. Low avidity anti-TT IgG_4 antibodies might be less efficient in clearing tetanospasmin and can lead to ineffective protection against tetanus. Proper keeping of TT vaccination records and proper spaced intervals between doses are vitally important to identify individuals with inadequate TT vaccination as well as to avoid hyper-immunisation.

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LIST OF ABBREVIATIONS

TS	tetanospasmin
Clostridium tetani	*C.tetani*
NT	neonatal tetanus
TT	tetanus toxoid
PHA	passive haemagglutination
ELISA	enzyme-linked immunosorbent assay
RA	radio immunoassay
TL	tetanolysin
WHO	World Health Organization
EPI	Expanded Programme on Immunization
NNT	non-neonatal tetanus
ng	nanogram
Kg	kilogram
kDa	kilo Dalton
VAMP	vesicle-associated membrane protein
GABA	γ-aminobutyric acid
*E*h	oxidation-reduction potential
HTIG	human tetanus immunoglobulin
DPT	diphtheria-pertussis-tetanus vaccine
MCH	maternal and child health
DT	diphtheria-tetanus vaccine
Td	tetanus-diphtheria vaccine

HIV	human immunodeficiency virus
CD	cluster of differentiation
Fc	fragment crystallisable
IU	International unit
Ml	millilitre
Lp	dose of tetanus toxin that cause paralysis
L+	lethal dose
MNH	Muhimbili National Hospital
ICU	intensive care unit
PBST	phosphate-buffered saline tween
BSA	bovine serum albumin
µl	microlitre
OD	optical density
EU	ELISA unit
N	normality
M	molarity
r^2	correlation coefficient
R^2	adjusted multiple coefficient of determination
SE	standard error
β	standardised regression coefficient
AIDS	acquired immunodeficiency syndrome

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Tetanus is caused by the action of a highly potent neurotoxin, tetanospasmin (TS), an exotoxin which is produced by *Clostridium tetani* (*C. tetani*). The disease usually occurs through infection of a skin injury with spores of *C. tetani*. *C. tetani* spores introduced into an area of injury germinate to tetanus bacilli in the presence of necrotic tissue with reduced oxygen tension. Contamination of umbilical stump with tetanus spores may lead to neonatal tetanus (NT) if the neonate has no passively acquired protective antibodies against TS.

Immunity to tetanus is antibody-mediated and depends on the ability of antitoxins/antibodies to neutralise TS either by blocking its binding to the receptor or clearance of immune complexes. Vaccination with tetanus toxoid (TT) stimulates production of antitoxins belonging to the IgG class, predominantly IgG_1 (1-3) and/or IgG_3 subclass (2). Tetanus antitoxin may be measured by *in vivo* and *in vitro* techniques. *In vivo* neutralisation assay has been regarded as the gold standard, but problems of animal protection, cost, biosafety, practical performance issues, including animal and toxin supplies, have hampered extensive application of these tests.

This assay is primarily a measure of serum IgG antitoxin. The *in vivo* neutralisation assay directly measures the biological activity of tetanus antitoxin by demonstrating the toxin-neutralising property of serum in laboratory animals, usually mice. With this test, the protective level of tetanus antibody is defined as ≥ 0.01 IU/ml (4).

The interaction between tetanus antitoxin and TS or TT may be measured *in vitro* by passive haemagglutination (PHA) test, enzyme-linked immunosorbent assay (ELISA), or radio immunoassay (RA). These techniques are simple, sensitive, and rapid, but they are generally less specific than the *in vivo* neutralisation method (4). Since the use of the indirect ELISA test to quantify tetanus neutralising antitoxins in human sera is limited by marked overestimation in low titre sera, a modified ELISA test has been developed. The principle of this new test, called the antigen competition ELISA, is the assumption that the binding of antitoxin to TT adsorbed to the surface of microtitre wells is different from the binding of antitoxin to TT or TS in solution (5). In the antigen competition ELISA test, the antitoxin to be tested is allowed to react with TT in solution, which prevents antitoxin binding to TT adsorbed to the insoluble carrier. The results of the antigen competition ELISA test correlate well with results of the *in vivo* neutralisation test.

Usually, a level of tetanus antitoxin of 0.1 IU/ml measured by an *in vitro* assay is considered as a safe estimation equivalent to 0.01 IU/ml as determined by the *in vivo* technique, both being considered minimum protective levels (4). However, experience with the antigen competition ELISA test is limited and further data is needed especially from sera with low antitoxin content (4). The proportion of women immunised with TT who have immunisation cards has remained low. Thus, TT coverage of women has been difficult to assess and methods have tended to underestimate the true coverage. Measuring tetanus immunity levels in a community using antigen competition ELISA offers the advantage of not depending on cards or recall memory for accuracy. Knowledge of the immunity to tetanus among male and female adults is of paramount importance in order to identify areas for improvement of the current TT immunisation schedule in Tanzania. Furthermore, distribution of anti-TT IgG subclass antibodies and their avidities are important functional characteristics of tetanus immunity that need to be explored as well.

1.2 Tetanus

1.2.1 Historical perspective

Mankind has known tetanus for thousands of years. The disease is ubiquitous, but it particularly kills many persons in tropical countries, where the disease is known by various local names (6). The belief that NT is linked with divine intervention is reflected in the Biblical story of the death of the son of King David and Bathsheba (Samuel 2, verses 11 and 12). In the 18th and 19th centuries NT was prevalent in the Scottish Islands, Iceland, the Westmann Islands, Faroe Island, and Greenland. In the St. Kilda, a Scottish island 40 miles west of the Outer Hebrides, the neonatal mortality reached 690 per 1000 live births and was passively accepted by the community (7-8). In 1764 Rev. Kenneth MacAuley wrote that "the St. Kilda infants are peculiarly subject to an extraordinary kind of sickness: on the fourth, fifth or sixth night after birth, many of them give up sucking; on the seventh, their gums are so clenched together that it is impossible to get anything down their throats. Soon after this symptom appears, they are seized with convulsive fits, and after struggling against excessive torments, till their little strength is exhausted, die generally on the eighth day". MacAuley's description of NT case is still relevant today.

In many industrialised countries, the decline in NT morbidity and mortality started well before TT was widely available. Improved obstetrical techniques and neonatal care were responsible for the reduction in NT mortality in the United States from 64 per 1000 live births in 1900 to about 1 per 100,000 live births in 1961-1964 (9). In the United States, NT accounted for 10% of all tetanus cases in 1965-1966 (9) and 1% in 1982-1985 (10). In Denmark improvements in hygiene resulted in a 45% reduction in NT morbidity between the 1920s and 1940s. The steady fall in NT morbidity in Denmark after 1950 coincided with an increasing degree of active immunisation of the population. The complete elimination of NT in Denmark at the end of the 1960s was probably due to the high tetanus antitoxin levels in pregnant women who had been immunised during their childhood (11).

1.2.2 Bacteriology of *C. tetani*

C. tetani is a gram-positive, obligate anaerobic, spore forming bacillus. Motility is rather sluggish although it has numerous peritrichous flagella. It bears round terminal spores that give the organism a drumstick appearance. Early spores are oval and subterminal. The spores are very resistant to a variety of deleterious agents including most disinfectants and extremes of temperature.

They resist boiling at neutral pH for several minutes. However, some strains survive boiling for some hours. Culture in semi-solid glucose medium encourages sporulation. On solid media, colonies spread early as fine feathery projections at the edge of a delicate film of growth.

The tetanus bacillus displays little biochemical activity. It is typically non-saccharolytic and is weakly proteolytic, hydrolysing gelatin but not other proteins. *C. tetani* is lecithinase-negative and lipase-negative. Many strains produce indole, but often only after 2 days. Hydrogen sulphide is not produced and the organism does not reduce nitrate. A neuraminidase is produced. Major end products of metabolism include acetic, propionic and butyric acids, and butanol. Haemolysis is noted on blood agar, which on occasion can be neutralised by tetanus antitoxin. Initially, the colonies are α-haemolytic but on prolonged incubation they become β-haemolytic. Tetanolysin (TL) is responsible for the haemolysis. Maximum yields of toxin occur in the stationary phase of growth and very little toxin is released into the medium until the cells are lysed. Phages have been demonstrated in *C. tetani* but they are not involved in toxin production.

1.2.3 Epidemiology

Tetanus has been recognised as a priority problem and has been one of the six target diseases of the World Health Organisation (WHO) Expanded Programme on Immunisation (EPI) since 1974. The main focus of the EPI is directed towards eliminating NT from developing countries (12). During the 1980s more than 40 developing countries conducted NT mortality surveys. They found NT mortality rates ranging from 1 to 2 per 1000 live births in Jordan and Sri Lanka to 67 deaths per 1000 live births in some areas in India (13). These early studies conducted before immunisation and clean delivery programmes were widely established, revealed that approximately 1 million newborns contract NT each year, of which 800,000 died. By uncovering the magnitude of NT mortality, the community surveys were critically important in changing health policy makers' perceptions and in generating political support for NT control and elimination (14).

Globally, the annual number of NT cases reported to WHO increased considerably between 1974 and 1989, probably reflecting improvement of the reporting systems (12). In 1989 there were 29,305 cases of NT reported globally, and NT accounted for 35% of all tetanus cases (12). Some downward trend was observed in 1990 and

1991. To estimate NT mortality in developing countries WHO takes into consideration expected mortality rates in the pre-vaccine era and the most recent percentage of pregnant women immunised with TT. NT is endemic in 90 countries throughout the world and in some of these countries it accounts for one-half of the neonatal mortality and one-fourth of infant mortality (15). In the developing world, NT cases account for half of the total tetanus cases reported (16). WHO estimated that in 1992 there were 578,000 infant deaths (Table I) due to NT (17).

Table I: Estimated number of actual and prevented NT deaths in developing countries, 1992 (17)

Region	Actual (%)	Prevented (%)
Africa	152,000 (26.3)	102,000 (14.9)
America	11,000 (1.9)	46,000 (6.7)
Eastern Mediterranean	90,000 (15.6)	53,000 (7.7)
Europe	1,000 (0.2)	300 (0.1)
Southeast Asia	210,000 (36.3)	467,000 (68.0)
Western Pacific:		
Without China	15,000 (3.1*)	16,000 (2.3*)
With China	114,000 (19.7)	18,000 (2.6)
Total: without China	479,000 (100)	684,000 (100)
with China	578,000 (100)	686,000 (100)

NT, neonatal tetanus; *Calculated from total without China

NT was reported to be the second leading, among the vaccine-preventable diseases in the world, to measles as a cause of childhood mortality (18). In 1996, the estimated deaths from NT were 438,080 with mortality rates of 4.8-10.7 per 1000 live births in high-risk developing countries (19). In 1997, WHO estimated the global number of NT cases to be 400,000 (20). In India a prospective community

study conducted in 1983-84 showed that NT was the second commonest cause (after septicaemia) of neonatal mortality, and that the NT mortality rate was 9.7 per 1000 live births (21). A community survey carried out in coastal Kenya in 1993 revealed NT mortality of 3.1 per 1000 live births (22). To date, NT continues to occur in several countries especially in the developing block, even though significant decline of NT cases in parallel with increased coverage of maternal vaccination with TT has been reported in several countries.

In the USA, the annual incidence rate of tetanus between 1985 and 1990 was about 0.02 per 100,000 people. Of the total 365 tetanus cases reported in the 6-year period, only one was NT. Approximately, 70% of the total cases occurred in persons 50 years of age or older (23-24). In 1994, only 36 cases of tetanus were reported in the USA (25). However, it is estimated that the actual number of cases is at least double the number reported.

Community-based NT mortality surveys have confirmed the importance of risk factors for NT, such as missing TT immunisation with TT during pregnancy, home delivery, unhygienic cutting of the umbilical cord, application of potentially infectious substances to the umbilical stump (22), and a history of NT in a previous child (26). A

case-control study in Pakistan showed that application of clarified butter (ghee) during the first few days of life was a significant risk factor (27). In another survey a high risk for NT was associated with unwashed hands, suggesting that a possible source of spores may be the hands of the birth attendant (28). The type of tool used to cut the umbilical cord did not seem to be an important risk factor (28-29). The main mode of contamination may be unhygienic dressing of the umbilical stump (28). Ritual practices of placing soil or cow dung on the umbilical cord has also been reported to play a role (29). Results of all these studies suggest that teaching mothers and birth attendants simple hygienic techniques have a significant impact on NT incidence.

Tetanus in age groups other than neonates has different morbidity and mortality pattern and different predisposing factors. There are no reliable data showing the real magnitude of non-neonatal tetanus (NNT) in the world. Existing surveillance data grossly underestimate the problem. Studies in industrialised countries show under reporting of NNT morbidity and mortality. Reported cases of tetanus did not represent more than 13% of the actual number of cases in Switzerland (30). The completeness of reporting tetanus deaths in the USA ranged between 40 and 76% (31). It has been estimated that in

the 1980s, 300,000-700,000 cases and 120,000-300,000 deaths due to NNT occurred each year in the world, excluding China (32). It was considered that the upper estimate of 700,000 cases is closer to reality. It is believed that in the beginning of the 1990s some 360,000 cases and 140,000 deaths could be attributed to NNT. The number of NNT cases reported to WHO from developing countries ranged from 90,335 in 1980 to 35,749 in 1990, whereas it was 4,812 in industrialised countries (17).

Tetanus spores are widespread in the environment (soil, animal faeces and surfaces of carpets) so that any breach in the skin (such as wounds, burns, animal or human bites, and even insect bites) may result in inoculation of the spores into the host. Portals of entry into the body for *C. tetani* are numerous, but the most common are traumatic and surgical wounds (about 65% of cases) and the umbilical stump after childbirth (33). The injury is often a minor one such as a wood or metal splinter or a thorn. Chronic skin ulcers account for 5% of cases and there is no obvious source in the remainder of cases (33). Traumatic wounds remain the main means of acquiring NNT, but in 8-31% of patients no portal of entry is detected (32). Tetanus bacilli have been recovered from the external auditory canal and it is thought that otogenic tetanus does occur in

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relation to ear infection (32). A significant number of cases of tetanus are cryptogenic. Lesions of the gastrointestinal tract occasionally are implicated as the source of tetanus, particularly in rural environments where the carriage rate of *C. tetani* may be 20 times higher than in urban dwellers (34). It is uncertain whether the tetanus bacillus multiplies in the human and animal intestine. Even if proliferation and toxin release does occur in the alimentary tract, tetanus, unlike botulism, does not arise as a result of absorption from the intestine. Among the small number of cases reported in recent years, older women figure prominently, often having received a trivial gardening wound. Athletes who get injuries from shoe spikes are also candidates for tetanus; injuries on playing fields have been shown to be a factor in the risk of tetanus in males aged 15-44 years (35). The incidence is higher in the non-white than white population of the USA, probably owing to greater exposure and lower immunisation rates (35). Men are less likely to be susceptible, the distribution of cases between the sexes depending largely on occupational habits and the active immunisation of men in the armed forces. The risk of acquiring tetanus in individuals over 80 years of age was more than 10 times the risk in persons aged 20-29 years (35). In agricultural countries the rural population suffers more than the urban, and more in the summer than in the winter. Infections of

the legs and feet are liable to occur in heavily manured areas. Those addicted to narcotic drugs are at an increased risk from tetanus. Of 142 cases in New York in 1955-65, 102 (71.8%) were drug addicts (36).

Maternal tetanus is defined as tetanus occurring during pregnancy or within 6 weeks after termination of pregnancy. This includes postpartum or puerperal tetanus (usually resulting from septic procedures during delivery), post-abortal tetanus (following septic manoeuvres during induced abortion), and tetanus during pregnancy (37). The incidence of maternal tetanus in developing countries is unknown. Most information comes from hospital studies, but because only a small proportion of women in developing countries deliver their infants in hospitals, these data are not representative of the population (38). In recent reviews on maternal morbidity and mortality, tetanus is rarely mentioned as a cause of maternal deaths or disease. Among the developing countries, only India, Malawi, Nigeria and Sudan reported between 2 and 10% of maternal deaths with tetanus as the cause (39). Few cases of tetanus during pregnancy have been reported (40-41).

Nevertheless, puerperal or post-abortal tetanus may be a significant cause of maternal morbidity and mortality in developing countries due to the unhygienic conditions often associated with home deliveries. In India maternal tetanus constituted 4-8.5% of all NNT (42-43). In 72 (18.6%) of the tetanus cases hospitalised for NNT in Ibadan, Nigeria, the uterus was the portal of entry. Of these 52 were due to puerperal tetanus, 12 occurred following an abortion, and in 5 women the infection was contracted through douching the vagina with native soap or using dirty rags as tampons. In the three remaining patients tetanus was associated with carcinoma of cervix, ulcerated prolapsed uterus, or cervical erosion (44). In Lagos, Nigeria, 10% of adult tetanus cases were attributed to maternal tetanus, largely following induced abortion (45). In a recent review it was estimated that between 15,000 and 30,000 cases of maternal tetanus occur each year (37). The latter estimate is considered to be more realistic.

1.2.4 Pathogenesis and pathophysiology

C. tetani produces two exotoxins; TL and TS, of which the latter is responsible for the clinical picture of the disease. Strains producing TS vary from completely non-toxigenic to very highly toxigenic ones. *In vitro,* maximum yields of TS are produced after the growth phase,

and little TS is released before the bacterial cells lyse. TS and the related toxins of *Clostridium botulinum* (46) are some of the most potent toxins known, with a lethal dose in humans below 2.5 ng/kg body weight (47). Their potency is primarily due to their absolute neurospecificity. These toxins are metalloproteases that enter nerve cells and block neurotransmitter releases.

TS is a protein with a molecular weight of 150 kDa, occurring in the intracellular form as a single polypeptide chain. The extracellular form is produced by proteolytic cleavage and comprises of a heavy chain (105 kDa) and an amino-terminal light chain (55 kDa) linked by a disulphide bond. Fragment C, the 50-kDa carboxyl-terminal portion of the heavy chain, has ganglioside and protein binding activities (Figure 1).

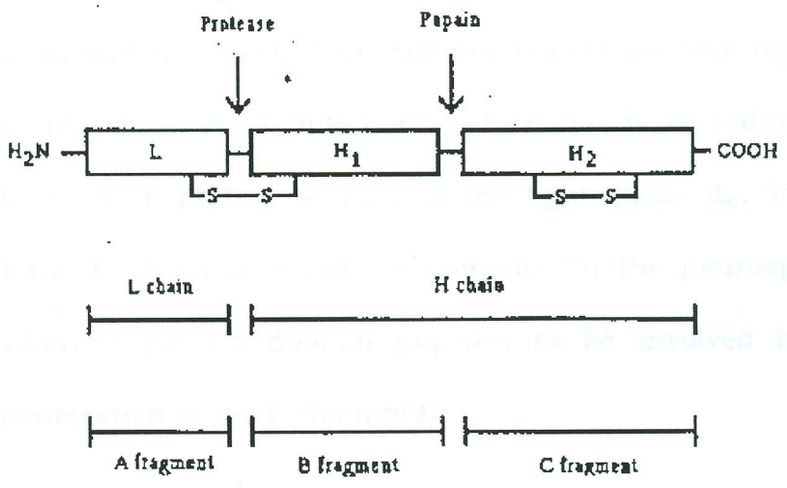


Figure 1: The various nomenclatures of TS fragments (48)

The light chain, a zinc-dependent protease, mediates the blockade of inhibitor release from neurons by proteolysis of synaptobrevin, vesicle-associated membrane protein (VAMP), a constitutive small-vesicle protein (Figure 2).

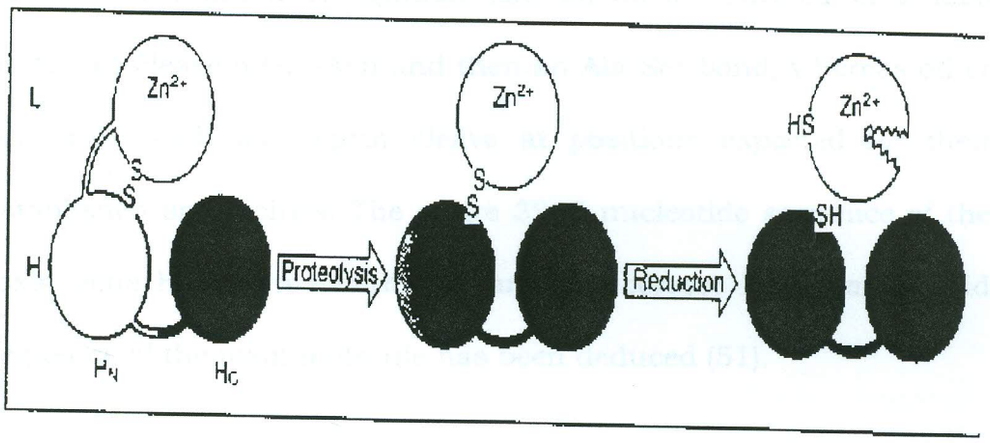


Figure 2: Scheme of the activation mechanism of the neurotoxins responsible for tetanus and botulism (49).

The toxin is depicted as a tripartite structure with the different domains connected by exposed protease-sensitive loops on the basis of biochemical and structural data. The two chains are held together by a single interchain disulphide bond, which has to be reduced to free the Zn^{2+} endopeptidase activity of the light chain (L). The H_c domain of the H chain is mainly responsible for the neurospecific binding, whereas the H_N domain appears to be involved in the neuronal penetration of the L chain (49).

Toxigenesis is plasmid mediated. Various bacterial and animal proteases cleave tetanus toxin; clostridial proteases cleave it within residues 445-461, which is part of a disulphide loop (Figure 3) formed by C-438 and C-466 (50). This region contains two Arg residues but does not contain a recognition site for furin. Purified clostridial proteases cleave a Glu-Asn and then an Ala-Ser bond, whereas other proteases such as trypsin cleave at positions expected for their established specificities. The entire 3945 nucleotide sequence of the toxin gene has been determined and the correct 1315 amino acid sequence of the toxin molecule has been deduced (51).

Evidence accumulated over time shows that the main site of action of TS is the central nervous system to which the TS gains access by ascending the motor nerve (52). TS seems to be taken up only through the neuromuscular nerve endings. It is generally agreed that intra-axonal transport is an important route for the movement of molecules along nerves. The TS is transported along α but not γ motor fibres (53), the reason for the association of TS with only α fibres is not known. TS also binds to preparations of synaptosomal membranes, which have been shown to contain gangliosides (54).

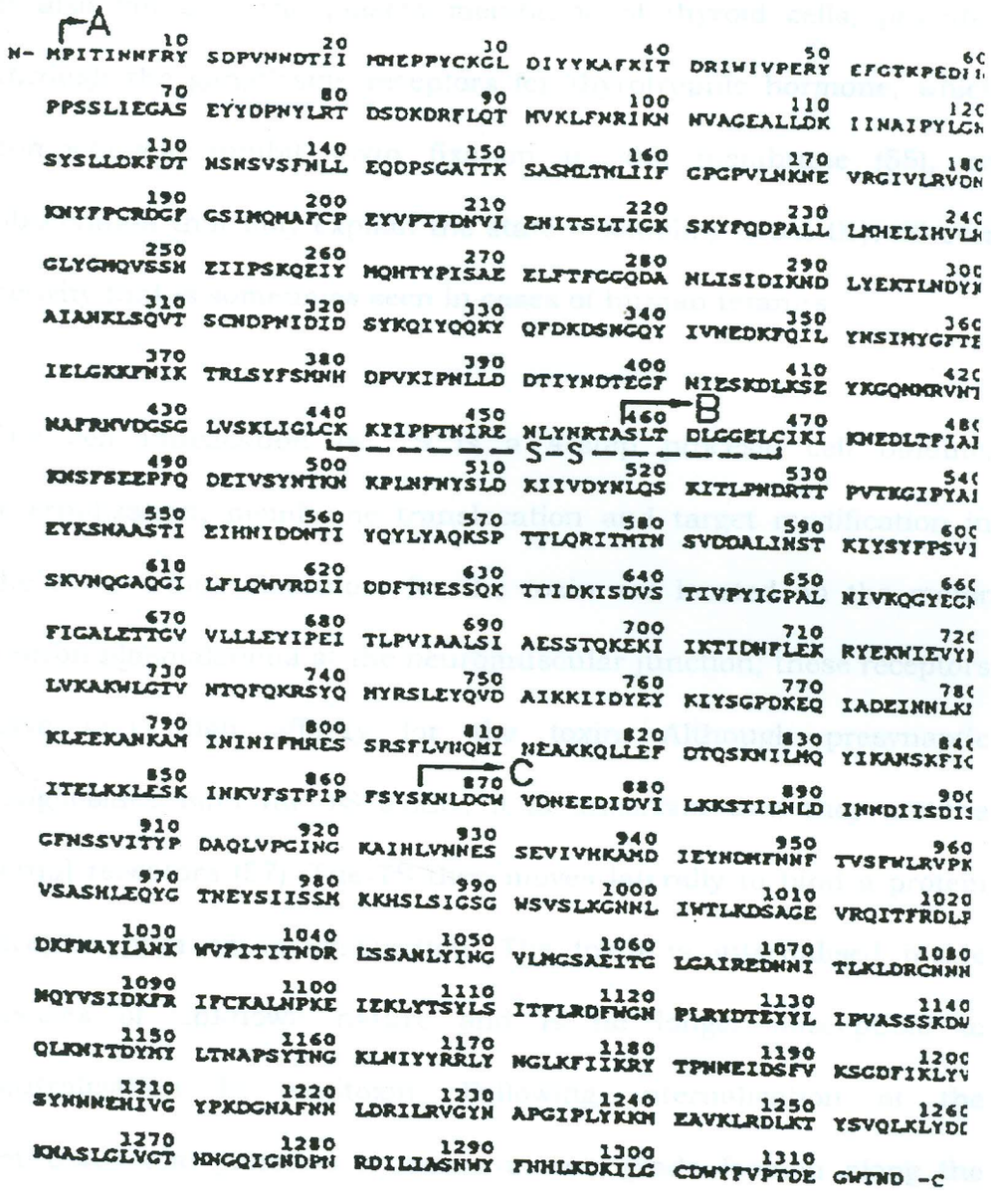


Figure 3: Amino acid sequence of tetanospasmin: A, origin of light chain; B, origin of the amino portion of the heavy chain (H_1); C, origin of the carboxyl portion of the heavy chain (H_2). The N-terminal methionine is removed during processing of the toxin and the amino acids are numbered starting with the adjacent proline. The disulphide bond connecting the light and heavy chains is illustrated (from cysteine 438 to cysteine 466). A second disulphide bond, connecting cysteine 1076 and cysteine 1092, is not shown (51).

It also binds to the plasma membrane of thyroid cells, probably through the ganglioside receptors for thyrotrophic hormone, which competitively inhibit toxin fixation by the membrane (55), an observation that may explain the state resembling acute thyroid over activity that is sometimes seen in cases of human tetanus.

The cell intoxication by TS is a 4-step process: cell binding, internalisation, membrane translocation and target modification in the cytosol (56). Receptors for the toxin are located on the motor neuron plasmalemma at the neuromuscular junction; these receptors have very high affinity for the toxin. Although presynaptic gangliosides bind the TS avidly, it is uncertain that they are the actual receptors (57). The TS then moves laterally to bind a protein receptor prior to internalisation. The toxin is internalised inside vesicles of unknown nature and is no longer susceptible to neutralisation by antitoxin. Following internalisation at the neuromuscular junction it moves in retrograde fashion along the axon. It is discharged in the intersynaptic space and enters the inhibitory interneurones and is confined to a very limited space where it reaches much higher concentrations than at the neuromuscular junction. The H chain seems to be involved in binding to ganglioside

receptors and internalisation into vesicles, and transported retroaxonally to the spinal cord to inhibitory interneurons (56).

TS is thought to cross the vesicle membrane following acidification of the lumen and subsequent transition to an acid conformation that can insert in the lipid bilayer and translocate the L chain. Indirect evidence indicates that an intracellular low pH step is required for intoxication; bafilomycin AI, a specific inhibitor of the vacuolar-type ATPase proton pump, protects spinal neurons from TS. The intracellular activity of TS is due to its L chain. The intraneuronal target that is cleaved is VAMP; the cleavage of a fragment from the N-terminal and the cytoplasm-facing end of the protein (57-58) leads to a sustained blocking of neurotransmitter release. Inactivation of transmitter release occurs after calcium entry during depolarisation (48). Highly specific inhibitors of zinc endopeptidases (49) inhibit the intraneuronal activity of the TS. The long duration of tetanus poisoning may relate to persistence of toxin in the intracellular location because of scarcity of free cytosolic proteases (59).

Local and ascending tetanus from locally injected TS is produced by toxin absorbed at the motor nerve endings and carried to the anterior horn cells. TS interferes at the synaptic junctions with the inhibitory

neurons governing the generation of motor impulses (Figure 4) and it permits motor hyperactivity and hence spasticity in the appropriate muscles.

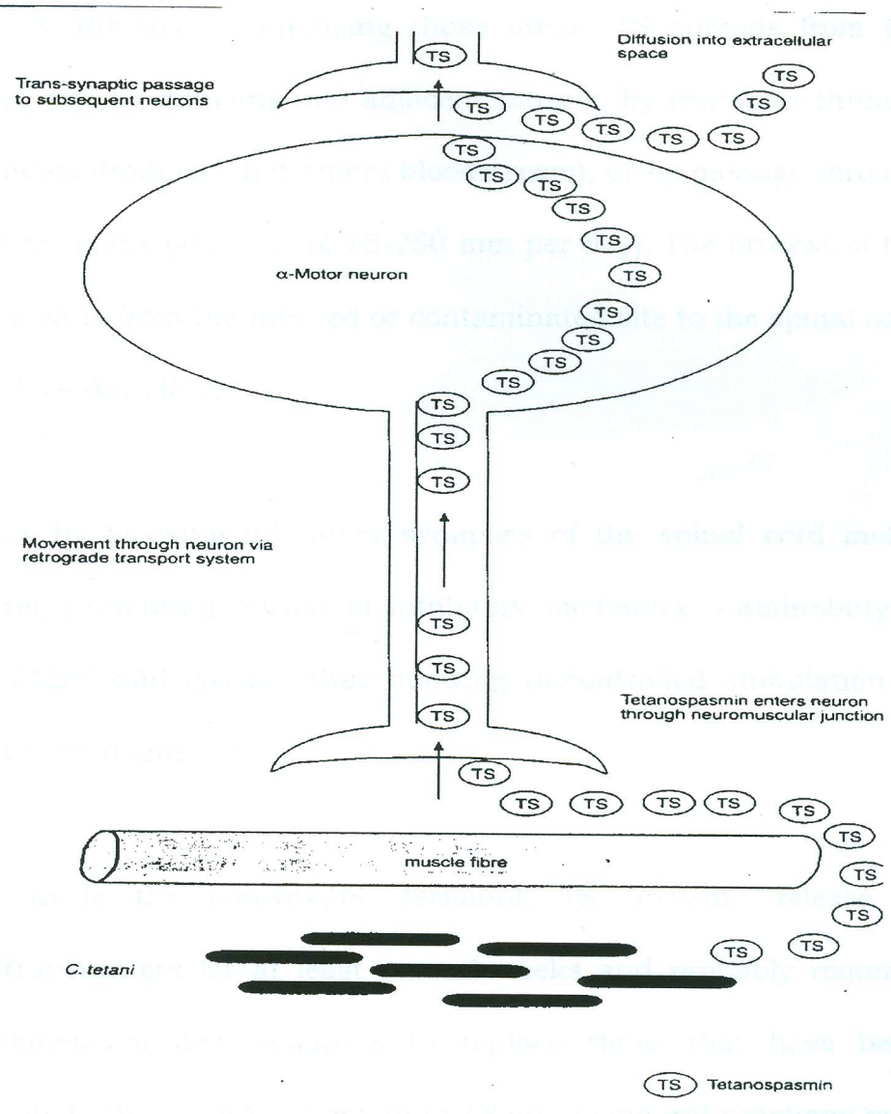


Figure 4: TS diffuses from the site of introduction of the α-motor neuromuscular junction. It moves via the retrograde transport system to the cell body, from which it diffuses out into the synapses and extracellular space within the spinal cord or brain stem (48).

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In descending tetanus the toxin appears to reach the brain stem from the bloodstream also by ascending the motor nerves, the onset of symptoms in the head and neck being accounted for by the shorter length of the nerves supplying those areas. TS spreads from the infected site by diffusing into adjacent muscle, by transport through lymphatics (from which it enters bloodstream), or by passage through the nerve axons (at a rate of 75-250 mm per day). The process of the toxin moving from the infected or contaminated site to the spinal cord takes 2-14 days (33).

TS acts by blocking inhibitory synapses of the spinal cord motor neurons, preventing release of inhibitory mediators, γ-aminobutyric acid (GABA) and glycine, thus allowing uncontrolled stimulation of the muscles (Figure 5).

Once inside the presynaptic terminal, TS prevents release of neurotransmitters for at least several weeks and probably requires development of new synapses to replace those that have been inactivated. Though a local action of TS on myoneural junctions may occur, central action of the toxin appears to account for most of the manifestations of natural and experimental tetanus.

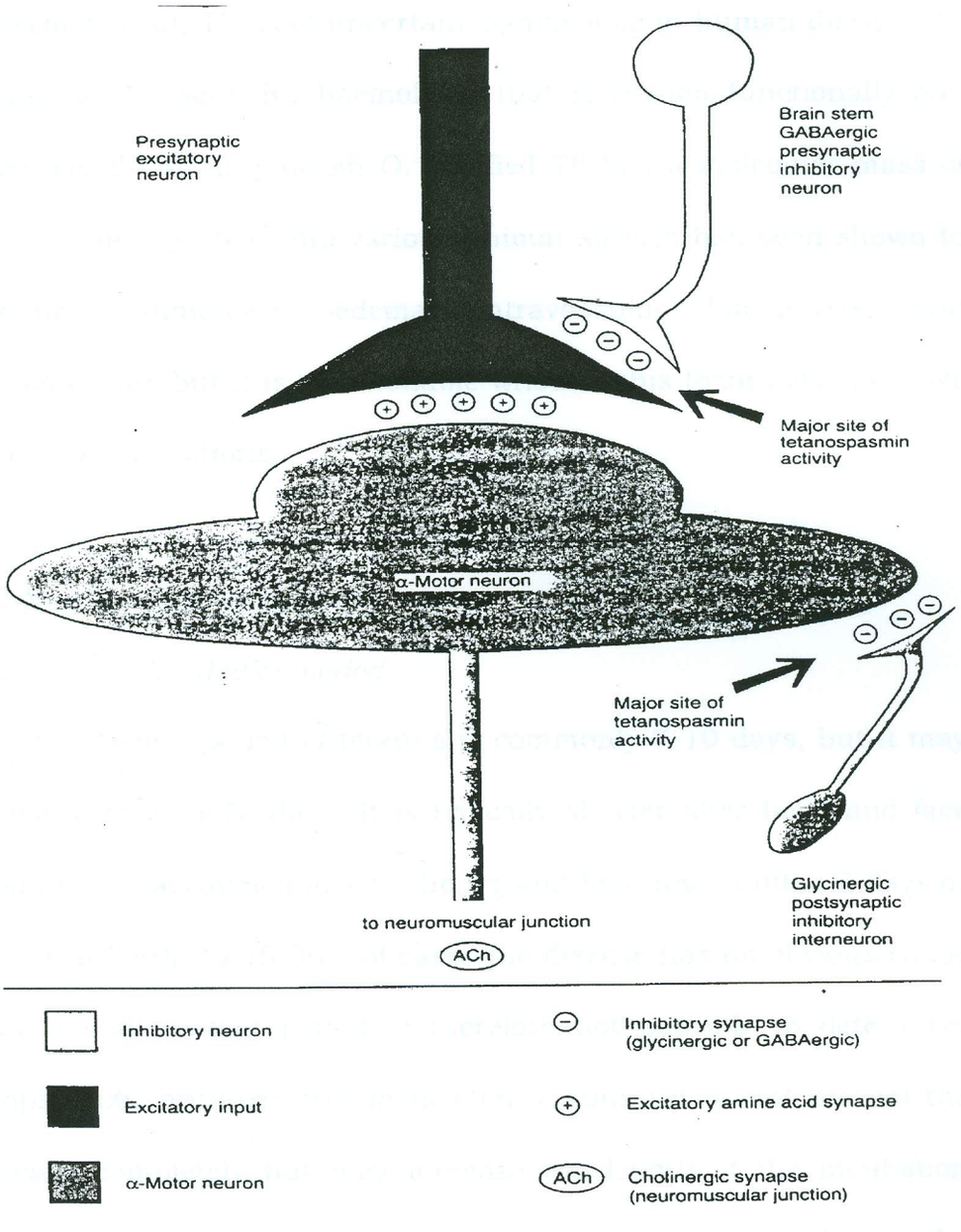


Figure 5: Sites of synaptic activity of TS. The major effects are at the synapse of the α-motor neuron with (1) the glycinergic interneuron, and with (2) the brain stem GABA-ergic neuron. The toxin can also inhibit the release of other transmitters, including those of excitatory amino acid synapses and of the neuromuscular junction (48).

A second toxin, TL, is of uncertain significance in human disease. TL is an oxygen sensitive haemolysin that is related functionally and serologically to streptolysin O. Purified TL has a molecular mass of 48± 3 kDa. Injection into various animal species has been shown to produce pulmonary oedema, intravascular haemolysis, and cardiotoxicity but it is questionable whether this toxin plays any role in *C. tetani* infections.

1.2.5 Clinical aspects

1.2.5.1 Incubation period

The incubation period of tetanus is commonly 7-10 days, but it may range from 2 to 30 days. It is typically shorter after head and face wounding than after injury to the leg and foot, and is often 7 days or less in NT (60). In 10-30% of cases the disease has no obvious cause and the incubation period is therefore not possible to determine. Prophylactic antitoxin treatment after a wound does not prevent the disease completely but may increase the length of the incubation period. Regardless of the clinical type of tetanus, the shorter the incubation period the poorer the prognosis.

1.2.5.2 Period of onset

This period, important to distinguish from the incubation period, is the time from the first symptom to the first reflex spasm.

1.2.5.3 Clinical presentation

Clinical tetanus is classified into four principal types: generalised, local, cephalic and NT. They appear to reflect variations in the site of toxin action, which is predominantly at the neuromuscular junction in some forms and in central inhibitory systems in others (48).

Generalised tetanus is the most common form of tetanus, at least in adults. Three striking elements seen in most patients are trismus (lockjaw), risus sardonicus and opisthotonus. The principal problems are airway obstruction, diaphragmatic compromise, and dysfunction of the autonomic nervous system. Since the toxin already in the α motor neurones is inaccessible to antitoxin, the disease progresses for 10-14 days after antitoxin is administered. Trismus is due to rigidity of the masseter muscles, which limits the patient's ability to open the mouth. Risus sardonicus (a sneering grin) may be subtle and recognised only by family or close friends of the patient. Abdominal rigidity is commonly present at the time of onset of the disease. With severe tetanus, all muscles contract with stronger

muscles overpowering the weaker. The patient is afebrile, conscious and anxious.

The generalised tetanic spasms, so characteristic of the disease are dramatic and extremely painful. Airway obstruction during spasms is common and may lead to apnoea, especially when the diaphragm and abdominal musculature are involved. Diaphragmatic or vocal cord paralysis may occur. Autonomic nervous system involvement with hypersympathetic activity is typically noted towards the end of the first week of illness. This appears to be mediated by disinhibition of sympathetic neurones in the intermediolateral cell columns. Features of this type of involvement include labile hypertension, tachycardia, cardiac arrhythmia, peripheral vascular constriction, profuse sweating, fever, increased carbon dioxide output, increased levels of plasma catecholamines, increased urinary catecholamine excretion, and at times hypotension. Parasympathetic function may be disrupted as well, leading to bradycardia, hypotension and even asystole. Gastric emptying may also be disturbed.

Localised tetanus is manifested by rigidity of the muscles at or near a site of injury. The involved muscles include the prime movers, agonists, antagonists and fixators. This condition may become stable

and chronic (for months) and then resolves spontaneously but most often it progresses to generalised tetanus. Neuromuscular transmission may be affected leading to weakness as well as rigidity. Partial immunity to TS may be responsible for the localised form of the disease and thus prevent progression to generalised tetanus.

Cephalic tetanus affects the face, most often the muscles innervated by the lower cranial nerves. Cephalic tetanus makes up only 1% of all cases of tetanus, but 17% of cases are related to injuries of the head or face (61). Initially, the cranial nerve abnormalities may be unilateral. Peripheral facial nerve weakness related to neuromuscular junction blocking is usually present and is typically the initial event noted. Dysphagia may also occur. When the muscles mediating extraocular movement are involved ophthalmoplegic tetanus results.

NT has two pre-requisites; a mother lacking an immunity and contamination of the umbilical stump with tetanus spores. Although certain obstetrical practices in some cultures may be responsible, most cases are related to unsterile equipment or dressings. The most common presentation is weakness and inability to suck usually noted during the second week of life. Later, classical findings of tetanus such as rigidity, spasm and opisthotonus are seen. A

hypersympathetic state is commonly seen and often leads to the death of the infant with NT. The prognosis is generally grim. Developmental retardation is common in survivors.

The differential diagnosis of tetanus includes strychnine poisoning, dystonic reactions to neuroleptic drugs (these typically involve torticollis and often protrusion of the tongue as well as oculogyric crises), hysteria or psychiatric disorders, hypocalcaemia or alkalotic tetany, the stiff man syndrome, status epilepticus, meningitis, dental or other infection leading to trismus, and dislocation of the mandible with simulated trismus. Rapid improvement of symptoms following intravenous benztropine or diphenhydramine indicates a dystonic reaction to a drug. Strychnine acts as a direct antagonist at the glycine receptor and thus produces the same lack of presynaptic inhibition seen in generalized tetanus. Lack of abdominal rigidity between spasms is probably more common in strychnine poisoning.

1.2.5.4 Diagnosis

The diagnosis of tetanus is made clinically and depends on clinical awareness. Absence of a precipitating wound does not exclude the diagnosis. Patients without an apparent source of infection or wound contamination should be questioned and examined for parenteral



drug abuse, otitis media or externa, rectal or vaginal instrumentation, and minor surgical procedures (48). Laboratory confirmation is often difficult, because the site of infection may be very small or inapparent (48).

Microscopic examination should be made, where possible, of pus or necrotic material in the wound, and special note taken of bacilli with round, terminal spores. The organisms may be present in such small numbers that they are overlooked.

Cultural methods have also been recommended. The pus or wound scrapings, or tissue taken from a necrotic focus in the wound, should be plated on blood agar for anaerobic incubation and seeded into one or more bottles of cooked meat medium. One bottle should be incubated unheated and the remainder after heating to 80°C in a water bath for various times from 5 to 20 min with the aim of killing non-spore forming bacilli and leaving *C. tetani* spores unharmed. Enrichment of the wound specimen in broth containing 1:100,000 crystal violet is said to be effective, and ascorbic acid polymyxin thioglycollate broth has also been used for this purpose. The inoculated liquid media are incubated at 37°C and subcultured on half of a blood agar plate daily for at least 4 days. The plates are

incubated anaerobically and examined for the swarming edge of growth of *C. tetani.* Colonies on blood agar are 4-6 mm in diameter, flat, translucent and grey with a matt surface and are surrounded by a narrow zone of β–haemolysis. The colony margins are irregular and rhizoid. The incubated cooked-meat medium broth should also be heated at 80°C and samples plated at short time intervals. A blood agar plate containing 4% agar to minimise swarming, one half of which has been treated with antitoxin, may be seeded on each half with growth presumed to be *C. tetani* and incubated for 2 days at 37°C. Colonies haemolytic on the untreated half, but not on the antitoxin half, are almost certainly toxigenic *C. tetani.* However, it should be appreciated that clinical decisions should not be based on culture results since valuable time would be lost, cultures are not uncommonly negative, recovery of the organism in an immune host is of no meaning, and some strains of *C. tetani* are non-toxigenic.

The toxigenicity of the bacilli is confirmed by demonstrating the production of TS in a toxin-antitoxin neutralisation test. Two mice, one unprotected and the other given, 1-2 hours earlier, a subcutaneous or intraperitoneal injection of 1000 units of tetanus antitoxin, are challenged by a subcutaneous injection in the hind leg of 0.5 ml of a 48 hours cooked-meat broth culture of the isolate

mixed with 2% calcium chloride. The protected mouse remains well; the unprotected animal shows typical ascending tetanus. Electrophysiological studies of patients with localised tetanus are said to give characteristic results that would be helpful diagnostically. The cerebrospinal fluid is normal in tetanus although elevation of the protein level may be seen. Serum and urine should be examined for the presence of strychnine in suspected cases. Even when strychnine intoxication is likely, it would be wise to continue to consider the diagnosis of tetanus (48).

1.2.5.5 Treatment

The clinical management of tetanus depends on the neutralisation of any unfixed toxin by antitoxin, the control of further bacterial proliferation and toxin production by antimicrobial therapy, wound toilet, excision of infected or devitalised tissue, and removal of foreign bodies and the provision of constant nursing care and supportive treatment, supplemented as necessary by anticonvulsive therapy and, in severe cases, by tracheostomy and intermittent positive-pressure ventilation (48). Airway and ventilatory control are critical. Tetanus requires aggressive intensive care. Any patient, regardless of age or severity of tetanus, has a chance for full recovery if management is optimal. In one study, patients over 70 years of age

fared as well as those under 70; this emphasises the importance of aggressive care in the intensive care unit setting, no matter how old the patient is (62).

Antispasticity drugs, particularly benzodiazepines, play an important role. Autonomic dysfunction is increasingly recognised as a serious problem; labetalol, clonidine, morphine, or epidural anaesthesia can usually manage it. It is important to remember to actively immunise all tetanus patients upon recovery to prevent recurrence. Remarkable quantities of protein and carbohydrate are required to maintain a positive nitrogen balance because of the muscle activity and excessive autonomic output. There is no evidence that corticosteroid therapy is beneficial in tetanus and it certainly might lead to complications.

Antitoxin neutralisation of toxin that has not yet entered the nervous system shortens the course of tetanus and probably reduces its severity. Human tetanus immunoglobulin (HTIG) is the preferred antitoxin preparation and should be given as soon as possible after the patient's spasms are controlled. Effectiveness of antitoxin has been demonstrated previously (63). A dose of 500 International units (IU) is as effective as the much larger doses originally recommended. Intravenous administration is probably preferable to intramuscular in

areas where the HTIG is suitable for intravenous use. Intrathecal therapy has also been considered desirable for use (64) but a meta-analysis of reports of such therapy concluded that such therapy is not of proven benefit (65). In a few cases of tetanus, there has been a recurrence of spasms within a few days after an initial response to antitoxin. A further dose of antitoxin may be of value in such cases (66).

The first dose of antitoxin and antimicrobial drugs and control of patient's spasms should precede surgical debridement of the wound. Surgery should be performed under local anaesthesia. The course of tetanus is not influenced by wound care and surgery so that there is still need for antitoxin.

C. tetani is susceptible *in vitro* to a number of antimicrobial agents including penicillins, cephalosporins, imipenems, erythromycin and other macrolides, metronidazole and tetracycline. Although antimicrobials may be considered of questionable value in a disease process that is intoxication, many in the field have recommended penicillin (48). However, it must be kept in mind that penicillin is a centrally acting GABA antagonist and it might therefore act in concert with tetanus toxin to worsen the hypertonic symptoms and perhaps

decrease the efficacy of benzodiazepines. A previous study compared metronidazole 2 g per day orally to procaine penicillin (4.5 million units i.m. per day); the metronidazole-treated patients showed significantly less progression of their disease, shorter hospitalisation period, and higher survival rate (67). This might mean that metronidazole was better than penicillin or, alternatively, that penicillin was detrimental. Metronidazole (and clindamycin) were shown to suppress toxin synthesis in *Clostridium perfringens* in an experimental model of gas gangrene (68). In any case, antimicrobial therapy must never be used as a substitute for antitoxin therapy and proper wound care.

The airway must be protected from the beginning because it frequently becomes occluded during tetanic spasms. An endotracheal tube may be placed with the patient under sedation and neuromuscular blockade and it is recommended at the same time that a soft feeding tube be placed into the stomach via the oesophagus. Tracheostomy may be required later as the endotracheal tube itself may serve as a stimulus for further spasms. Careful pulmonary hygiene and management of ventilation will help minimise the incidence of pneumonia and pneumothorax, which are common problems in these patients (48).

Benzodiazepines are the agents of choice for control of rigidity and spasms in tetanus patients; these drugs are GABA agonists and therefore indirectly antagonise the effect of toxin. These drugs, however, do not restore glycinergic inhibition. The greatest experience is with diazepam, but lorazepam may be more desirable because of its longer duration of action. Very large doses may be required and parenteral preparations contain propylene glycol that may lead to metabolic acidosis. Use of a feeding tube for administration of these agents avoids this problem. Another approach is to use midazolam by continuous intravenous infusion at a rate of 5-15 mg per hr since it has a short half-life and it does not require propylene glycol as a solvent. Withdrawal symptoms may follow discontinuation of benzodiazepines, so doses should be tapered over a period of two weeks. Tachyphylaxis may lead to increased dosage requirements over time. Just enough benzodiazepine compounds should be given to prevent spasms and induce adequate sedation. Intrathecal baclofen, a GABA agonist, is another therapeutic option but has no advantages over the benzodiazepines, except in less developed countries since it may reduce the need for mechanical ventilation. There may be a place for propofol, a non-barbiturate sedative, but it should not be used as a single agent because it does not have GABA agonist activity (48).

If GABA agonists cannot control the effects of tetanus, neuromuscular junction blockade is necessary. Vencuronium, by constant infusion, or pancuronium, by intermittent injection, are effective for this purpose. Patients should also receive sedation while getting neuromuscular blockade; this is important to keep them from perceiving their situation and to help block out the memory of what they have been going through. Autonomic signs cannot be relied on to indicate inadequate sedation because patients may also have sympathetic, and sometimes parasympathetic dysfunction; accordingly, electroencephalographic monitoring is necessary.

The therapy of choice for the hypersympathetic signs and symptoms of tetanus is combined α– and β–adrenergic nervous system blockade with labetolol. β-adrenergic blockade should not be used alone because of the risk of hypertension. Clonidine is a useful alternative to labetolol and morphine can be effective. Epidural anaesthesia is also effective. Hyperfunction of the parasympathetic nervous system is rare. It may lead to bradycardia or asystole in which case a pacemaker may be required.

The management of tetanus-prone wounds depends on clinical features (Table II).

Table II: Clinical features of tetanus-prone wounds (69)

Clinical features	Tetanus-prone wounds	Non-tetanus-prone wounds
Age of wound	>6 h	= or <6 h
Configuration	Stellate wound, avulsion, abrasion	Linear wound
Depth	>1 cm	=or <1 cm
Mechanism of injury	Missile, crush, burn, frostbite	Sharp surface (e.g. knife, glass)
Signs of infection	Present	Absent
Devitalised tissue	Present	Absent
Contaminants (dirt, faeces, soil, saliva)	Present	Absent
Denervated and/or ischaemic tissue	Present	Absent

The perfusion and oxygenation of tissue adjacent to a wound are likely to be impaired for some days and the oxidation-reduction potential (*E*h) within a wound is reduced until healing is established. The impaired oxygenation is exacerbated by the presence of a foreign body or of devitalised tissue. The growth of aerobic and facultative organisms within the wound makes additional demands on the oxygen supply, further reducing the *E*h and encouraging the germination of anaerobic spores with toxin production. Wounds requiring special attention are lacerations, stabs, bites (animal and human), wounds that are deep and penetrating, and those that receive delayed attention or are heavily contaminated with agricultural or horticultural materials or soil. In general, such wounds should not be sutured; packing, with consequently delayed primary closure, or grafting may be preferable, and frequent follow-up inspection is necessary. Prompt and thorough surgical attention is of paramount importance. Foreign materials and devitalised tissue must be removed and the wound edges excised if the tissues are not viable.

Immune status should be determined if possible, but when a patient is unconscious and there are no records or other reliable data it is advisable to assume that immunity is lacking. Protective immunity depends upon a full course of adsorbed TT completed or boosted within the previous 5-10 years. If the patient has received a full course of TT or a booster injection within the previous 5 years, a further TT injection should not be given unless the risk of tetanus is assessed as high; unnecessary TT injections may lead to painful local reactions. If 5-10 years have elapsed since the last full course of TT or booster injection, a further booster injection of adsorbed TT is advisable. If the patient has not been immunised, or if the matter is in doubt, the first dose of a full course of adsorbed TT injections should be given immediately, along with an injection of antitoxin. The prophylactic dose of HTIG is 250 units intramuscularly. Adsorbed TT should be given at the same time as HTIG to start a course of active immunisation in a non-immune patient. TT should be given with a separate syringe and injected into a contra lateral site. The second and third injections of adsorbed TT are given at monthly intervals (48).

1.2.5.6 *Complications*

Hypoxia is one of the common complications of tetanus despite the general availability of intensive care units in developed countries. It is due to delay or inadequate treatment of respiratory problems. Cardiovascular problems related to hypersympathetic activity are still seen. Other complications include phrenic and laryngeal nerve palsies, other mononeuropathies, rhabdomyolysis, acute renal failure, myositis ossificans circumscripta, and vertebral compression fractures. Serious psychological after effects are seen in 40% of patients and many patients feel that their general health has worsened after recovery from tetanus (70).

1.2.5.7 *Prevention and control*

The prevention of tetanus depends primarily on immunisation and on prompt and adequate attention to wounds. Hygienic precautions in midwifery are important in the prevention of NT. An effective programme of active immunisation can largely control tetanus occurring in children and adults in the general population. Active immunisation with TT is one of the most effective preventive measures in medical practice and passive immunisation is now readily carried out with HTIG.

The elimination of tetanus is an attainable goal. Strategies to control and eliminate tetanus should take into consideration differences between NT and NNT. NT may be eliminated by combining two approaches: (a) increasing immunisation coverage among women of childbearing age, especially pregnant women, with TT, and (b) improving maternity care, with particular emphasis on increasing proportion of deliveries that are attended by trained personnel (12). In 1989 WHO declared its commitment to the global elimination of NT by the year 1995 by reducing its incidence to less than one case per 1000 live births for each district of every country in the world (71). To achieve and maintain NT elimination the percentage of children protected at birth through TT immunisation of mothers or through clean delivery should be above 80%. Improvements in clean cord care practices, as well as the establishment of surveillance systems for early detection of NT cases, were deemed necessary (71). In Africa region, coverage with TT 2+ increased from 25% in 1989 to 40% in 1993 (71).

Although progress has been made towards eliminating NT as a public health problem, present resources and commitments must be increased and activities greatly accelerated if the 1995 goal is to be achieved by all countries. WHO/EPI Global Advisory Group identified

four constraints to NT elimination: (1) insufficient funds to purchase TT in selected high-risk countries, (2) lack of adequate health care infrastructure in many countries, resulting in limited TT vaccination activities and poor access to clean birth practices, (3) civil unrest in some high-risk countries and (4) high levels of NT underreporting (71).

To reach the global elimination goal for NT, each country must identify areas where the incidence rate is higher than 1 per 1000 live births, coverage levels are low, or there is limited access to clean deliveries or trained birth attendants. These high-risk areas must be targeted for intensified vaccination efforts, including the use of mass vaccination campaigns (71). In addition, surveillance activities in all areas must be strengthened. Finally, because NT is not a communicable diseases, and *C. tetani* can not be eradicated from the environment, ensuring long-term elimination of NT will require the development of adequate health care delivery systems to reach those at greatest risk-infants of poor women residing in rural areas in developing countries.

For control of NNT, immunisation of the whole population is essential. Other measures such as prevention of trauma and

preventive care of the wounded are useful but not sufficient to control tetanus. There are three main elements in the strategy to control NNT: (a) active immunisation of the whole population with the main emphasis on primary immunisation of infants and children and on properly spaced booster doses for adults to maintain immunity throughout the whole life (b) wound care and prophylactic measures in wounded persons and (c) health education to make the public aware of the risk of tetanus and the importance of its prevention (31).

1.3 TT immunisation

1.3.1 TT immunisation schedule

In most developing countries the immunisation schedule for infants recommends three primary doses of diphtheria-pertussis-tetanus (DPT) vaccine at 6th, 10th and 14th week of age. The EPI recommends starting the primary series of DPT vaccine early to achieve early protection against pertussis and tetanus. The primary series should also be completed early, as more infants will be immunised when they attend child clinics.

In most developing countries routine immunisation in infancy has been implemented since the middle 1980s. The cohorts of women entering childbearing age in the 1990s were not immunised with TT

in their infancy or adolescence, and they represent a large group of susceptible persons. Since 1987, the EPI Global Advisory Group recommended TT immunisation for all women of childbearing age (72). For previously unimmunised women of childbearing age in developing countries WHO recommends a five-dose TT schedule through maternal and child health (MCH) clinics, school health programmes or special outreach services (Table III).

Table III: TT immunisation schedule for women of childbearing age by the EPI (4)

Dose	When to give	Expected duration of immunity (yr.)
TT 1	At first contact or as early as possible in pregnancy	None
TT 2	At least 4 weeks after TT 1	1-3
TT 3	At least 6 weeks after TT 2	5
TT 4	At least one year after TT 3 or during subsequent pregnancy	10
TT 5	At least one year after TT 4 or during subsequent pregnancy	Whole childbearing age

TT, tetanus toxoid

The first three doses are basic. In women who are not immunised the first dose should be given as early as possible during pregnancy. Usually, three doses of DPT vaccine received as an infant are counted as equivalent to two doses of TT received as an adult. Women who can provide evidence of 3 doses of DPT vaccine in childhood will need only two doses of TT in the index pregnancy and one additional dose of TT in the next pregnancy.

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It has previously been reported that hyper-immunisation with TT occurs in the range of 11-17% among tetanus immune subjects (73-74). Thus proper documentation of TT immunisation is essential because it is important in guiding the spacing of subsequent booster doses without hyper-immunising previously vaccinated individuals. Guidelines for TT immunisation of women who were previously immunised are summarised in Table IV.

Table IV: Guidelines for TT immunisation of women who were immunised in the past (4).

Age at last vaccination	Previous immunisations	Recommended immunisations: At present contact/ Pregnancy	Recommended immunisations: Later (at intervals at least one year
Infancy	3 DPT	2 doses of TT*	1 dose of TT
Childhood	4 DPT	1 dose of TT	1 dose of TT
School age	3 DPT + 1 DT/Td	1 dose of TT	1 dose of TT
School age	4 DPT + 1 DT/Td	1 dose of TT	None
Adolescence	4 DPT + 1 DT at 4-6 years + 1 TT/Td at 14-16 years	None	

TT, tetanus toxoid; DPT, diphtheria-pertussis-tetanus; DT, diphtheria-tetanus; Td, tetanus-diphtheria; *At least 4 weeks between doses

Some developed countries recommend the routine use of tetanus-diphtheria (Td) vaccine every 10 years to maintain tetanus immunity throughout life. Previous studies suggest that revaccination every 20 years may be sufficient to maintain immunity (5, 75). However, some studies have reported the possibility of an insufficient response to a booster dose given 17-20 years after the primary series (76). The booster response may vary individually, and a pronounced dispersion

of antibody levels can be expected with time following a primary series. Giving a dose of Td vaccine to wounded persons is an important measure to maintain immunity against tetanus in adults. Nevertheless, such emergency booster doses are needed no more often than once every 10 years.

1.3.2 Effectiveness of TT

TT is a monovalent vaccine obtained after inactivation of TS by formaldehyde. TT vaccine became first available in 1938. It is used as a vaccine either singly for adults or in a combined form (DPT) for children. TT is stable and can remain potent if stored at room temperature for months or at 37°C for a few weeks without a significant loss of potency (77) thus, making it suitable for the field conditions existing in developing countries. In most studies, TT vaccine efficacy ranged from 80 to 100% (78-81).

1.3.3 Safety of TT

TT is considered to be safe for use in pregnancy and it has so far not shown to pose any risk to the mother or foetus (82). In the USA, tetanus and diphtheria toxoids are the only vaccines recommended for administration during pregnancy (83-84). Local minor reactions such as erythema and wheal have been reported in a small

proportion of vaccinees, usually in individuals immunised with multiple doses in the past and showing high antibody levels at the time of injection (85-87) and are due to local immune complex mediated reactions due to antibody excess (Arthus reaction). Some study reported hypersensitivity reaction due to induction of specific IgE response following booster immunisation (88). Overuse of TT can also lead to polyneuropathy (89-90) with an estimated incidence of 0.4 cases per million doses of TT (91).

1.3.4 Reported failures of TT immunisation

There are a number of reports from India (92-93), Egypt (94), and Nigeria (95-96) describing the failure of TT to prevent NT in infants of immunised women. This problem becomes increasingly prominent as immunisation coverage rises, since the proportion of mothers of NT cases in infants of immunised mothers will increase, even with high TT vaccine efficacy. There are several reasons that could explain this observation, including inaccurate immunisation schedule, inappropriate immunisation schedule, low potency vaccine, poor maternal immune response, inadequate placental transfer and excessive toxin exposure.

1.3.5 Factors influencing the response to TT

Indications from some studies suggest that diseases such as malaria and infection with human immunodeficiency virus (HIV) may influence response to TT. A decreased response to TT has been observed in individuals infected with HIV (97). Since loss of responsiveness to soluble antigens like TT is one of the first immunologic consequences of HIV infection (98), HIV infected patients may not become immune after TT administration. Both HIV-1 and HIV-2 interfere with the proliferation of TT-specific T lymphocytes *in vitro* (99), raising further concern that some HIV infected patients may become rapidly unprotected even if their previous immunisations were adequate. Abnormalities of the immune response become more severe with advancing disease (100). In HIV-infected individuals the amount of antibodies formed after vaccination with T-cell-dependent recall antigens, such as TT, is impaired in proportion to the number of CD4+ T cells and to the *in vitro* proliferative response of T lymphocytes to anti-CD3 monoclonal antibodies (101-102). A recent study reported that the avidity of the IgG1 anti-TT antibodies formed by HIV-infected adults was within the range for healthy controls, irrespective of their CD4+ T-lymphocytes (103). However, studies in Africa and Haiti showed that HIV infected women had the same levels of tetanus antibody after two doses of TT

given during pregnancy as seronegative women (104). In spite of the reported decrease in response to TT in some HIV infected people, this vaccine, like all other inactivated bacterial and viral vaccines, is recommended in HIV infected individuals.

1.4 Immunity to tetanus

1.4.1 Naturally acquired immunity

It has been proposed that naturally acquired immunity against tetanus can be induced by a sublethal dose of tetanus toxin or by fragments of tetanus toxin released from tetanus bacilli located in the digestive tract, as a result of ingesting tetanus spores (105-106). Some studies reported finding tetanus antitoxin in the sera of persons who claim not to have been immunised with TT (105-107). Proponents of the naturally acquired immunity hypothesis believe that naturally acquired immunity to tetanus occurs in developing countries due to the presence of tetanus bacilli in the intestinal tract. Thus, the theory of naturally acquired immunity against tetanus has been based on the observed colonisation of the intestinal tract with tetanus bacilli and spores in many animals including human beings as well as the observed rise in tetanus antibody level in experimental animals following ingestion of a suspension of tetanus spores or tetanus toxin. However, evidence obtained from other studies (108-

110) does not support this theory. It is therefore conceded that occurrence of naturally acquired immunity to tetanus is quite uncertain and perhaps of no epidemiological significance in the prevention and control of tetanus.

1.4.2 Immunity after infection

There is no residual immunity against tetanus following recovery from the disease. This is a consequence of the fact that the lethal dose of tetanus toxin is far below the optimal concentration required to stimulate antibody production. Immunity to tetanus can only be achieved through vaccination with TT and this should be given to both adults and children including individuals who have suffered from clinical tetanus (4).

1.4.3 Immunity following TT immunisation

Tetanus antitoxins in vaccinated individuals are present in the blood stream as well as in extravascular spaces. Antitoxins to TT are very effective in neutralising TS, thus provide protection to the host against tetanus. Antitoxins to TS are able to neutralise even though they do not bind to the ganglioside-binding site of the TS (111). Four antigenic determinants on both TS and formol TT have been described, all of the corresponding 4 anti-toxins were capable of

neutralising TS *in vivo* (112). The role of cell-mediated immunity to tetanus is unknown.

Vaccination with TT stimulates the immune system to produce IgG antitoxins against TS. In a previous study it was observed that multiple booster doses of TT may not only enhance serum antitoxin, but could also lead to an ineffective immune response (113).

1.4.4 Placental transfer of tetanus antitoxin

IgG antibodies are transferred across the placenta from mother to foetus. This transfer process is believed to be mediated by receptors on placental syncytiotrophoblast specific to the Fc portion of the IgG molecule and the transfer priority of the IgG subclasses seems to be in the order of IgG_1=IgG_3>IgG_4>IgG_2 as reviewed recently (114). The order of transfer of the IgG subclasses has been reported to be similar in both Caucasians and Africans (115) and does not therefore appear to be influenced by racial or geographical factors.

Progressive rise of foetal IgG antibody level occurs from the fourth month of gestation until term, and the bulk of the transfer of IgG occurs from 26th week to birth (114). In the neonate, antibodies to TT decline at a rate similar to that of antibodies to many other infectious

agents, and it has been estimated that about 80% of the passively acquired anti-toxin is still present in the newborn circulation at the age of one month. A previous study has documented that the foetal immune response to maternal TT immunisation does not confer active protection against tetanus (116). However, some studies have reported a higher cord/maternal ratio of tetanus antibodies in European when compared to Africans (117-118). It has been proposed that this difference could be due to the high immunoglobulin levels often found in African mothers exposed to multiple antigenic stimuli, since some studies have shown that placental transfer of tetanus antibodies is more efficient in the low range of maternal total IgG than in the high range of maternal total IgG (4).

The interval between doses of TT given to pregnant woman influences the level of tetanus antitoxin in cord serum. Optimal immune response is achieved when the intervals between the TT doses are long. Unfortunately, in developing countries many pregnant women report to antenatal clinics when pregnancy is already advanced, hence the interval between the first and second dose of TT is less than optimal. The cord/maternal ratio of tetanus antibodies increases, as the interval between the second dose and delivery is

prolonged (119). In order to assure long enough intervals between TT doses it is necessary to start immunisation as early as possible in pregnancy.

1.4.5 Protective level of tetanus antitoxin

Attempts have been made to establish the level of antitoxin that provides protection against tetanus. These attempts have failed to establish this hypothetical level due to several problems including lack of knowledge on the real dose of TT that stimulates protective antibody response and methodological problems associated with antitoxin determination using *in vitro* techniques such as PHA, ELISA and RIA. The amount of circulating antitoxins needed to ensure complete immunity against tetanus is not known for certain. Therefore, there is no absolute protective level of antitoxins and protection results when there is sufficient toxin-neutralising antibody in relation to the toxin load (66).

Antitoxin activity is expressed in International units (IU) and the potency definition is primarily based on the results obtained by *in vivo* toxin neutralisation assays in mice. A convenient test dose of toxin is the smallest quantity which, when mixed with one IU of the standard antitoxin (or a fraction of one unit), causes clearly

recognisable paralysis within 4 days but does not cause significant suffering; mild but definite paralysis of the hind leg is suitable for this purpose. This Lp dose of toxin is preferable to the L+ dose i.e. that which kills the animal by the fourth day, as it minimises the illness to which the mice are subjected (120-122). Tetanus antitoxin level of 0.01 IU/ml of serum is generally considered the minimum protective level based on studies that correlate antitoxin level with tetanus symptoms or death in experimental animals.

While this level is generally believed to represent the optimal protective concentration, some few cases have been reported where clinical tetanus occurred in persons with antitoxin levels higher than the protective level (66, 113, 123-124). Overwhelming toxin level as well as presence of non or poorly neutralising antibody subclasses in the cases could explain the occurrence of disease in such individuals who have the protective antitoxin level.

A previous study showed great discrepancies between indirect ELISA and *in vivo* assay levels of antibodies, especially in the low range of titres. The lowest ELISA value reliably predictive of protective antibody level in the serum has been found to be 0.16 IU/ml (125).

1.4.6 Antibody avidity

Avidity refers to the combined functional affinities of antibodies formed during a polyclonal humoral immune response whereas affinity refers to a measure of the binding constant of a single antigen-combining site with a monovalent antigenic determinant. Antibody affinity is an important parameter of the immune response, which plays a major role in governing the biological effectiveness of antibody (126). Association between low antibody affinity and anti-TT IgG_4 subclass in man has been reported (127). Results from another study suggested that IgG_4 antibodies to TT are of lower functional affinity than IgG_1 antibodies and that, in addition, IgG_4 responses to TT display a more restricted affinity heterogeneity (128). Indeed, a tendency to produce a predominantly low affinity antibody response may result in defective antigen clearance and predispose to immune complex disease (128).

IgG and other classes of antibodies produced in the secondary response tend to be of much higher affinity than in primary response. The avidity and affinity of IgG are initially low after primary antigenic challenge, and it matures slowly within weeks and months (129-130). High-avidity antibodies are superior to low-avidity antibodies in their bactericidal activity (131-133). It has been argued that qualitative

characteristics of the antibodies, such as avidity and opsonophagocytic activity, should be considered in evaluating the efficacy of vaccines such as pneumococcal vaccine (131, 134-136).

1.4.7 Duration of immunity

The degree and duration of immunity increases with the number of TT doses given (137). Three primary doses of DPT vaccine induce an antibody level above the minimum protective level of 0.01 IU/ml as determined by the *in vivo* neutralisation assays, with a mean level of between 0.1 and 1 IU/ml (138-141). Immunisation of an infant with three doses of DPT vaccine provides tetanus immunity for one to three years. Reinforcing the infant immunisation with a fourth dose given between 15th and 24th month of life prolongs tetanus immunity until 6 or 7 years of age. A fifth dose of TT at school entry will provide immunity until 17 or 18 years of age. An additional dose at school leaving or during military service will assure sufficient immunity for at least two more decades (75, 142-143). In adults, one dose of TT ensures little, if any protection. Two to four weeks after the second TT dose, the mean level of tetanus antitoxin usually exceeds the minimum protective level of 0.01 IU/ml. A third dose of TT induces antitoxin production with mean levels of between 1 and 10 IU/ml and immunity lasts for at least five years (4). However, some study

demonstrated a limited-dose regimen of maternal TT that provides significant protection for up to 12 or 13 years (144).

1.4.8 Tetanus immunity by age and sex

Only immunised persons are protected against tetanus; those not immunised are at risk of tetanus from contaminated wounds, including the umbilical stump. With an increasing proportion of women immunised with TT, they will be immune and more infants will have protective levels of passively acquired tetanus antitoxin as demonstrated by previous studies (145-150). However, the acquired passive immunity has also been shown to suppress the development of active immunity to the first dose of DPT given at 3 months but not the two subsequent doses given at 4 and 6 months of age (151). Absence of suppression has also been documented in a previous study (147). These observations suggest that the booster doses of DPT vaccination are essential in stimulating the production of optimal level of antitoxin for the protection of the infant against tetanus.

In addition to dose dependence, tetanus immunity is age related; the level of tetanus antibodies is highest in persons under 30 years of age. Immunity subsequently decreases with increasing age and falls to the lowest levels in those aged over 50 years (152-157). Studies

done in developed countries revealed 72-100% tetanus immune blood donors (158-160). There are geographical differences in tetanus immunity levels. In Italy the prevalence of non-immune response was higher in the south than north. This probably reflects a difference in health care delivery (161-162).

There is a clear difference between levels of tetanus immunity in men and women. In industrialised countries, more men are immune than women, probably due to additional immunisations given during military service or professional activities (35, 160, 163). More than 80% of military recruits had acquired protective levels of tetanus antitoxin through past immunisation (162, 164-166). Women in industrialised countries, especially older women, often belong to the most susceptible groups (167). In India, a country where TT is given to women of childbearing age, and especially to pregnant women to prevent NT, the immunity status of women is better than that of men (168).

1.4.9 Impact of TT immunisation programme

1.4.9.1 Impact on immunity

Limited serological data from developing countries show the impact of immunisation programmes on the pattern of tetanus immunity. On

the Turks and Caicos Islands, where an immunisation programme for children had been offered for 6 years, some 75% of children 6-15 years old had protective titres of antibodies, while only 18% of those above 20 years of age were protected (169). Another study reported an increase in immunity among Indian women (168). In Peru over 60% of pregnant women had protective levels; the percentage of immune women was similar among women presenting in labour in two capital city hospitals (serving poor and lower middle class populations) and a regional hospital (in the Amazon jungle). The proportion in the jungle hospital was comparable to that in the two urban hospitals probably reflecting recent vaccination efforts in the jungle region of Peru, an area traditionally endemic for tetanus. However, the survey was limited to hospitalised women and the immunisation coverage among women giving birth outside of hospital may be lower owing to decreased access to health care (170). A recent study in Peru showed that 54% of pregnant women are protected against tetanus (171). Studies done in two Kenyan sublocations showed that 69% of women at childbearing age had protection against tetanus (172). In developed countries, studies to assess the impact of TT immunisation on tetanus immunity documented 90% or more of immune persons (173-176).

1.4.9.2 Impact on tetanus incidence

There are spectacular examples of the impact of immunisation on the tetanus incidence in developing countries. A reduction in NT mortality following the implementation of programmes to immunise women of childbearing age and especially of pregnant women has been observed in Bangladesh (177-178) and Haiti (179). Data from a relatively good surveillance system in Sri Lanka showed a significant decline in NT and NNT, which was associated with increasing immunisation coverage of pregnant women and children with vaccines containing TT (17). Data from Egypt showed a long-term reduction in the incidence of NNT. In 1980-1986 a steady increase in the reported number of NT cases reflected improvements in surveillance, while the downward trend in NT incidence in the past six years was attributable to the immunisation programme (17). Immunisation coverage of pregnant women in Egypt with two or more doses of TT increased from about 10% in 1987 to about 70% in 1991-1992; the immunisation coverage with three doses of DPT vaccine in infants exceeded 80% during the past 6 years. The reduction in tetanus morbidity observed in national surveillance data in Egypt is confirmed by hospital study data (94).

High coverage with TT can also be remarkably successful in preventing NT. A good example of this is in the city of Maputo, Mozambique. When 95% coverage with two doses of TT was achieved, the annual number of reported hospital cases of NT dropped from 170-250 cases before 1979 to 0 cases in 1986 (180-181). NT mortality declined dramatically in a district in Aceh Province, Indonesia, between 1984 and 1987. During 1985 a TT immunisation crash programme was implemented in Aceh Province that resulted in 84% of women 10-45 years of age receiving two doses to TT (182). Baseline and follow-up data confirmed 85% reduction in NT mortality during this period, from 32 to 5 per 1000 live births. The neonatal mortality attributable to causes other than tetanus remained unchanged. In Venezuela NT mortality decreased by 93% from 1970-1974 to 1985-1991 (183). Such a downward trend in NT morbidity was also reported from Thailand (184) and in some states in India (185). Similarly, the clear fall in the reported incidence of NNT in developing countries since the mid-1980s coincided with the introduction of immunisation programmes (17). In other countries, such as Uganda and Nigeria, the impact of immunisation programmes is not yet visible in the hospital-based data (186-187).

In industrialised countries the introduction of immunisation with TT in the 1940s and 1950s led to a significant reduction in tetanus incidence. Tetanus has now become a disease of the elderly. In Switzerland (30) and the United States (24, 188-189) persons below 20 years of age constitute only 2-6% of all tetanus cases, and 64-93% of tetanus cases occur in persons over 50-60 years of age. Such a trend has also been observed in England and Wales (190), and in Australia (191). In developing countries, tetanus is still a serious health problem in neonates and school age children (44-45, 192). However, available data on the age distribution relate to earlier periods before the implementation of immunisation of children and women of childbearing age. In some of these countries, where a reduction in the total number of tetanus cases has already been seen, there may be a change in age distribution of tetanus cases.

2.0 EXPERIENCES IN TANZANIA

2.1 Immunisation programme

EPI was established in Tanzania in 1974. TT immunisation programme has been integrated into the MCH clinics since 1975. Initially, pregnant women were vaccinated with TT at the 28th, 32nd, and 36th weeks of gestation during the first pregnancy and a single TT booster dose was recommended during the third trimester in subsequent pregnancies. Currently, a five-dose TT schedule is used for previously unimmunised women of childbearing age as recommended by the EPI Global Advisory Group (71). DPT doses are given to infants at 4, 8, 12 weeks of age and a TT booster is given to the child either during outreach services or following injury/wound infection in individuals who seek treatment from hospitals. Noteworthy, is the fact that there is no existing immunisation schedule for regular TT booster vaccination of male adults after the DPT vaccination during childhood.

2.2 TT/DPT immunisation coverage

The immunisation coverage of 3rd dose of DPT vaccine for children under one year in Dar es Salaam region in 1993 was reported to be 88% (193). The reported immunisation coverage of 3rd TT dose for women of childbearing age in Dar es Salaam in 1994 was 19% (193).

The estimated immunisation coverage of TT 2+ for pregnant women in 1996 was 31% (19). However, the reported TT immunisation coverage for women of childbearing age is surprisingly very low despite the nation-wide TT immunisation programme since 1975. The observation suggests either under-reporting or improper records.

2.3 Incidence of tetanus

Tetanus still remains a public health concern in Tanzania. In the early seventies tetanus had a mortality rate of 19.6%-20.6% (194-195). In Dar es Salaam, the incidence of NT was reported to be 22 per 1000 live births in 1979 (196). Between September 1983 and July 1984, 68 babies with NT were admitted to Muhimbili National Hospital (MNH) Dar es Salaam alone (197). Admissions due to NT accounted for 1% of all admissions of under 5 in 1996 and 1997 (198). Deaths due to NT were 8% in 1996 and 5% in 1997 of all deaths of under 5 with case fatality rates of 86.2% and 66.7%, respectively (198). To date, tetanus still occurs and the mortality has increased despite the country's wide immunisation programme. It is still common among residents of Dar es Salaam and it is the second commonest cause of admission (1994-1996 Hospital Registry) at MNH intensive care unit (ICU) with relatively high morbidity and mortality (199). A previous retrospective study of 106 cases of NNT



treated over 3-year period in the ICU of MNH revealed that 73 (68.9%) patients had no history of TT vaccination, 27 (25.5%) in the age group of 41-50 years, males being predominant (87.8%) with a mortality rate of 73.6% (199). Recent retrospective analysis of ICU admissions at MNH over a one-year period from January to December 1997 showed that NNT patients accounted for 34 (10.3%) of all admissions with mortality rate of 76.5% (200).

2.4 Impact following TT immunisation programme

A progressive decline in the number of NT cases seen in health care facilities since 1975 has been recorded in Tanzania when revamping of TT immunisation in MCH clinics was initiated. The incidence of NT had fallen to 6 per 1000 live births in 1989, though it is estimated that 40% of the cases occur in neonates whose mothers were vaccinated with at least two doses of TT in pregnancy (197). These observations suggest possible failure of immunisation. Another study also reported occurrence of NT in neonates of vaccinated mothers (113). A study done in Dar es Salaam revealed that following antenatal immunisation, antibody levels in mothers and newborns were higher than the minimum protective level of 0.01 IU/ml of serum, even with a single dose of TT. It is therefore possible that prevention of NT may require neutralising antibody concentrations

greater than the accepted protective level (201). Recent studies using indirect ELISA technique showed that the current DPT/TT immunisation schedules provide adequate tetanus immunity for women of childbearing age (202) and 1-5-year-old children though about half of the 6-15-year-old children studied had no immunity against tetanus (203). These findings endorsed the need for regular boosters with TT after primary immunisation in the 6-15-year-old children.

3.0 STATEMENT OF THE PROBLEM

In Tanzania, serosurveys may help clarify the tetanus immunity in situations where routine administrative data are not available, as for example where TT mass campaigns have been conducted either without recording the data or improper recording. TT immunisation has been conducted vigorously in Tanzania since 1975, but reported TT coverage in women of childbearing age is much lower than expected. Serosuveys may also be useful to clarify concerns related to low efficacy of TT, as for example when large numbers of NT cases are reported in children of adequately immunised mothers. It was therefore considered of interest to conduct a serosurvey to investigate immunity to tetanus among pregnant women attending MMC antenatal clinic.

Data on immunity to tetanus following DPT/TT immunisation in male adults are still not available. It was also of interest to determine tetanus immunity among male adults in order to have adequate knowledge in this group as well.

Protection against tetanus not only depends on the total amount of antitoxin but also the IgG subclass distribution and the binding avidities of the subclass antibodies that are formed. The ultimate goal would be to use the data obtained to help EPI identify high-risk groups and areas where TT delivery needs to be targeted and intensified, and to adjust the vaccination programme accordingly.

4.0 OBJECTIVES OF THE STUDY

4.1 Broad objective

To investigate immunity to tetanus among pregnant women and male blood donors in Dar es Salaam, Tanzania.

4.2 Specific objectives

4.2.1 To determine tetanus antitoxin levels in pregnant women and in male blood donors using an antigen competition ELISA technique.

4.2.2 To determine the influence of HIV on tetanus antitoxin levels among blood donors.

4.2.3 To determine distribution of anti-TT IgG subclass antibodies in pregnant women and in male blood donors.

4.2.4 To determine avidities of anti-TT IgG subclass antibodies among pregnant women.

5.0 HYPOTHESES

5.1 There is no difference in immunity to tetanus between pregnant women and male blood donors in Dar es Salaam, Tanzania.

5.2 There is no difference in immunity to tetanus between HIV-seropositive and HIV-seronegative individuals

5.3 There are no differences in the binding avidities of anti-TT IgG subclasses.

6.0 MATERIALS AND METHODS

6.1 Study design

This was a cross-sectional study conducted in September 1999.

6.2 Ethical considerations

The study was carried out in accordance with existing ethical guidelines. Ethical and research clearance was obtained from the Higher Degree Research and Publication Committee, Muhimbili University College of Health Sciences, Dar es Salaam. Informed verbal consent was obtained from each participant before enrolment into the study.

6.3 Sample size

Sample size was calculated using the following formula:

$$n=\frac{4\Pi (100-\Pi)}{\epsilon^2}$$

Where, n=sample size

Π=Seroprevalence for tetanus immunity for pregnant women/male blood donors was assumed to be 50%

ϵ=Standard error-5%

Four hundred participants from each group (pregnant women and blood donors) were supposed to be included in the study.

6.4 Serum samples

Three mls of blood samples were drawn from each pregnant woman and male blood donor, serum was separated after centrifugation, inactivated at 56°C for 30 minutes and stored at –20°C. All sera were then transported on dry ice from Dar es Salaam to Bergen, Norway and were stored at –20°C until the time for assay.

6.5 Tetanus antitoxin assay

Tetanus antitoxin level was determined by an antigen competition ELISA assay as previously described (5). The assay is suitable for quantifying sera with low levels of tetanus antitoxin. The results of

the assay correlate well with those of *in vivo* neutralisation assay and they are reliably predictive below 0.16 IU/ml. Tetanus antitoxin level of 0.1 IU/ml was considered protective as documented previously (4). Briefly, TT at 0.75 LF/ml was bound to standard ELISA plates after it had been diluted in carbonate buffer pH 9.6. The plates were incubated at 4°C overnight then stored at 4°C for up to 4 weeks for subsequent use.

Before use, a plate was washed three times with phosphate-buffered saline containing 0.05% Tween 20 (PBST) and was blocked with 100 µl of 2% bovine serum albumin (BSA) in PBST per well. The plate was incubated at 37°C for 1 hour and washed three times with PBST. Twelve two-fold serial dilutions of each test serum were made in PBST beginning with 1:8. In parallel, there were equivalent dilutions of the test serum diluted in BSA-PBST with 0.1 LF/ml of TT. Similar dilutions of tetanus immunoglobulin, 3.3 IU/ml and 1.1 IU/ml (WHO International laboratory for biological standards, Copenhagen, Denmark) for pregnant women and male blood donors, respectively, were added to each plate. Known positive and negative control sera were also included in every assay. The plate was incubated at 37°C for 1 hour. After washing three times with PBST, 100 µl of horseradish peroxidase conjugated goat anti-human IgG (Dako A/S,

Copenhagen, Denmark) diluted 1/1000 in PBST were added to each well and incubated at 37°C for 1 hour. Fifty µl of substrate, orthophenylene diamine (Dako A/S) were added to each well. To stop the reaction 50 µl of 4 N sulphuric acid were added to each well. Optimal dilutions of TT (1:640) for coating, serum samples and conjugate (1:1000) for uses were determined earlier by a checkerboard titration. The absorbance value was determined at 492 nm in an ELISA plate reader (Emax™, Sunnyvale, CA, USA). A 4-parameter standard curve was constructed by plotting the average optical density (OD) of the duplicate for each dilution of the standard against its respective concentration. The lowest detection limits of the assays were 0.002 IU/ml and 0.0005 IU/ml for pregnant women and male blood donors, respectively.

6.6 Anti-TT IgG subclass antibody assay

All serum samples with tetanus antitoxin level ≥ 0.1 IU/ml were tested for anti-TT IgG subclass antibody levels using indirect ELISA technique. The plates were coated and blocked as for tetanus antitoxin assay and PBST was used as a wash buffer. Briefly, 100 µl of each serum sample diluted 1:50 and 1:100 in PBST was dispensed in duplicate and incubated at 37°C for 1 hour. On the assay plate, the standard positive control, tetanus immunoglobulin (WHO

International Laboratory for Biological Standards), which demonstrated high levels of IgG_1, IgG_3, and IgG_4 subclass antibody activities and low activity of IgG2 subclass antibody, was included. By endpoint titration, this standard positive control was arbitrarily assigned a value of 120 ELISA units (EU)/ml. The wells were washed three times with PBST. The specificity of the monoclonal antibodies to IgG_1 was verified by demonstrating its reaction with purified IgG_1 myeloma protein, but not with purified IgG_2, IgG_3, and IgG_4 proteins. Monoclonal antibodies to other IgG subclasses were similarly shown to react only with the corresponding myeloma protein as described previously (204). Mouse monoclonal antibody to human IgG_1, IgG_2, IgG_3 and IgG_4 subclasses which were supplied in the form of ascitic fluid containing 0.1% sodium azide (Zymed Laboratories, inc. San Fransisco, CA, USA) were diluted in PBST, 1:8000 for IgG_1, 1:16,000 for IgG_2, 1:64,000 for IgG_3 and IgG_4. The dilutions of the monoclonal antibodies were adjusted to obtain the same OD values in a comparable range. Thus, with the same amount of antigen used for coating, these antibodies at the proper dilution were expected to give the same OD value allowing comparison among subclasses (204). Each monoclonal antibody was added in duplicate per plate and incubated at 37°C for 1 hour. All values for each of the four subclasses were determined in one assay.

After washing three times with PBST, 100 μl of peroxidase-conjugated rabbit anti-mouse immunoglobulins (Dako A/S) diluted 1:1000 in PBST were added in each well and incubated at 37°C for 1 hour. The plate was washed three times with PBST. One hundred μl of 1,2-phenylenediamine dihydrochloride (Dako A/S) were added and incubated in the dark for 5-10 minutes. Adding 50 μl of 2.5 N sulphuric acid to each well stopped the enzymatic reaction. Optimal dilutions of TT (1:640) for coating, serum samples (1:50 and 1:100) and conjugate (1:1000) for uses were determined earlier by a checkerboard titration. The absorbance value was read immediately at 492 nm in a microplate reader (Emax™). A standard curve was constructed by plotting the average OD of the duplicate for each dilution of the standard positive control against the respective concentration of the standard. The values for test samples were read from linear parts of the standard curves and corrected for sample dilutions. The final individual anti-TT IgG subclass level of activity was calculated quantitatively in EU/ml of serum.

6.7 Anti-TT IgG avidity assay

ELISA technique that has been previously tested (205) was used with a minor modification, to determine avidities of anti-TT IgG subclass antibodies. Serum samples with detectable anti-TT IgG subclass

antibody levels from pregnant women were tested. Plate coating and blocking procedures were similar to those described for tetanus antitoxin assay. Carbonate buffer and urea were used as a wash buffer and a denaturing agent, respectively.

Briefly, 100 μl of each serum sample diluted 1:50 and 1:100 in carbonate buffer were added in quadruplicate and incubated at 37°C for 1 hour. After washing three times with carbonate buffer, mouse monoclonal antibody to human IgG_1, IgG_2, IgG_3 and IgG_4 subclasses were added as described above. Sera for each of the four subclasses were measured in one assay. The plate was washed thrice and 100 μl of 8 M urea were added in between three washings in the first duplicate alternating with carbonate buffer in the next duplicate of serum samples and incubated at room temperature for 15 minutes each time.

The plate was washed thrice and 100 μl of alkaline phosphatase goat anti-human IgG conjugate (Vector lab, inc., Burlingame, CA, USA) diluted 1:500 in carbonate buffer were added and incubated at 37°C for 1 hour. After three washings, 50 μl of substrate, *p*-nitrophenyl phosphate (Sigma®) were added into each well and incubated in the dark at room temperature for 20 minutes. Adding 50 μl of 3 N sodium

hydroxide solution to each well stopped the enzymatic reaction. A microplate reader (Emax™) was used to read the absorbance value at 405 nm. Antibody avidity of individual serum samples was expressed as avidity index (%), calculated from: OD (urea)/OD (carbonate buffer)×100. Avidity index was classified as used previously (205) into 3 categories: low, <30%; medium, 30-50%; and high, >50%.

6.8 HIV-1 serology

All serum samples from blood donors were screened for anti-HIV-1 antibodies by Behring Enzygnost anti-HIV-1/2 plus ELISA (Behringwerke, Germany). Reactive samples were further tested by Wellcozyme anti-HIV-1 ELISA (Murex Diagnostic Ltd., Dartford, UK). Samples reactive on both assays were considered seropositive for HIV-1 antibodies and those with discordant reactivity were confirmed by a Western blot.

6.9 Statistical analyses

The data obtained were analysed on SPSS for Windows version 10.0 (206). Due to differences in immunisation schedules used, age distribution, TT vaccination status and TT doses received between the pregnant women and male blood donors, the data of the two groups

were treated separately in order to allow appropriate analysis for the proper interpretation of the results in each group.

Since the distribution of tetanus antitoxin and anti-TT IgG subclass antibody levels were skewed, log transformation was performed and geometric mean tetanus antitoxin and anti-TT IgG subclass levels were determined. Independent sample t-test was used to evaluate the differences in geometric mean tetanus antitoxin levels with respect to age, number of pregnancies, TT vaccination status, time of vaccination, number of TT doses received and time after last DPT/TT vaccination.

Correlation coefficient (r^2) between tetanus antitoxin level and time after last vaccination, and anti-TT IgG subclass antibodies were determined. Correlation coefficient (r^2) was also determined between anti-TT IgG subclass binding avidities and tetanus antitoxin level, time after last vaccination, and anti-TT IgG subclass antibodies.

Multiple regression analysis (linear and stepwise) was then performed to study the independent contribution of age, number of pregnancies, TT vaccination status, time of vaccination, TT doses received, HIV status and time after vaccination to the prediction of tetanus

antitoxin levels. Multiple regression analysis was also performed to study the independent contribution of individual anti-TT IgG subclass antibodies to the prediction of respective binding avidities. The goodness of fit of each linear model was assessed and expressed as adjusted multiple coefficient of determination (R^2). The limits of the confidence in the prediction from a regression equation were determined by standard error (SE) of the estimate. The model was tested for statistical significance by *F*-ratio test. The effect of each independent variable was adjusted for the possibility of distorting influences from other independent variables and was expressed as standardised regression coefficient (β). Multicollinearity of independent variables was assessed by tolerance that when it was close to zero, there was a possibility of multicollinearity. The alpha level for rejecting the null hypothesis was 0.05.

7.0 RESULTS

Due to time constraint and limited resources, 376 participants were included in the study. One hundred and seventy six apparently healthy pregnant women attending antenatal care at MNH with verbal histories or documentation of having been vaccinated under the current five-dose TT schedule in Dar es Salaam, Tanzania were studied. Two hundred apparently healthy male blood donors at the time of donation from MNH blood bank were also examined.

The age of all study participants as well as the number of pregnancies conceived by pregnant women were recorded. History of maternal and male blood donors vaccination with TT (in current or previous pregnancy) and DPT/TT respectively, was obtained either from mothers/blood donors, or extracted from TT documentation held by the participant/vaccination records available, and recorded in data sheet (Appendices 1 & 2, respectively). The date/year of last DPT/TT immunisation was recorded in order to determine time after vaccination.

The median age of pregnant women was 25 years (range 15-41 yr.). The mean number of pregnancies was 2.7 (range 1-9). Among participants, 13.1% gave verbal histories of TT vaccination, 58% had

been vaccinated currently and 42% previously. Pregnant women who had been vaccinated with two TT doses were 38.7%, whereas 57.9% had 3-5 previous TT doses and 3.4% reported > 5 TT doses (range 2-8).

The median age of blood donors was 31 years (range 18-70 yr.). Forty-three blood donors (21.5%) did not report a vaccination history. Among those who reported vaccination histories, 60 (30%) had been vaccinated with DPT vaccine during childhood, whereas 97 (48.5%) had only vaccination of 1-3 TT doses as prophylaxis following injury/wound infection. None had received both DPT and TT vaccination and documentation/vaccination records were not available.

Three (1.7%) pregnant women had undetectable tetanus antitoxin and six (3.4%) had levels below that considered protective (≥ 0.1 IU/ml). All these women (previously unvaccinated) had been vaccinated in the current pregnancy with only two TT doses at an interval of 4 weeks and the 2nd TT dose was received one month prior to inclusion. The number of women and the levels of tetanus antitoxin (IU/ml) were 1 (0.001-<0.01), 5 (0.01-<0.1), 15 (0.1-<1.0), 92 (1.0-<5.0), 36 (5.0-<10.0), and 24 (≥ 10.0). Age had no significant

effect on tetanus antitoxin levels but the levels measured were highest among 35-44-year-old women (Table V). Women who had 2-4 pregnancies had significantly higher mean tetanus antitoxin levels than those who had conceived once. Women who had TT documentation had higher mean tetanus antitoxin levels than those who had verbal histories. No significant difference was observed between current and previous vaccination. Women who had 4-5 TT doses had significantly higher mean tetanus antitoxin levels than those with 2 TT doses. The mean time after the last TT vaccination was 12.7 months (range 1 mo.-8 yr.). Mean tetanus antitoxin levels were significantly lower 2 years after last TT vaccination. Time after last vaccination also correlated significantly with tetanus antitoxin levels (r^2=–0.283, p=0.01). Time after last vaccination seemed to be affected by TT vaccination status and TT doses as long duration after last vaccination might be a risk factor for not retaining TT documentation and also affect the number of TT doses to be administered. After controlling for TT vaccination status and TT doses, time after last vaccination still correlated significantly with tetanus antitoxin levels (r^2=–0.208, p=0.006).

Table V: Geometric mean tetanus antitoxin levels according to various categories among pregnant women attending antenatal care (*n*=176) in Dar es Salaam, Tanzania

Variable	No.	Protected (%)	Mean antitoxin (IU/ml)	Mean difference (95% CI)	*p*-value
Age (yr.)					
15-24	81	77 (95.1)	2.91		
25-34	82	77 (93.9)	2.69	0.22 (0.71; 1.64)	0.712
35-44	13	13 (100)	5.23	–2.32 (0.28; 1.12)	0.099
<25	81	77 (95.1)	2.91		
≥25	95	90 (94.7)	2.95	–0.04 (0.66; 1.47)	0.946
No. of pregnancies					
1	52	46 (88.5)	2.02		
2-4	100	97 (97.0)	3.32	–1.30 (0.38; 0.96)	0.034
>4	24	24 (100)	3.79	–1.77 (0.25; 1.13)	0.101
Vaccination status					
Verbal history	23	20 (87.0)	1.21		
Documentation	153	147 (96.1)	3.36	–2.15 (0.21; 0.63)	0.001
Time of vaccination					
Previous	74	69 (93.2)	2.88		
Current	102	98 (96.1)	2.96	–0.08 (0.65; 1.45)	0.890
TT doses					
2	68	61 (89.7)	1.94		
3	23	23 (100)	2.66	–0.72 (0.36; 1.47)	0.372
4	38	38 (100)	4.56	–2.62 (0.25; 0.74)	0.003
5	41	40 (97.6)	4.29	–2.35 (0.26; 0.80)	0.007
>5	6	5 (83.3)	1.93	0.01 (0.25; 2.93)	0.999
Time after vaccination (yr.)					
<2	138	134 (97.1)	3.28		
≥2	38	33 (86.8)	1.92	1.36 (1.06; 2.76)	0.029

TT, tetanus toxoid

Tetanus antitoxin was undetectable in 47 (23.5%) male blood donors and the levels were below that considered protective (≥ 0.1 IU/ml) in 25 (12.5%). The number of blood donors and the levels of tetanus antitoxin (IU/ml) were 2 (0.001-<0.01), 23 (0.01-<0.1), 90 (0.1-<1.0), 34 (1.0-<5.0) and 4 (≥ 5.0). Among those with undetectable level, 43 (91.5%) had no vaccination history. Tetanus antitoxin level was age dependent and fell to the lowest levels in those aged ≥48 years (Table VI). Those that reported TT vaccination had significantly higher mean tetanus antitoxin levels than those who had DPT vaccination. Individuals who had 2-3 TT doses had significantly higher antitoxin levels than those with a single TT dose did. Mean tetanus antitoxin levels were similar in HIV-seropositive and seronegative individuals. The mean time elapsed after last vaccination was approximately 6 years (range 1 mo.-24 yr.). Time after last vaccination had significant effect on tetanus antitoxin levels. There was also significant correlation between time after last vaccination and tetanus antitoxin levels (r^2=–0.331, p=0.001).

Table VI: Geometric mean tetanus antitoxin levels (IU/ml) according to various categories among male blood donors with reported vaccination (*n*=157*) in Dar es Salaam, Tanzania

Characteristics	No.	Protected (%)	Mean antitoxin (IU/ml)	Mean difference (95% CI)	*p*-value
Age (yr.)					
18-27	59	49 (83.1)	0.38		
28-37	64	55 (85.9)	0.36	0.02 (0.62; 1.77)	0.877
38-47	19	17 (89.5)	0.30	0.08 (0.61; 2.63)	0.521
≥48	15	7 (46.7)	0.08	0.30 (0.74; 3.72)	0.002
Vaccination status					
DPT	60	40 (66.7)	0.33		
TT	97	88 (90.7)	0.72	−0.39 (0.31; 0.93)	<0.001
TT dose (s)					
1	35	28 (80.0)	0.15		
2	46	44 (95.7)	0.53	−0.38 (0.07; 0.82)	0.001
3	16	16 (100)	1.95	−1.80 (0.23; 2.17)	<0.001
HIV status					
Positive	10	7 (70.0)	0.26		
Negative	147	121 (82.3)	0.41	−0.15 (0.21; 0.73)	0.135
Time after vaccination (yr.)					
<10	123	109 (88.6)	0.48		
≥10	34	19 (55.9)	0.16	0.32 (0.79; 2.58)	0.001

TT, tetanus toxoid; DPT, diphtheria-pertussis-tetanus; CI, confidence interval ; *Of 200 male blood donors, 43 with no reported vaccination were excluded from the analysis.

In multiple regression analysis, time after last TT vaccination, TT doses and TT vaccination status were significant predictors (F=12.089, p<0.001) for the variations in tetanus antitoxin levels among pregnant women (Table VII). Multicollinearity was not observed.

Table VII: Predictors for tetanus antitoxin level among pregnant women attending antenatal care (*n*=176) in Dar es Salaam, Tanzania

Independent variables	Adjusted R^2	SE	β	p-value	Tolerance
Time after last vaccination	0.075	0.548	–0.221	<0.001	0.705
TT doses	0.057	0.531	0.262	0.001	0.715
TT vaccination status	0.023	0.508	0.189	0.020	0.817

TT, tetanus toxoid; R^2, multiple coefficient of determination; SE, standard error; β, standardised regression coefficient

In blood donors, multiple regression analysis revealed that TT doses received and time after last vaccination predicted significantly the variations (F=34.934, p<0.001) in tetanus antitoxin levels (Table VIII). Multicollinearity was not observed.

Table VIII: Predictors for tetanus antitoxin level among male blood donors with reported vaccination (*n*=157) in Dar es Salaam, Tanzania

Independent variables	Adjusted R^2	SE	β	*p*-value	Tolerance
Time after last vaccination	0.294	0.526	–0.352	<0.001	0.973
TT doses	0.048	0.315	0.249	0.004	0.849

TT, tetanus toxoid; R^2, multiple coefficient of determination; SE, standard error; β, standardised regression coefficient

The distribution of anti-TT IgG subclass antibodies is summarised in Table IX. Anti-TT IgG_1 and anti-TT IgG_3 were the most dominant subclasses detected in the majority of pregnant women and male blood donors.

Table IX: Distribution of anti-TT IgG subclass antibodies among pregnant women (*n*=167*) and male blood donors (n=128*) in Dar es Salaam, Tanzania.

		Anti-TT IgG subclass levels (EU/ml)	
Groups	Subclass	No. (%)	Geometric mean ±SD (Range)
Pregnant women	IgG_1	162 (97.0)	5.85 ±0.48 (0.56-95.50)
	IgG_2	29 (17.4)	2.52 ±0.31 (0.58-13.49)
	IgG_3	152 (91.0)	3.27 ±0.43 (0.10-33.11)
	IgG_4	25 (15.0)	4.95 ±0.63 (0.10-39.81)
Blood donors	IgG_1	105 (82.0)	4.05 ±0.45 (0.76-61.70)
	IgG_2	18 (14.1)	1.43 ±0.11 (0.18-5.05)
	IgG_3	90 (70.3)	2.63 ±0.19 (0.20-13.79)
	IgG_4	3 (2.3)	1.53 ±0.54 (0.21-4.08)

TT, tetanus toxoid; SD, standard deviation; *Individuals with tetanus antitoxin level ≥ 0.1 IU/ml

The distribution of binding avidities of anti-TT IgG subclass antibodies in pregnant women is summarised in Table X. The binding avidities of Anti-TT IgG_1 and anti-TT IgG_3 subclass antibodies were found to be high whereas the binding avidity of anti-TT IgG_2 and anti-TT IgG_4 subclass antibody was found to be low.

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Table X: Avidities of anti-TT IgG subclass antibodies among pregnant women in Dar es Salaam, Tanzania.

		Avidity indices (%)			
		< 30	30-50	>50	Avidity indices (%)
Subclass	n	No. (%)	No. (%)	No. (%)	Geometric mean ± SD (Range)
IgG_1	162	0 (0)	0 (0)	162 (100)	75.28 ±0.06 (53.70-99.80)
IgG_2	29	20 (69.0)	9 (31.0)	0 (0)	46.03 ±0.05 (28.18-52.48)
IgG_3	152	0 (0)	12 (7.9)	140 (92.1)	70.23 ±0.07 (47.86-97.72)
IgG_4	25	23 (92.0)	2 (8.0)	0 (0)	25.28 ±0.06 (16.60-28.84)

TT, tetanus toxoid; SD, standard deviation

There was significant correlation between tetanus antitoxin level and respective anti-TT IgG_1 (r^2=0.350, p<0.001), anti-TT IgG_3 (r^2=0.166, p=0.01) and anti-TT IgG_4 (r^2=0.182, p=0.01) binding avidities. No significant correlation was found between antitoxin level and anti-TT IgG_2 avidity (r^2=0.066, p=0.210). Significant correlation was also observed between time after last vaccination and respective anti-TT IgG_1 (r^2=–0.273, p=0.001), anti-TT IgG_3 (r^2=–0.239, p=0.01) and anti-TT IgG_4 (r^2=–0.115, p=0.01) binding avidities. There was also significant correlation between anti-TT IgG_1, anti-TT IgG_3 and anti-TT IgG_4 subclass antibodies and corresponding anti-TT IgG_1 (r^2=0.256, p=0.01), anti-TT IgG_3 (r^2=–0.157, p=0.05), and anti-TT IgG_4 (r^2=0.124, p=0.01) binding avidities. No significant correlation was found

between anti-TT IgG_2 and corresponding anti-TT IgG_2 avidity (r^2=–0.041, p=0.417).

Multiple regression analysis showed that anti-TT IgG_1, Anti-TT IgG_3 and anti-TT IgG_4 (after excluding tetanus antitoxin level and time after last vaccination) predicted significantly the variations in the binding avidities of anti-TT IgG_1 (F=25.862, p<0.001), anti-TT IgG_3 (F=13.163, p<0.001) and anti-TT IgG_4 (F=11.979, p<0.001), respectively (Table XI). Multicollinearity was not observed.

Table XI: Predictors for binding avidities of anti-TT IgG subclass antibodies among pregnant women in Dar es Salaam, Tanzania

Independent variables	n	Adjusted R^2	SE	β	p-value	Tolerance
Anti-TT IgG_1	162	0.152	0.057	0.373	<0.001	0.994
Anti-TT IgG_3	152	0.054	0.065	0.117	0.036	0.890
Anti-TT IgG_4	25	0.040	0.062	0.311	0.010	0.730

TT, tetanus toxoid; R^2, multiple coefficient of determination; SE, standard error; β, standardised regression coefficient

8.0 DISCUSSION

The World Bank (207) has reported that vaccination is the most cost-effective public health intervention for infectious diseases. Immunisation of women with TT during pregnancy provides protection to the newborns against NT as well as maternal tetanus. With an increasing proportion of women immunised with TT, women will be immune and more infants will have protective levels of passively acquired tetanus antitoxin. The present study has bias in the sampling method because all the pregnant women were users of MCH services and they were more likely to have been vaccinated with TT. In fact, all the pregnant women studied had verbal histories or documentation of having been vaccinated either in current or in previous pregnancies under the current five-TT schedule. However, women of childbearing age who have had pregnancy are likely to have similar anti-TT immunological profile as the study sample. The present study also investigated immunity to tetanus in male blood donors. This aspect of the study also has a selection bias because self-selection of male blood donors can often mean that they are healthier than the general population.

Our findings showed that 3 (1.7%) pregnant women had undetectable tetanus antitoxin and six (3.4%) had levels < 0.1 IU/ml. These

findings could probably be explained by inadequate immune response to two TT doses received by these women. These findings compare with those reported previously (150, 208). It has been documented (4) that optimal immune response is achieved when the intervals between the TT doses are long. The first three TT doses are basic and in women who are not immunised the first dose should be given as early as possible during pregnancy. Unfortunately in Tanzania, like other developing countries, pregnant women as observed in our study, report to antenatal clinic when pregnancy is already advanced, hence the interval between the first and second dose of TT before delivery is too close for the mother to develop an immune response that will protect the newborn. The interval between doses of TT given to pregnant women influences the level of tetanus antitoxin in cord serum as well. A previous study reported that the cord/maternal ratio of tetanus antibodies increases as the interval between the second dose and delivery is prolonged (119). In order to assure long intervals between TT doses it is therefore necessary to start vaccination as early as possible in pregnancy. The proportion of protected women observed in our study is comparable to those reported elsewhere (146-150). Age had no significant effect on tetanus antitoxin levels possibly because of small number of pregnant women in the age group of 35-44 years. The proportion of protection is much

higher when compared to findings by other workers (167-168, 170-172). Early introduction of TT immunisation programme and massive campaigns might have impacts on the observed tetanus immunity among pregnant women.

Our findings showed that tetanus antitoxin was undetectable in 47 (23.5%) male blood donors and the levels were below 0.1 IU/ml in 25 (12.5%). This could be explained by the fact that a substantial proportion of individuals (21.5%) did not report a primary or any vaccination at all, which is important in stimulating early immune response to TT. Since the introduction of TT immunisation programme in 1975, only individuals who were less than 25 years had an opportunity to get early DPT vaccination. Among those, 60 individuals reported to have been vaccinated during their childhood. Our findings do reveal higher proportion of tetanus immunity when compared to a study done in Burma (209) but lower compared to those reported elsewhere (158-160) which revealed 72-100% of tetanus immunity among blood donors as well as male adults (154, 173). The higher proportions of tetanus immune persons in developed countries are most likely due to early introduction of vaccination programmes since 1940. The findings of non-protective levels with age in the present study are comparable to those from studies by

other workers (173, 175-176), which reported increasing susceptibility to tetanus with increasing age. Younger vaccinated individuals were more likely to be protected than older people. The predominant occurrence of clinical tetanus in the majority of male adults with no TT vaccination in Dar es Salaam (199) may reflect that the affected individuals lacked immunity to tetanus. Thus, immunity to tetanus can only be achieved through vaccination with TT and this should be given to both adults and children including individuals who have suffered from clinical tetanus (4). However, some studies have reported occurrence of clinical tetanus in persons with antitoxin levels higher than the arbitrary protective level (66, 113). Overwhelming toxin level as well as presence of non or poorly neutralising antibody subclasses in the cases could explain the occurrence of disease in such individuals who have the so called protective antitoxin level. Improved vaccination programmes are required to reduce the number of non-immune persons particularly those at great risk of acquiring tetanus.

Our findings also showed that pregnant women who had ≥ 2 pregnancies had higher mean tetanus antitoxin levels than those who had conceived once. This is due to the fact that most women get vaccinated during pregnancy. As the number of pregnancies

increases, the number of TT doses received by a woman increases. Targeting pregnant women for at least three TT doses is the most effective strategy. The present findings differ from those reported by other workers (148, 171) who observed no effect of the number of pregnancies on tetanus immunity level. May be different immunisation schedules adopted could explain these different observations.

TT vaccination status was found to be a significant predictor for the variation in tetanus antitoxin levels. Vaccination status based on verbal history and not written documentation could lead to inaccurate immunisation history. Women who had no TT documentation could not retain the vaccination documents they were given and the records were also not available from antenatal clinic sources. Among blood donors, susceptibility to tetanus as shown by non-protective levels of antitoxin was higher (88.4% *vs* 18.5%) among those who did not report vaccination. However, among those who did not report vaccination, 5 (11.6%) were immune to tetanus. This could be due to failure to recall the previous vaccination received, as there is no residual immunity following recovery from clinical tetanus. Without documented records, it is difficult to know whether previous vaccinations were adequate or not. Some previous studies (75, 159)

have reported the association between lack of written TT documentation and non-protective levels of tetanus antitoxin. It has also been reported (4) that proper documentation of TT vaccination is essential because it is important in guiding the spacing of subsequent booster doses without hyper-immunising previously vaccinated individuals. Thus, this underscores the importance of proper keeping of vaccination records.

Our findings also showed that blood donors with TT vaccination had significantly higher mean tetanus antitoxin levels than those with childhood DPT vaccination. This could be due to waning of tetanus antitoxin levels among blood donors who had primary vaccination alone as none had secondary series of vaccination. Secondary vaccination is important in boosting the primary immune response. In addition, TT doses received also predicted 4.8% of the variation in tetanus antitoxin levels. Individuals born before the introduction of immunisation programme had no opportunity to receive childhood DPT doses and they reported to have been vaccinated with 1-3 TT doses as prophylaxis following injury/wound infection instead. It was not possible to determine the intervals in which the TT doses were administered in these individuals. Among pregnant women, number of TT doses received also predicted 5.7% of tetanus antitoxin levels.

The degree and duration of tetanus immunity increased with increasing number of TT doses, a finding that has been observed previously (137). However, women who reported to have been vaccinated with > 5 TT doses had relatively lower antitoxin levels than those who had 3-5 TT doses. Waning of tetanus antitoxin levels and possibly inaccurate vaccination history could explain the findings. Doses given at properly spaced intervals will not only achieve the higher levels of tetanus antitoxin but also will prolong tetanus immunity. It has also been reported (4) that long intervals between doses of TT are best for achieving the optimal immune response.

Mean tetanus antitoxin levels were similar in HIV-seropositive and seronegative individuals, a finding that has been reported previously (104). HIV infection interferes with antibody responses to antigen encountered after infection has occurred, but affects less severely the antibody responses of lymphocytes educated prior to infection (97). It is possible that these HIV-seropositive individuals were vaccinated before they became infected with HIV. TT as a monovalent vaccine or as a component of combined vaccines is recommended for HIV-infected adults and children regardless of the presence or absence of symptoms of AIDS (4).

Time after last vaccination explained 7.5% of the variation in tetanus antitoxin levels among pregnant women. This could be explained by waning of tetanus antitoxin that led to non-protective levels in some women. Following vaccination with 5 TT doses at recommended intervals, immunity to tetanus is expected to last for the whole childbearing period (4). In blood donors, mean tetanus antitoxin levels were significantly lower 10 years after last vaccination. Time after last vaccination also explained 29.4% of the variation in tetanus antitoxin levels. Waning of tetanus antitoxin levels could explain our findings. According to TT immunisation schedule adopted, there is no regular revaccination with TT after primary vaccination. Thus, none of the blood donors had received both primary and secondary vaccination. Individuals who received only three DPT doses in early infancy or in whom the interval between doses is more than 10 years could have diminished capacity to respond to a TT booster dose (4). A previous study (75) suggested that revaccination every 20 years is sufficient to maintain immunity. Another study suggested the possibility of an insufficient response to a booster dose given 17-20 years after primary vaccination (76). Thus, some developed countries recommend the use of Td vaccine every 10 years to maintain tetanus immunity throughout life. Our findings support the need for a regular TT booster dose and appropriate timing of immunisation.

Our present study also showed that, in addition to predominant anti-TT IgG_1 and anti-TT IgG_3 subclass antibodies, anti-TT IgG_2 and anti-TT IgG_4 were also detected in both pregnant women and male blood donors. Our findings are comparable to those who reported the predominant occurrence of anti-TT IgG_1 (1-3), and/or anti-IgG_3 (2) subclass antibodies. The occurrence of Anti-TT IgG_2 and anti-IgG_4 subclass antibodies has been reported previously (2-3).

The present study also showed that binding avidities of anti-TT IgG_1 and anti-TT IgG_3 were high. This is in line with finding that reported the high binding avidity of IgG1 (103). However, the avidity of IgG_4 was found to be remarkably low. Thus, our results show a pattern of decreasing avidity in the order of IgG_1>IgG_3>IgG_2>IgG_4 of antibodies to TT. Association between low antibody affinity and the IgG_4 subclass in man has been reported previously (127-128). Low affinity antibody response in man has been shown to reflect probably the differences in the regulation of isotype switching and affinity maturation at the T cell level (127). Indeed, the association of restricted heterogeneity and low affinity for IgG_4 anti-TT responses can be interpreted as indicative of differential isotype-specific T cell help. A previous study has speculated that multiple TT booster doses may enhance serum anti-TT antibody levels that could lead to

ineffective immune response (113). Furthermore, hyper-immunisation that has been previously reported (73-74) also commonly results in muscle soreness (local reaction) as documented previously (85-87). This reaction is due to the presence of antibody, which forms complexes with the injected TT. The antibody-TT complexes attract complement and leukocytes to produce localised vascular damage, with ensuing local swelling, pain and malaise. A previous study has also reported hypersensitivity reactions (88). In addition, overuse of TT can also in rare instances lead to polyneuropathy (89-90) with an incidence of 0.4 cases per million doses of TT (91). These side effects might have negative impacts on compliance to vaccination and consequently on the immunisation programme.

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9.0 CONCLUSION AND RECOMMENDATIONS

In conclusion, 9 (5.1%) pregnant women with non-protective levels of tetanus antitoxin pose great risks of NT to their newborns and are also susceptible to maternal tetanus, whereas 72 (36%) male blood donors are susceptible to tetanus and the susceptibility is highest from 48 years of age. Anti-TT IgG_1 and anti-TT IgG_3 were the most dominant subclass antibodies detected in the majority of individuals. Low avidity anti-TT IgG_4 antibodies detected in some individuals might be less efficient in clearing TS and can lead to ineffective protection against tetanus. A regular TT booster dose at 10 yearly intervals is recommended to ensure adequate and long lasting immunity among male adults. Proper keeping of TT vaccination records and proper spaced intervals between doses are vitally important to identify pregnant women and male adults with inadequate vaccination and to avoid hyper-immunisation. Strategies to increase TT immunisation coverage need to be strengthened.

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