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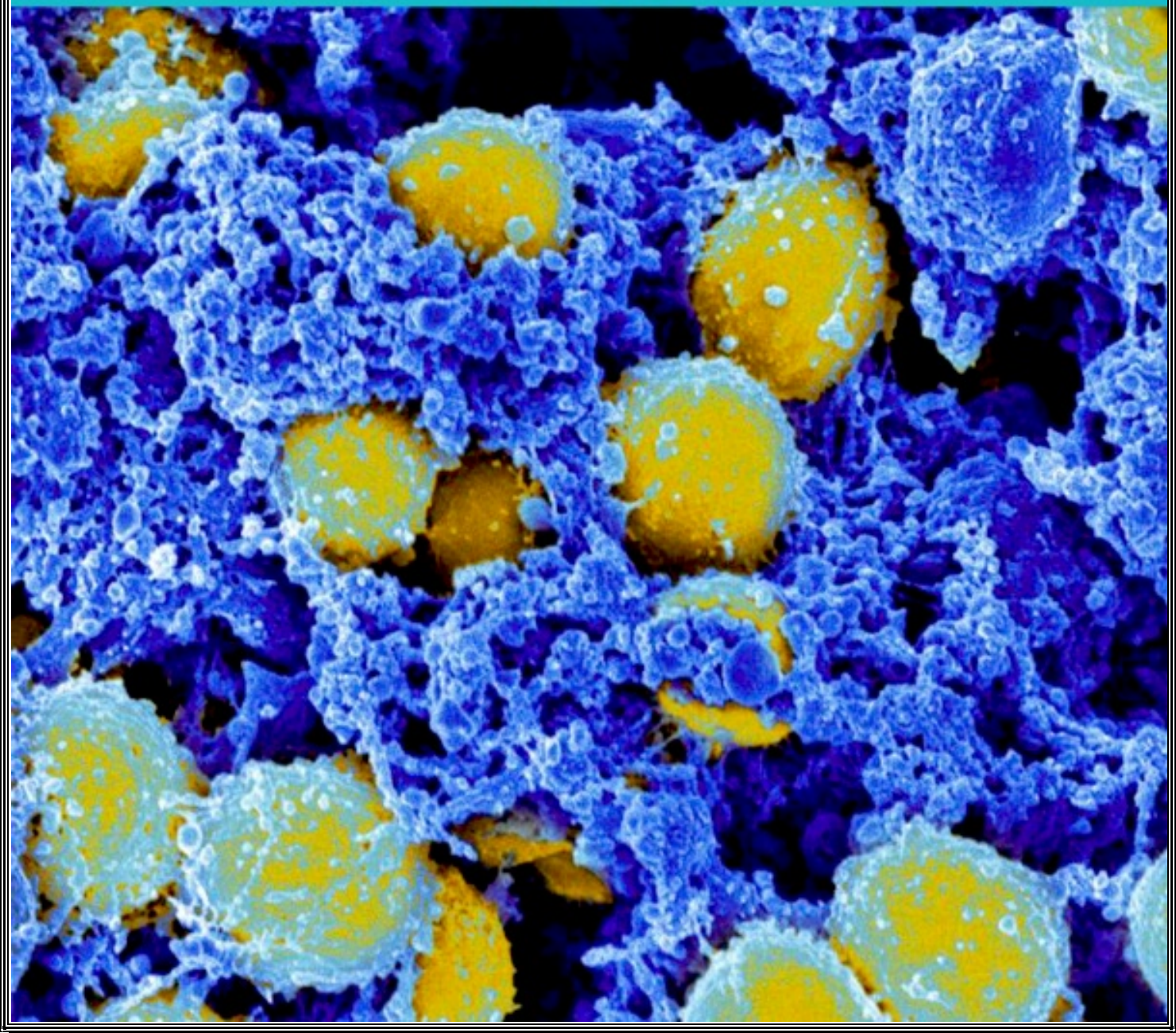

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Cover Image: *Digitally-colorized scanning electron micrograph (SEM) depicts a clump of yellow-colored, spheroid-shaped Staphylococcus aureus bacteria that were enmeshed in a blue-colored, filamentous extracellular matrix, which normally binds cells together within the body's various tissue types.*

Photo Credit: *National Institute of Allergy and Infectious Diseases (NIAID)*

Intelligent Plants

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Plants are the only creature in this universe which prepares their food not only to fulfill their own needs but also for all the hetero- trophs on the planet. They also provide us with fresh oxygen for our survival along with absorption of carbon dioxide. Plants provide shelter for birds and also serve as a source of food for our animals. Beside all of this we think that plants are feeling less, visionless and they don't have ability to think about their environment in which they live. But facts are really contrasted to our thinking, as plants monitor their environment all the time, they know when someone comes near them; they know when someone stands over them. They even know when someone moved their pots from one place to another. They know if light is coming from their right side or left. They know if another plant has grown over them and block the incoming light for them. So it is the plant vision, off course plants don't see in picture as we do.

Plants have feelings when someone breaks their twigs, branches or flower or even when attacked by certain pathogens, in response to attack they produce signal mes- sages to warn the other plants. When some- one cut grass from his lawn to make beautiful layouts, special smell produces from cut grass due to trauma or injury tells the other plants that they are in trouble now. Dr. Conseulo De Moraes from Penn State University performed an experiment to check out the plant intelligence, she took daughter wine (a plant which doesn't prepare its food but de- pendent on other plants) and put it in the middle of tomato and wheat plant which were grown in pots. She finds that daughter wine moves towards tomato plant and catch the tomato plant's juicy stem rather than hard wheat plant's stem. But the question is that how daughter wine finds that tomato plant is the best source of food not wheat plant, to answer this question she carried out.

Further experiment and found that plants produce volatiles in their environment and daughter wine from these chemicals found that it is a tomato plant.

Plants have behaviors similar to those of animals like defense and attack. Plants produce certain types of toxins when pathogens attack them; along with these toxic chemicals they also form some physical barriers to re- strict the enemy from attack like thorn, production of gummy material around stem and formation of corky layers. Some plants pro- duce chemical signals to invite the predators to catch their prey which attacks on plants, so plants are so smart that they use others organisms to kill their enemy . Some plants lack the ability of preparation of some nutrients essential for their growth and development, so like animals they behave as predators e.g. trap plants. Trap plants are very beautiful, attract bees, butterflies and other small organisms towards them by producing sweet smell, when these organisms land on them they catch them and get essential nutrients by killing them. Plants show some others behaviors like animals as search of food. Dr. James Cahil experimental plant ecologist from University Of Alberta proved this by his experiments that roots of plant show behavior just like some animals do for search of food (Cahill et al. 2010). The roots move to- wards the area where foods present by means of sensory part present on the roots of the plant.

Recently a very interesting research was carried out (Appel and Cocroft 2014). In this research the sound of leaf chewing of caters- pillar was recorded by using very sophisticated micro- sensor. During the chewing the induction of defense related metabolites was observed in the leaves of Arabidopsis. This chewing recording was played near healthy plants, and interestingly healthy plants started to express same metabolites' which were expressed during chewing process. This concluded that plant has ability to listen because after listening to the chewing sound, plants were able to induce the expression of defense related proteins and metabolites.

Plants are social as animals, they think not for themselves but also for their community; they share happiness and grieve with each other and find solution of problems as animals and humans do. Plants also have memory to recall hard conditions when again come in their lives to manage them properly. Plants enjoy songs as humans, research shows that plants placed in classical music show better growth than grow along road side, example is the dancing plant which like humans shows dancing movements as music plays near it. Plants also have sleeping and working period like animals, if there is any disturbance in these periods results directly in bad growth and even death in severe cases.

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Pseudomalaria- A Cryptic Infection of Domestic and Wild Birds

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Introduction

Nature has endowed mankind with a magnificent asset of avian flora and it's our liability to maintain it in healthy condition and surpass it to next generation. Success in achieving the above target is highly questionable, as most of our birds are suffering from some cryptic haemoprotozoan infections. The aim of the manuscript is to create awareness about these cryptic infections in birds so some future strategies can be laid down before witnessing any field outbreak. In comparison to mammalian malaria, avian protozoan diseases such as malaria and pseudomalaria received the least attention of parasitologist. Avian malaria caused by *Plasmodium spp*. and pseudomalaria caused by *Haemoproteus spp.* predominantly found in tropical conditions and are transmitted by dipteran flies. Avian malaria is a common mosquito-transmitted disease, however, pseudomalaria is a midges and hippoboscid transmitted disease. Infection is commonly known as pseudomalaria, primarily because of the parasite similarities with *Plasmodium spp*., although, they lack asexual stages of multiplication (schizogony) in erythrocytes. In India, the majority of wild pigeons and doves are found to be affected with *Haemoproteus columbae*. Although it's high prevalence, the disease often faced negligence due to under-estimation of potential pathogenicity resulting in sudden outbreaks on introduction in non-endemic regions and a major cause of death of young birds. There are more than 140 species of *Haemoproteus* affecting birds which includes, 4 major economically important species of

Haemoproteus viz., *H.columbae* (pigeon, dove), *H.meleagridis* (turkey), *H.nettionis* (duck, swan) and *H.sacharovi* (pigeon, dove), which are more frequently encountered by veterinary parasitologist. Although the disease condition is not much pathogenic, mortality upto 20% can be observed in affected bird flock. Organisms are transmitted by blood-sucking flies in which developmental stages, comparable to those of genus *Plasmodium spp*. occurs. The clinical form appears more often in young nestlings than the adult.

History

The term *Haemoproteus* was first used in the description of *Haemoproteus columbae* in the blood of the rock pigeon (*Columba livia*) by Kruse in 1890. In 1897, a medical student, William MacCallum and his colleague, Eugene Opie at the United States reported a similar haemoprotozoan in the blood of crows. McCallum in 1897 studied the life cycle of *H.columbae* which still in a present day lacking for other *Haemoproteus spp*. A pioneer researcher, Adie reported dipteran as a biological vector for transmission of prseudomalaria.

Vector

Pseudolynchia genera of hippoboscid fly are incriminated as the vector of *H. columbae* in rock pigeon (*Columba livia*) and dove. In a similar fashion, *Culicoides spp*. has been reported to be a vector of *H.meleagridis* and *H.nettionis*. In India, several researchers have mentioned the presence of pigeon louse (*Pseudolynchia canariensis*) underneath the affected bird plumage which can be observed on keen observation. Both sexes of the vector are blood feeder so can transmit the parasite and it is worth to note that fly does not lay eggs rather pupiparous. The pathogen has a negative effect on the fertility of vector itself. Adult flies are around 10mm long reddish brown with yellow spots on indistinctly segmented abdomen and clearly visible while feeding. Apart from midges and hippoboscids, few researchers suspect on Tabanids as a potential vector of pseudomalaria.

Host Range

Pigeon, Dove, Turkey, Swan, Goose, Ducks etc.

Clinical Signs

The heavily vector-infested bird becomes restless (due to irritation caused by fly bite), emaciated and prone to secondary infections. Generally adult birds in endemic regions are considered resistant due to evolutionary resistance and remain asymptomatic, although, young birds and new acquaintance suffers heavily with high mortality rate. Disease condition often aggravates in immune-compromised condition as our personal experience in Ranikhet (New Castle Disease) affected pigeon. In certain cases, it can result in corneal opacity as we observed in one case of white collar dove; however, it needs to be further studied to draw a conclusion.

1. Anorexia
2. Anaemia
3. Dullness and depression
4. Reluctant to move
5. Ruffled plumes
6. Torticollis (few cases)
7. Prostration and Death

Post-Mortem and Histopathological Lesions

On post-mortem examination observed the enlarged gizzards, enlargement of the spleen, liver and kidneys, yellow –white necrotic lesion were observed on breast region muscles and wings and petechiae and ecchymotic hemorrhages were observed at affected areas.

On histopathological examination, observed the multi focal areas of necrotic changes in muscles and also sometimes the fibers contained basophilic calcified material in the affected areas. The megaloscizonts were filled with RBC. Schizonts or loose merozoits were often present with in

blood vessels. Gizzard wall was thickened due the merozoits in muscular layer. The alveolar capillaries and pulmonary vessels were severely congested. Several rounded or elongated thin-walled schizonts were evident in capillaries or larger bronchial vessels. Occasionally schizonts were peripherally situated and appeared to be in endothelial cells

Diagnosis

Presently, both conventional and molecular diagnostic tools are available for diagnosis of *Haemoproteus spp*. A conventional method like microscopy, though highly efficient, for confirmation of species molecular tools need to be adopted. The presence of macrogametocytes and microgametocytes of variable shape and localization of their pigment granules are recorded for morphological identification of species, although it is not highly reliable.

1. Based on the clinical sign like corneal opacity (Fig. 1)
2. On blood smear examination, observed the halter shaped gametocytes in RBC (Fig. 2) and hemozoin granules in RBC.
3. Post-mortem lesions
4. On histopathological examination, observe the presence of a large number of schizonts in endothelial cells of blood vessels of lungs.
5. Molecular tests

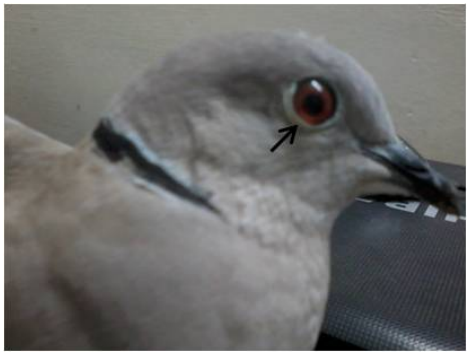

Figure 1: Bird showing corneal opacity

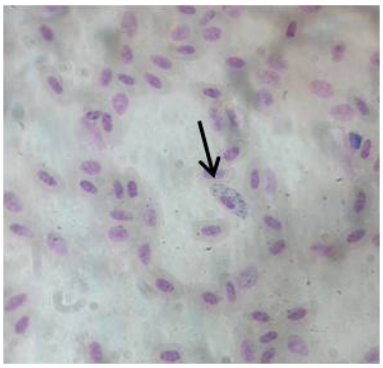

Figure 2: Blood smear examination revealed the halter shaped gametocytes in RBC

Treatment

Pigeons (*Columba livia*) that had recovered from previous infections are susceptible to reinfection, whereas pigeons with chronic infection acquire immunity (premunition).until and unless blood parasitemia is high or host is showing clinical sign and symptoms treatment is not advisable. Antimalarial drugs chloroquine (2000 mg/l in water for 1 day) found to be useful along with vitamin supplements for 5 days. Apart from the *Pseudolynchia canariencis*, slender pigeon louse *Columbicola columbae* also commonly seen in Indian rock pigeons, therefore, application of topical insecticide like permethrin (0.25%) on the pigeon's body can control of vectors.

Prevention and Control

1. By eliminating the vector population
2. Regular removal of litter
3. Habitat management(decrease vector breeding sites)
4. Application of insecticides on birds as well as bird house
5. Use of ventilation fans

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Mesenchymal Stem Cells: A potent candidate for Virus Isolation and pathogenesis studies

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Many reports are available showing susceptibility and replication of different human and animal viruses in different MSCs.

Human viruses

The Kaposi sarcoma (KS) -associated herpesvirus (KSHV), the etiological agent of Kaposi sarcoma (KS), is known to infect bone marrow cells. The differentiation and proliferation of hematopoietic cells in bone marrow is controlled by mesenchymal stem cells (MSCs). Study conducted by Parsons et al., (2002) presents the first report of direct KSHV infection of primary human bone marrow cell, indicating its susceptibility in fetal derived MSCs. This study suggested the possible role of MSCs in KSHV related pathology and possibility of using this system for studying the long term effects of the virus. Both cell-free and cell-associated Kaposi sarcoma–associated herpesviruses (KSHV) are found susceptible to primary MSCs and infection can be maintained in nearly half of the cells for up to one and a half month. Thus, this study guarantees the use of MSCs in KSHV-related human disease. In another study also suggests the role of human mesenchymal stem cells in pathogenesis of Kaposi sarcoma.

Herpes simplex virus type-1 (HSV-1) is known to cause opportunistic infection subsequent to allogenic stem cell transplantation. HSV-1 which is highly contagious is known to infect humans, produce cold sore. HSV-1 is able to infect hMSCs and heparin sulphate (HS) and 3-O sulfated heparan sulfate (3-OSHS) serve as its entry receptors during this process. HS is known to mediate the proliferation and differentiation in mesenchymal stem cells. MSCs are known to

have immunomodulatory action. It has been anticipated that infusion of human mesenchymal stem cells (hMSCs) in patients with HSV-1 viremia may compromise the host's defense against infectious agents.

In a study by Sundin et al., 2006, proposed that MSC can be infected in vitro with both CMV (Cytomegalo virus) and HSV-1(Herpes simplex virus – 1). Mesenchymal stem cells cannot be infected with EBV in vitro, even if a small subpopulation of MSC expresses CD21, the receptor for its uptake.

Hepatitis B virus (HBV) infection is one of the most common blood borne liver infection. It has been estimated that about 2 billion people have been infected with hepatitis B virus (HBV) and also millions of people have chronically infected with HBV. Earlier studies have shown the capacity of human BMSCs to differentiate into functional hepatocyte-like cells in vitro and its ability to restore the function of liver function in hepatic failure in animal models. Ma et al. (2011) demonstrated for the first time the infection, replication of HBV in human bone marrow MSCS (BMSCs). These in -vitro system of MSCs can be used to assess the complex interactions between HBV and its host cell. Moreover, this finding may help to explain the cases of HBV reinfection in liver transplants recipients.

Freisinger et al. (2010) demonstrated the hematopoietic differentiation (HD) of CD34- adipose tissue derived MSCs (ASC) clones (CD90+, CD105+, CD45- and CD34-) under specific invitro stimulations and exhibits macrophage-like characteristics. Macrophages are well known in infectivity and pathogenesis of HIV-1 This Mesenchymal Stem Cell derived hematopoietic cells are permissive to HIV-1 infection. However the undifferentiated ASCs were non-permissive to HIV-1 infection.

The bone marrow serves as a reservoir for heterogeneous population of cells concerned with the regeneration and repair of many organs including the lungs. Epithelial cells derived from swine bone marrow express various stem cell markers like octamer-binding transcription factor (Oct 4) and stage-specific embryonic antigen-1 (SSEA-1), the alveolar stem cell marker Clara cell secretory protein (Ccsp), and the epithelial cell markers pan-cytokeratin (Pan-K), cytokeratin-18 (K-18), and occludin and support influenza virus replication in vitro. These exclusive populations of progenitor epithelial cells in the bone marrow might have airway reconstitution potential and may be a useful model for cell-based therapies for infectious and non-infectious lung diseases.

Recently, Cheung et al. (2013) demonstrated the capacity of Respiratory Syncytial Virus (RSV) to modify the MSC function. The reported modulation of IL6, an important immune regulator, provides evidence of an RSV mediated affect on MSC proliferation and immunomodulatory functions that may be relevant to RSV-associated lung disease

Animal viruses

Bovine herpes virus 4 (BoHV-4) is a gamma herpes virus. Despite the fact that BoHV- 4 has been isolated from normal cattle's and cattle's with different disease manifestations, yet the pathogenicity of this virus is no clear. Even if BoHV-4 has been isolated from various tissues, evidence suggested the persistence of BoHV -4 in both natural and experimental cases are demonstrated in monocyte/ macrophage lineage. Macrophages can harbour persistent infection with BoHV-4. The differentiation and proliferation of haematopoietic precursors, such as macrophages are controlled by nearby mesenchymal stem cells. The chance of interaction between BoHV-4 and MSCs was studied by Donofrio et al., (2005). They concluded that primary bovine mesenchymal stem cells were highly permissive to full multiplication of BoHV-4. The etiological agent of Infectious bursal disease (IBD) is IBD virus, a member of family birna viridae Infectious bursal disease (IBD) is an important immunosuppressive viral disease affecting chickens worldwide. The target of virus attack is Bcells of bursa (RODENBERG et al.,

1994). Due to the strain variation in vvIBDV field viruses, its isolation and propagation in primary or secondary cell cultures of chicken embryo origin were found to be very difficult. (Mannan et al., 2009).

In a study by Mohammed et al. (2012a) confirmed the susceptibility of the chicken MSCs to very virulent infectious bursal disease (vvIIBDV) after five consecutive passages. Cytopathic effect such as rounding up of cells and monolayer detachment, intracytoplasmic brownish colouration was observed at 24hr post infection. Virus replication was monitored by cytopathic effect observation, indirect immunoperoxidase, and reverse transcription polymerase chain reaction (RT-PCR). This study suggested the possibility of using the chicken MSCs for vvIBDV propagation.

Infectious Bronchitis is a corona virus infection affecting respiratory tract, kidneys and oviduct. Various diagnostic methods of acute IBV include immunoperoxidase test, enzyme-linked immunosorbent assay, virus isolation or serological detection. On the other hand very rapid diagnostic methods are available such as detection of viral RNA. Generally, IB vaccines have been produced from embryonated chicken eggs. This method has some disadvantages of being takes long time and requires large area for the incubation of eggs. Therefore there is a need for an alternate system for vaccine production, different cell cultures will provide this. In a study by Mohammed et al. (2012b), successfully adapted Infectious Bronchitis virus (IBV) in chicken MSCs. In this study, IBV was first adapted in SPF embryos by blind passage and then adapted to the chicken mesenchymal stem cells upto 20th passages. The cytopathic effects (CPEs) was observed

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Immune Evasion Strategies- A Boon for Parasites

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Parasites bathes in the sea of antibody and immunoguards, still succeed in giving life to new parasite remains always a mystery for a biologist. Parasites cause direct damage to host by competing for nutrients, disrupting tissues (hydatid disease), reducing economic value of hides, destroying cells (*Babesia* sp., *Theilaria* sp. etc) and mechanical blockage (*Toxocara* sp. and *Parascaris* sp. in intestine), they also cause indirect losses to host through decrease in milk production, wool production, reduced fertility and weight loss. There are reports of high prevalence of helminth parasite and latent carrier of protozoan parasite in livestock, however, they opt clinical shape under transportation stress, overcrowding, or under conditions of compromised nutrition, in short all those conditions which make the host immunosupressed. Thus manipulating immune system is one of the most economic ways of controlling parasitic problem when compared to chemotherapeutic measures. Additionally, use of drugs results in residue problem and environmental contamination. To deal with immunomodulation, sound knowledge on parasitic immunity and immune evasion strategies are required. Thus the present manuscript is aimed toward introduction of various parasitic immune evasion strategies to the readers so some future strategy can be built up with inter-sectoral collaboration to avoid economic losses due to parasitic burden.

The immune system is one of the most complex systems of an organism which prevents infection and keeps the parasites in check. Susceptibility of a host to parasites is dependent on several factors viz. genetic background, age, nutrition and hormonal status. In bacteria or viral infection immune response delivered at the right place and at the right time but in parasitic infection this is not the case because of-

- Complex structure & different life cycle stages of parasites
- Incomplete elimination of parasites
- Immune evasion or survival strategies of parasites: Parasite immune evasion is the process by which parasites counteract the immune system of the host.

The studies on host-parasite relationship have shown that, parasites unlike other disease causing agents have coevolved with their host immune system and Barry (1997) has opined that parasites during the course of co-evolution with their hosts have captured genes from their hosts to produce molecules that disarm host immunity. The host parasite relationship is based on the subtle interplay between parasite survival strategies and host defence mechanisms. Successful parasites have evolved strategies for survival & development in both invertebrate and vertebrate hosts. The goal of a parasite is to propagate within the host and be transmitted to the next host.

Immune evasion strategies

Parasites use diverse array of mechanisms to avoid and antagonize the immune response of their hosts. Different mechanisms of immune evasion is dependent on-

- Life cycle stage of parasites
- Route of penetration and
- Microenvironment inside the host

Specific immune evasion strategies used by various helminths and protozoa are briefly depicted in Table 1 and 2, respectively.

1) Sequential antigenic variation

The strategy of evading the immune response by the way of producing variant surface glycoprotein (VSG) was discovered for the first time by Paul Ehrlich in African trypanosomes. The VSG's forms a thick coat over the surface of the trypanosomes. Research has revealed that there are about 1000 silent VSG genes are present in trypanosomes and only one is transcribed at a time. The variation is brought about by DNA rearrangements at the expression site or through activation of a new expression site. With the every change in VSG type, different waves of parasitaemia are produced, as trypanosomes with older VSG are selectively killed by antibodies generated against that particular VSG.

Various other parasites have also known to show rapid and repeated surface antigenicity within the host (*Giardia lamblia*, *Babesia* spp., *Eimeria, Plasmodium* spp.) which allow them to survive and multiply within host body despite the active immune response. Unlike VSG of anterior station trypanosomes, *Giardia* sp. bears variant specific surface protein (VSP) which ensures their survival in gut lumen, however, immunomodulator like metronidazole has high success rate in treatment of giardiosis.

2) Molecular mimicry: The friendly fire

Molecular mimicry (eg. *Trypanosoma cruzi*) refers to the property of a parasite to share antigenic determinants with the host by capping with the host antigens or synthesizing host mimicking antigens onto their surface. Parasites that mimic host antigens can evade immunity, because self-tolerance mechanisms eliminate or anergize autoreactive T-cells. However, depending on the degree of structural resemblance between parasite and host epitopes and/or the repeated activation of autoreactive T-cells during infection, mimicry can stimulate the destruction of host tissues the resulting condition is termed as autoimmune response. Apart from adopting host machinery, certain helminth like *Fasciola hepatica* generate proteins which are biochemically

and functionally related to host protein and these are often termed as helminth defence molecules (HDM).

- *Schistosoma matheei* : Adherence of host leucocytes, dendritic cells and adsorption of host immunoglobulins(Igs) such as IgG1, IgG2a, IgG2b, IgA & IgM.
- *Taenia solium* cysticerci and other metacestodes including hydatid cyst: Adsorb MHC molecules.
- *Trypanosoma theileri* & *T. lewisi* : Adsorb host serum proteins.

3) Proteases and surface antioxidants

Invading parasites secretes certain proteases like hyaluronidase, cystatin, cyteine protease, cathepsin, collagenase etc to penetrate host tissues. In order to survive inside host under challenging conditions, parasites synthesize certain antioxidant molecules like ferritin, peroxidase, superoxide dismutase, glutathione transferase etc to prevent attack on their metabolic machinery. These proteases and surface antioxidants prolong their survival in host body even after preliminary elimination using several anthelmintic drugs.

(i) *Leishmania* sp. of protozoa produce various proteases and surface antioxidants which enable them to survive with in host even after clinical healing.

- Leishmanolysin – Cleaves albumin, haemoglobin, IgG & lysosomal protein.
- Lipophosphoglycan (LPG) and leishmanial superoxide dismutase: scavenging of reactive oxygen intermediates.
- Leishmanial protein kinases (LPK-1 and c-LPK2): inactivates complement components by phosphorylation.
- Ovathiol- A: antioxidant molecule.
- gp63 metalloproteinase: protease catalyzed conversion of C3b to C3bi on the parasite surface.

Note: LPG, gp63 and LPK are subsequently explored for their vaccine potential and presently they are part of promising future *Leishmania* vaccine like Leish 111f.

(ii) *Fasciola* spp.: Inspite of regular use of anthelmintics, high prevalence rate of *Fasciola* sp. has been recorded in Indian subcontinent probably because of highly evolved immmuno evasion strategies.

- Produces superoxide dismutase (SOD) which neutralizes super oxide radicals which are toxic for juvenile flukes.
- Cathepsin L proteases: Apart from digestion of blood components, they cleave immunoglobulins (IgE and IgG) precisely in the hinge region (thus separating the Fab from the Fc regions) and prevent the antibody-mediated attachment of eosinophils to the parasite surface and hence may aid in protecting the parasite from immune attack.

(iii) African trypanosomes contain cysteine proteases that may be released into the bloodstream of their infected hosts.

T. brucei- trypanopain

T. cruzi- cruzipain

Table 1: Specific immune evasion strategies to helminths

S.No	Parasite	Mechanism of action
1.	*Fasciola* sp.	Antigen shedding and renewal Proteases and surface antioxidants
2.	*Schistosoma* sp.	Antigen shedding and renewal Anti-complementary effects Parasite protease inhibitors
3.	*Taenia* sp.	Parasite protease inhibitors Molecular mimicry

		Anatomical seclusion
4.	*Echinococcus* sp.	Anti-complementary effects Immunosuppression Anatomical seclusion
5.	*Haemonchus* sp.	Antigen shedding and renewal
6.	*Dirofilaria* sp.	Immunosuppression
7.	*Ancylostoma* sp.	Antigen shedding & Inhibitory factor

4) Antigenic shedding and renewal

In an immunocompetent individual, host humoral immune system immediately respond to parasitic antigen and build up immunoglobulins, hitherto, the time immune system required to prepare Igs, parasites evade immune attack by shedding of surface coat/antigens.

- Frequent shedding of the 'Glycocalyx ' layer of the tegument
  Eg: *Fasciola hepatica* & *Fasciola gigantica*
- Shedding of the surface antigens from tegument
  Eg: *Schistosoma mansoni*, *Ancylostoma* sp.
- Shedding of the cuticle and epicuticle
  Eg: *Toxocara canis* larva
- Shedding of a 70 to 90 kDa larval glycoproteins
  Eg: *Haemonchus contortus*

5) Parasite protease inhibitors

Schistosoma sp. secrete Serpins (serine protease inhibitors) viz. ShSP1, Smpi-1 & contrapsin which act on thrombin and elastase and make them inactive. *Taenia taeniaeformis* produces

Taeniastatin that acts by inhibiting neutrophil chemotaxis, T-cell proliferation and IL-2 production.

6) Anticomplementary effects

(i) *Schistosoma mansoni*: Acquire host Decay acceleration factor (DAF), it may dissociate C3 convertase and thereby impede the complement cascade. Schistosomula are sensitive to complement mediated killing, however, they become refractory to complements after 24hr and they enters circulation to face complement system 48-72 hrs post infection.

(ii) *E. granulosus*: The hydatid cyst is pretected by acellular lamimnated layer which coinatins certain molecules like myoinositol hexakisphosphate (InsP6), which Inhibits the activation of factor B and factor H of alternate compliment pathway thereby impairs the C3b deposition.

(iii) Ticks: Anticomplement proteins have been identified in the saliva of different tick species. 16.77 kDa proteins termed OmCI was characterized in salivary gland extracts of the soft tick *Ornithodoros moubata*. This protein targets the terminal pathway of the complement system by inhibiting C5 cleavage. OmCI belongs to the lipocalin family and is the first member of this protein family reported to inhibit complement activation.

Calreticulin (CRT) secreted by haematophagus parasites viz. ticks, *Haemonchus contortus*, *Necator americanus*, is well known as a multifunctional and abundant protein of the endoplasmic reticulum where it acts as a molecular chaperone and Ca^{2+}-signaling molecule. In relation to the complement system, it has been demonstrated that extracellular CRT can bind to C1q, and furthermore, can compete with antibodies for binding to C1q and inhibition of C1q mediated hemolysis. It has been suggested that through this mechanism CRT might contribute to the progression of autoimmune disease by preventing immune complex clearance.

Table 2: Specific immune evasion strategies of protozoa

S.No	Parasite	Mechanism of action
1.	*Trypanosoma* sp.	Antigenic variation- **VSG** Molecular mimicry Immunosuppression Proteases and surface antioxidants Production of inhibitory and cytotoxic factors
2.	*Leishmania* sp.	Proteases and surface antioxidants
3.	*Babesia* sp.	Antigenic variation Anatomical seclusion
4.	*Theileria* sp.	Molecular mimicry Resistance to lysosomal killing
5.	*Anaplasma* sp.	Antigenic variation
6.	*Eimeria* sp.	Antigenic variation
7.	*Toxoplasma gondii*	Reduced antigenicity Resistance to lysosomal killing
8.	*Ehrlichia* sp.	Antigenic variation

7) Parasite's modulation of host immunity

Parasites modulate the host immune response in various ways for their survival and propagation. The classical example is the egg granuloma formation in schistosoma infection. In schistosomiasis it has been proven that Th1 response induces protection, however, eggs released after patency by female worms causes skewed Th2 response. This is brought about by the soluble egg antigens (omega proteins, SEA) released by developing miracidium inside egg. Though, Th2 response limits the pathology caused but will not eliminate the schistosomes and makes individual susceptible to bacterial and viral attacks.

Parasites such as *Diaofilaria immitis, Trypanosoma* sp. cause immune-suppression by inducing the production of specific suppressor cells, by stimulating lymphotoxicity, by producing blocking antibodies and through neutralization of the components of the immune system. Excretory secretory products of *Fasciola hepatica* inhibits lymphocyte proliferation. *Theileria annulata* initially causes lymphocytosis followed by exhaustion of lymphnode leading to lymphopenia.

8) Resistance to lysosomal killing

Toxoplasma gondii, a coccidian parasite with cats as the definitive host and warm-blooded animals as intermediate hosts survive within phagocytic cells by inhibiting the fusion of parasitophorus vacuole with lysosomes.

Theileria annulata infected macrophages produce IFN gamma, which in turn produce IL-1 and TNF alfa. These act as an autocrine growth factor or apoptosis rescue factor and stabilizing the developing macroschizont infected cell. The overproduction of these molecules are responsible for pathogenesis in theileriosis.

Conclusion

Immune evasion can take several forms, as hiding form and suppressing the immune response. Therefore, parasite interference with the normal immune response poses a novel problem to the immune system, since parasite manipulation of the immune response is likely to alter the balance that exists between immunoprotection and immunopathology. One of the major hindrances in producing effective vaccine against parasites, particularly helminths is the scant information on functional characterization and identification of antigens responsible for immunomodulation. Immune evasion though considered as hindrance in formulation of appropriate control measures against parasitic diseases, rather it can serve as prime target for development of strategies meant for sustainable parasitic control.

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Clostridium tetani: - An Overview

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Introduction

Bergy's Manual of systemic Bacteriology has classified *clostridium* in the phylum Frimicutes and class clostridia . It is one of the important genus in order clostridiales and family clostridiaceae. Kitasto (1889) isolated *C tetni* in pure culture and established the etiological role of the bacillus by reproducing the disease in animals by inoculation of pure culture .Rosenbach (1886) detected a slender bacillus with round terminal spore from a patient with tetanus.

Epidemology

Tetanus was first described in Egypt over 3000years ago. It is world wide in distribution but the incidence is very much higher in the developing countries due to warm climate ,unhygienic practices and poor Medical services .There are between 800000and 1million death due to tetanus each years,80%of deaths occurs in Africa and south East Asia .It remains endemic in 90 countries world-wide .Tetanus neonatrum and postabortal and purpueral tetanus have got high fatality rates. At least half the deaths due to tetanus occur in neonates.

Morphology

Clostridium tetani is long thin Gram-positive organisms, 4.0to8.0 *0.5 to 1.7µm though there may be considerable variation in length(as much as 15µm).They occurs singly and occasionally in chain. The organism is motile (except Type VI *C tetani)* with peritrichous flagella and non-capsulated. *Clostridium tetani's* on Gram stain is said to resembles tennis rackets or drumsticks.

Found in nature as spores in soil or parasitizing the gastrointestinal tracts of animals, these bacterium causes serious toxicity in humans.

The spores of *clostridium tetani* are spherical , terminal and bulging ,giving bacillus the characteristics, racket shaped or drumstick appearance. Yong spore is oval rather than spherical.

Biochemical characteristics

Biochemical characteristics used for the identification of *clostridium tetani* includes :positive Indole -test and liquifies gelatin slowly. It dosen't form H2s and MR, VP negative. *clostridium tetani* is slightly proteolytic and don't ferment carbohydrates. The organism produces a green fluorescence when grown on media containing neutral red. Eg ;MacConkey's medium. It is catalase and oxidase negative.

Cultural characterstics

Clostridium tetani is obligate anaerobes ,grown in ordinary medium but the growth is improved by addition of blood and serum in the media but not glucose .Unlike *C perfringes*, *C tetani* is extremely difficult to cultivate as it is sensitive to oxygen toxicity. They produces a very faint growth on FAA plates .single colonies can be hardly observed, but a thin flim of bacteria can often be discerned ,because bacteria swarms on moist agar plates .on dryer blood agar single medium sized colonies may be formed (4-6mm in diameter).these colonies are flat ,transculent and grayish with irregular margin. A thin zone of haemolysis can be often observed around colonies on blood agar.

Table no1:Colony characterstics of *Clostridium tetani* on different culture media

Culture Media	Colony Characteristic
Robertson`s cooked meat or Thioglyclollate medium	Turns black on prolonged incubation
Blood agar	Translucent and grayish with irregular margin

	,α-hemolytic colonies ,which later developed into β-hemolytic due to production of haemolysin.

Virulence factor and Pathogenesis

The pathogenicity of *Clostridium tetani* may be associated with virulence factor sucha as toxins. *Clostridium tetani* produce two exotoxin , tetanolysin and tetanospasmin which are encoded by plasmid born genes .Tetanolysin has local necrotizing effect, which may facilitate the spread of *C tetani* .Tetanolysin is a heat and oxygen labile haemolysin .Its pathogenic role Is not clear.It may act as leucocidin.serologically,tetanolysin is related to streptolysinO and haemolysin of *C perfringens,C novyi,and S pyogens*.

Tetanospasmin is a neurotoxin, oxygen-stable and heat labile protein and rapidly gets destroyed by proteolytic enzymes.Tetanoplasmin ,which is similar to botulism toxin in structure and mode of action but they act on different parts of nervous system. Tetanoplasmin blocks the inhibitory nerve impulses by interfering release of neurotransmitters (glycine and y-aminobutric acid)and leads to spastic paralysis.

Clostridium tetani is not an invasive organism –it appears to be derived primarily from the faeces of animals and indirectly via soil .The spores are common in dust and soil contaminated by horse or cow dungs. Infection occurs in deep penetrating wounds ,such as wound inflicted by lacerated injury or by street accident which are soiled by contaminated dirt. Sometimes ,trivial injuries caused by contaminated splinters ,rusty nails and prick thorn may cause tetanus. When a person is harbouring *C tetani* in intestine, endogenous tetanus may result following septic abortion and puerperal sepsis. Merepresence of spores of *C tetani* in wound dose not result in tetanus. The spores remain dormant till favourable environment is available .Introduced foreign bodies in

deeper tissue create a nidus of devitalised material and anaerobic environment enabling tetanus spores to germinate and grow.

Growth of *C tetani* is completely local.They mulitiply locally and produce toxin (tetanospasmin). Tetanospasmin is relased on autolysis of the bacterial cells. The toxin initially binds to peripheral nerve terminals .It is transported within the axon and across synaptic junctions until it reaches the central nervous system.That becomes rapidly fixed to gangliosides at the presynaptic inhibitory motor nerve endings ,and is taken up into the axon by endocytosis. The effect of toxin is to block the relase of inhibitory neurotransmitters glycine and gamma-amino buytric acid(GABA)across the synaptic cleft ,which is required to check the nervous impulse.Iif nervous impulses cannot be checked by normal inhibitory mechanisms, the generalised muscular spasms characteristics of tetanus are produced. The toxin appears to act by selective cleavage of a protein component of synaptic vesicles , synaptobrevin II, and this prevents the relase of neurotransmitters by the cells.

Clinical Significance

A powerful toxin elaborated by *C tetani* ies responsible for causing a nerologic disorder characterised by increased muscle tone and generalised spasms I.e tetanus. The incubation period of tetanus varies from four days to serval weeks, but is commonly 6- 12 days.Tetanus occurs in several clinical forms including generalised ,neonatal and localised form.

Generalised tetanus

It is common form.The spasm of the masseter muscles is sign in early stage.The characteristics of tetanus result from sustained contraction of facial muscles known as risus sardonicus. Other symptom includes persistent back spasms (opisthotonos).Gradually, other voluntary muscles are also affected .In more serve disease ,autonomic nervous system became involved leading to

sweating, hyperthermia, cardiac aarrhythmias ,and fluctulation in blood pressure. Death occurs in 15-60%cases usually due to paralysis of chest muscles which leads to respiratory failure.

Neonatal tetanus

Infection is occasionally encountered and typically originated from the umbilical stump ,which then progress to generalised disease .Prognosis is very poor in infants whose mothers are non immune and mortality rate exceeds 90%.

Localised tetanus

It is uncommon form and characterized by persistent spasm of the musculature at the site of primary infection and disease remain confined at the injury site .Cephalic tetanus is rare variant of localised tetanus which follows head injury or ear infection .It is associated with dysfunction of one or more of carnial nerves, most commonly carnial nerve vII .Incubation period is a few days ,and mortality is high.

Laboratory Diagnosis

The laboratory diagnosis of tetanus involved the isolation and identification of *C tetani* and the detection of toxigenicity is isolated by mouse toxicity testing. The latter is the definitive test for the laboratory diagnosis of tetanus. Sero diagnosis is not relevant because the clinical disease dose not result in the production of tetanus antitoxin.

Isolation and Identification of *C tetani*

Suitable Specimen : Wound swab ,exudates or tissue from wound.

Diagnostic Method

1. Direct smear and Gram's –staining: Gram-positive bacilli with terminal spore and drum-stick appearance.

2. Culture: Culture is done in blood agar or in Robertson's cooked meat medium. C tetani produces swarming growth on anaerobic blood agar after 1-2 days of incubation .Culture result are positive in about 30% cases. The specimen is also inoculated into three tubes of Robertson's cooked meat broth. One of these tubes is heated at 80®c for 15 mins ,the second for 5 mins and the third left unheated. The ideas of heating the inoculated tubes is to destrory vegetative bacteria but the tetanus spores remains viable. The inoculated Robertson's cooked meat are incubated at 37®c for about 4-5 days and sub cultured on one half of blood agar plate daily for up to 4 days.

3. Toxigenicity test: For demonstration of toxigenicity animal inoculation is done .Two mice are taken,0.2ml of 2 to 10days old cooked meat broth culture filtrate is injected subcutaneously into right side of tail of one mouse (test animal);same amount of toxin filtrate is injected into other (control).In positive case, the test animals develops symptoms with stiffness and spasm of the tail and inoculated hind limb within 12 to 24 hrs which subsequently spreads to the rest of the body .Rigidity start on leg on the opposite trunk ,forelimbs, in that order .Death occurs in 1-2 days. The control animals shows no change.

Treatment

Treatment must be initiated immediately after clinical diagnosis without waiting bacteriological diagnosis. The treatment of tetanus includes:

Mild tetanus: Mild cases of tetanus can be treated with

- Tetanus immunoglobulin (TIG) also called tetanus antibodies or tetanus antitoxin. It can be given as intravenous therapy or by intramuscular injection.
- Metronidazole IV for 10 days.
- Diazepam oral or IV

Severe tetanus: Severe cases will require admission to intensive care. In addition to the measures listed above for mild tetanus.

- Human tetanus immunoglobulin injected intrathecally (increases clinical improvement from 4% to 35%)
- Tracheotomy and mechanical ventilation from 3 to 4 weeks .Tracheotomy is recommended for securing the airway because the presence of an endotracheal tube is a stimulus for spasm.
- Magnesium ,as an intravenous(V) infusion to prevent muscle spasm.
- Diazepam as a continuous IV infusion
- The autonomic effect of tetanus can be difficult to manage and may require IV Labetalol, magnesium, clonidine or nifedipine.

In order to survive from tetanus infection the maintenance of an airway and proper nutrition are required. An intake of 3,500 to 4,000 calories at least 150 g of protein per day is often given in a liquid form through the tube directly into the stomach or through a drip into a vein. The high-caloric diet is required because of increased metabolic strain brought on by the increased muscle activity .Full recovery takes 4 to 6 weeks because body must regenerate destroyed nerve axon terminals.

Prevention and control

Tetanus is fully preventable disease by active immunization with tetanus toxoid.

1. Active immunization: usually immunization with tetanus toxoid is carried out in all children during first year of life(beginning at 6-8 weeks of age)by DPT vaccine containing diphtheria toxoid , pertussis vaccine and tetanus toxoid, which confers immunity for 7 to 10 yrs. Immunisation in adult consists administration of 3 doses of tetanus toxoid ,IM: 1st and 2nd doses are administered 4-8 weeks apart, and 3rd dose is given 6-12 month after the 2nd dose. A booster dose is given after 5 yrs on entry into school and after booster dose is given every 7 to 10 yrs throught life.

2. Combined immunization: In emergency after an injury it is ideal to immunize the individual with tetanus toxoid in one arm along with administration of 1,500 I.U of ATS or 250 units of HTIG in another arm. Penicillin is administrated before wound cleaning. The other doses of toxoid are injected at appropriate intervals.

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Lymphatic Filariasis

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Introduction

Lymphatic filariasis, commonly known as elephantiasis is a neglected tropical disease. It refers to a parasitic infection that causes extreme swelling of legs and arms. The disease is caused by filarial worm which is transmitted from human to human via the female mosquito when it takes a blood meal.

Elephantiasis is typically characterized by a thickening of the skin and subcutaneous tissue that gives rise to the groosly enlarged and swollen limbs that earn the condition its name.the painful and profoundly disfiguring visible manifestations of the disease, lymphooedema, elephantiasis and scrotal swelling occur later in life and lead to permanent disability.

Epidemiology

Lymphatic filariasis affects more than 120 million people in 80 countries worldwide and is an extremely painful, debilitating and disfiguring disease. The disease causing agent is found mainly in tropical and sub-tropical climates of Asia, Africa and South America.

Causative agent and transmission

The disease is caused by the thread –like parasitic filarial worms Wuchereria bancrofti, Brugia malayi, Brugia timori which live in the lymphatic system and can cause extreme swelling of the extremities and genitals. Wucheria brancrofti is responsible for 90% of the cases.

The disease is transmitted to humans by mosquitoes. Mosquitoes are infected with microfilariae by ingesting blood when biting an infected host. Microfilariae mature into infective larvae within the mosquito. When infected mosquitoes bite people, mature parasite larvae are deposited on the skin from where they can enter the body. The larvae then migrate to the lymphatic vessels where they develop into adult worms, thus continuing a cycle of transmission. Culex and Anopheles mosquitoes mediate the transmission. Adult worm lodge in the lymphatic system and disrupt the immune system. The worms can live for an average of 6-8 years and during their life time produce millions of microfilariae.

Clinical Manifestations

Lymphatic filariasis infection involves asymptomatic, acute and chronic conditions.

- Majority of the infections are asymptomatic showing no external signs of infection but still cause damage to the lymphatic system and the kidney and alter the body's immune system.
- Acute phage or the inflammatory phase is characterized by filarial fever, lymphoedema, lymphadenitis and adenolymphangitis(inflammation of lymph channels).
- Chromic condition leads to lymph varices, hydrocele(scrotal swelling), elephantiasis (skin/tissue thickening) and chyluria.involvement of breasts and genital organs are common.

Pathogenesis

Fourth stage larvae(moulting stage from L3)and adult worm are pathogenic. They are responsible for inducing various pathological changes in filariasis. The following stages occur in pathogenesis of lymphatic filariasis:

- Dilatation of lymphatic vessels
- Infection of the lymphatic vessels(lymphangitis)
- Obstruction of the lymph nodes.

Diagnosis

Definitive diagnosis is made by demonstration of the microfilariae in the peripheral blood. Chylous urine and hydrocele fluid are the other less important specimens. Blood is collected as follows:

Nocturnal periodic *W. bancrofti*: between 10pm and 4 am at night.

Subperiodic nocturnal : between 8pm and 10 pm during the night.

Subperiodic diurnal : between 2pm and 6 pm in the afternoon.

Methods of examination for parasitic diagnosis include:

1. Blood microscopy
2. Diethylcarbamazine (DEC) provocation test
3. QBC
4. Urine microscopy
5. Microscopy of hydrocele fluid and lymph node aspiration

A newer immunodiagnostic test, based on the detection of antigens of *W. bancrofti*, is highly specific and sensitive. An added benefit of this test is that blood samples do not have to be taken at night.

The serological tests include:

- Demonstration of circulating antibodies
  - Indirect haemagglutination assay
  - Indirect fluorescent antibody
  - Enzyme linked immunosorbent assay
- Demonstration of circulating antigens
  - Two monoclonal antibody based ELISA.

Cellular assay include filarial skin test and invitro lymphocyte response to filarial antigen but neither of these are specific. PCR can also be used but it is positive only when circulating microfilariae are found in the peripheral blood.

Ultrasound of the scrotal area is the only noninvasive method for the detection of live adult worm in the affected lymph nodes. The live adult worms are identified by a distinctive pattern of their movement known as filarial dance sign.

Management

The medical management of a filarial infection should be specific and based on the microfilariae isolated or antigenemia detected. Mass drug administration reduces the transmissionof filarial infection and filarial morbidity by decreasing the burden of microfilaremia resulting in suboptimal levels for transmission by disease vector. The World Health Organization's Global Programme to eliminate lymphatic filariasis aims to stop the spread of transmission and to lessen the severity of the disease in those already infected. In those areas suspected to be highly disease endemic, DEC and albendazole are distributed once a year for upto six years to help stop the spread of infection. Hygiene education efforts are also aimed at alleviating acute episodes of filarial fevers and have been particularly effective at lessening the social stigma associated with the most prominent outward manifestations of the disease.

Preventive measures

The prevention and control depends upon mosquito control and chemotherapeutic control. He Mosquito control is aimed to break to break the cycle of transmission by controlling mosquito vectors which includes the following :

- Environmental control by effective drainage and sewage management system.
- Clinical control by insecticides like DDT, malathion etc.
- Biological control by use of carnivorous bacteria(*Bacillus sphericus)*,fish (*Poecilia reticulate mollinensis*)and spore forming bacteria (*Bacillus thurengenesis).*

The objective of chemotherapeutic control is to:

- Lowering the transmission by treating clinical cases.
- Interrupt the transmission of infection.

Elephantiasis in Nepal

In Nepal, out of total population (approximately 23.2 million), 13.9 million (60%) are estimated to be at risk of infection. It is endemic in different regions of Nepal. Similar studies were also carried out by Sherchand et al. (2003), Ghimire et al. (2003), Manandhar (2001), and Pradhan et al.(1997). Eleven persons have been found suffering from elephantiasis in five VDCs of Beni (Myagdi), according to a survey. The survey was conducted recently at different five VDCs of the district- Belkhola, Histan, Ghar, Ratnechaur, Pulachaur. The survey was held under the elephantiasis elimination campaign 2015. According to the District Public Health Office, Panchthar, elephantiasis infection has been found in 80 people in the district. A mass anti-filariasis medicine administration campaign has been conducted in the district to prevent this disease. The three-day anti-elephantiasis campaign was conducted in 36 districts of the country from 12th March,2016. It is said that a total of 13 million people would be distributed the anti-elephantiasis medicines.

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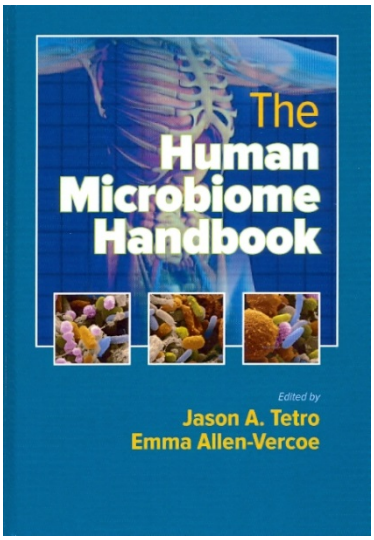
Book Review: The Human Microbiome Handbook

The Human Microbiome Handbook, edited by **Jason Tetro** and **Emma Allen-Vercoe**, is published by DEStech Publications, Inc., best known for advanced publications in engineering and health science. March 2016, 370 pages, 6x9, Hardcover, ISBN: 978-1-60595-159-1, **Price: $142.50**. www.destechpub.com. Go to the link above to examine 20% of the book.

For the first time, a group of the world's leading scientists in human microbiome research have come together to provide an extensive examination of the past, present and future of the microbial effects on health. This compilation offers any reader an opportunity to jump in at any chapter any learn how bacteria and other species influence our digestion, immunity, metabolism, genetics, and psychology.



This book offers a concise technical reference covering human microbiome research and its ramifications for medicine and nutrition. The initial chapters furnish a scientific explanation of the microbiome in general and its ecology. The book then provides a detailed investigation of microbial populations as these pertain to physiology, metabolism and immunology. The final portions are devoted to exploration of the microbiome's effects on chronic and autoimmune diseases and include assessments of clinical therapies and nutritional interventions designed to alter the microbiome to mitigate chronic health conditions.

Some of the highlights of the book include:

- Critical reference explains strategies of microbiome research in humans
- Evaluates medical and nutritional therapies for modifying the microbiome

- For healthcare researchers, nutritionists, microbiologists, and medical professionals

Chapters Included

- Ecology of the Human Microbiome
- From Birth to Old Age: Factors that Shape the Human Gut Microbiome
- Microbial Biochemical Processes Critical to Human Health
- The Gut Microbiome: Pathways to Brain, Stress, and Behavior
- Effects on Immunity
- Fecal Microbiota Transplantation in Gastrointestinal Disease
- Probiotics and the Microbiome

About the Editors

Emma Allen-Vercoe completed her BSc. (Hons.) In biochemistry from the University of London, UK, and her PhD in molecular microbiology. Allen-Vercoe's lab at the University of Guelph in Ontario, Canada, houses an innovative platform that facilitates culturing of human microbiota ecosystems in vitro and permits study of microbial ecosystem responses to stressors affecting human health.

Jason A. Tetro earned his BSc. (Hons.) in Applied Biochemistry from the University of Guelph, Canada, and continued his research at the University of Ottawa as a member of the Centre for Research on Environmental Microbiology and the Emerging Pathogens Research Centre. His research primarily focused on the interruption of pathogen spread in food, blood, and both animate and inanimate surfaces.

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Immunomodulatory agents for Bacteria and Bacterial Vaccine

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Introduction

Immunomodulators/ Immunomodulatory agents are those extrinsic or intrinsic substances that elicit the immune response of antigens. In vaccines these substances are called as adjuvants. In animal health, the dependence on modified live antigens were reduced by the use of adjuvants, which have the potential to prevent disease in immunocompromised animals. Subunit vaccines doesn't have the features of an original pathogen and often poorly immunogenic. In such a situation the preferred criteria for the new-generation vaccine development is to add highly purified synthetic adjuvants. Vaccines that include attenuated live organisms or whole inactivated organisms usually not require adjuvants. Immunomodulatory agents derived from bacteria are potent stimulators of the innate immune system. Agents mainly include microbial derivatives, monophosphoryl lipid A, CpG oligonucleotides, cholera toxin and heat labile toxin from *Escherichia coli*. A wide variety of microbes and their products act as pathogen associated molecular patterns and stimulate TLRs. Which inturn activates macrophages and dendritic cells to produce cytokines such as IL12 and IL1. Thereby increase the T cell pool and mount up the adaptive immune response. Depending on the specific microbial product, they may enhance either Th1 or Th2 responses.

Commonly used bacterial derived immunomodulators in veterinary vaccines

Most potent immunostimulator of bacterial origin is *BCG* (Bacillus Calmette-Guerein), the live attenuated vaccine strain of *Mycobacterium bovis*. It elicits both B and T cell mediated immune responses, phagocytosis, allograft rejection, and also used to treat upper respiratory tract infections, equine sarcoids and ocular squamous cell carcinoma in horses. Freund's Complete

Adjuvant (FCA) is an emulsion adjuvant which is effective in potentiating cellular and humoral antibody responses to injected immunogens. Cord factor/P3 which is chemically trehalose dimycolate from the mycobacterial cellwall and muramyl di peptide which is a glycopeptides from Mycobacteria cell wall. Both cell wall constituents causes tumour regression and non specific immunity to bacterial infections. Killed anaerobic corynebacterium (*Propionibacterium acnes*) enhances antibody production and activates macrophage through TLRs and stimulates cytokine synthesis. It is used for treating staphylococcal pyoderma, malignant oral melanoma in dogs, feline leukemia in cats, and respiratory disease in horse. The bacterial (18 *Escherichia coli* strains) extract Broncho-Vaxom (OM-85BV) used in recurrent respiratory tract infections is an immunomodulator.

Bacterial toxins such as cholera toxin, from *Vibrio cholerae* and heat labile toxin from *E. coli* are potent mucosal adjuvant. It increases antigen presentation by B cell and differentiation to IgA secreting cells & serum IgG. It also makes interaction with T cells and increases cytokine synthesize. Bacterial DNA (CpG motifs) acts as an immunomodulator. It is 20 times often potent than mammalian DNA. Unmethylated CpG dinucleotides of bacteria act through TLR 9 and trigger plasmacytoid dendritic cell and B cells of humans and higher mammals to increases the production of various kind of cytokines and proinflammatory cytokine. Up-regulation of MHC II and co-stimulatory molecules enhances Th1 response. Cyclic dinucleotides like c-di-GMP can inhibits *Staphylococcus aureus* infection and can be used in animals as an immunomodulator, immunotherapeutic, immunoprophylactic, or vaccine adjuvant. TLR4 ligands such as bacterial lipopolysaccharides (or their derivatives) enhance antibody formation and have no effect on cell mediated responses, but they can break T cell tolerance, and they have a general immunostimulatory activity. It stimulates macrophages and activates production of IFN γ, direct B cell mitogen activity, increase both T and B cell pool and also activate complement by alternative pathway. In low acid condition it hydrolyses Lipid A and form a derivative called MPL (Monophosphoryl Lipid A). It is a derivative of *Salmonella Minnesota* R595 LPS. AS04,

one of the licensed adjuvant is a combination of MPL and Aluminium hydroxide or phosphate. Action is mediated through activation of dendritic cell and production of cytokines and co stimulatory molecules. The TLR5 ligand bacterial flagellin is an adjuvant that promotes mixed Th1 and Th2.

In most of veterinary bacterial vaccines like HS vaccine, Raksha HS+ BQ vaccine, ET vaccine, Botuthrax, Pulpyvax and Rotavec Corona vaccines, the most widely used adjuvants are Aluminum salts. Commonly used ones is Alum (aluminium potassium sulphate) and Aluminium hydroxide. Aluminium adjuvants activate an intracellular innate immune response system called Nalp3 inflammasome. Production of the pro-inflammatory cytokines IL-1β and IL-18 by macrophages in response to alum *in vitro* required intact inflammasome signaling. Its mode of action is by forming a "depot" at the site of injection. The antigen is released slowly from the depot, leading to a prolonged exposure to antigen-presenting cells and lymphocytes. Also promote antigen phagocytosis by APCs - dendritic cells, macrophages, and B cells. It also induce inflammation resulting in the recruitment of neutrophils, eosinophils, & macrophages. This also boosts Th2 type of immune response – IgG1, IgE. Another inorganic salt is Calcium phosphate - $Ca_3(PO_4)_2$, which is a normal constituent of the body. So get better tolerated and absorbed than other adjuvants. It also entraps Ags and releases slowly and elicits high amounts of IgG Abs & much less of IgE Abs. It is mainly used with DPT vaccine.

Conclusion

One of the main key for the success of the vaccine depends on the selection of the adjuvants. Understanding the complex mechanism of immune system and the clinical success of existing immunomodulators are needed for the discovery of future immunomodulatory agents. An effective, safer and more cost effective immunomodulators help in the development of superior vaccines and anti-infective therapeutics. Subunit or inactivated bacteria/viruses vaccines are going to mainly comprise the next generation veterinary vaccines. These vaccines would require

proper immunostimulators and delivery systems to afford a long-term protection from infectious diseases in animals. There is an urgent need for the development of new and improved adjuvants for veterinary and human vaccines.

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