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Review on Diagnosis of Tuberculosis in Animals and Humans

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ABSTRACT

Tuberculosis (TB) is an infectious disease that has a major impact on health of human and animals. Mycobacterial infections have been difficult to study due to lack of diagnostic facilities. Diagnosis of TB in cattle and other species is often made based on history, clinical findings of capricious appetite, fluctuating temperature, chronic cough, tuberculin skin test which could be single intradermal or comparative intradermal tuberculin skin test and necropsy findings of caseous or calcified foci in varies tissues of the body. In addition to these modern diagnostic techniques such as blood-based laboratory tests which include Lymphocyte proliferation assay, Gamma interferon assay, Enzyme linked immuno sorbent assay, have been developed for the detection of Mycobacterial infection. In humans diagnosis is performed in better way than in animals and it is often based on history, clinical findings of sputum examination, Chest X-ray and tuberculin skin test. In laboratory, all the specimens colleted from human TB patients and tuberculin positive cattle will be processed and cultured. Culture is the golden standard technique for diagnosis of TB and the specimen which could be milk, sputum or tissue is inoculated into two slants of Lowenstein-Jensen media one with pyruvate and the other with glycerol. Histopathological examination of the tissue from tuberculin positive slaughtered cattle will also be conducted to appreciate the TB lesion under microscope. Finally, Biochemical test such as Niacin producing tests, Nitrate reduction tests, Pyrazinamidase deamination tests, drug sensitivity tests and Molecular techniques which include The insertion sequence, Direct repeats region, Variable number tandem repeats and the Polyguanine-cytocine rich sequence could be carried out to characterize Mycobacterial species.

Keywords: Tuberculosis, Animals, Humans, Diagnostic techniques.

1. INTRODUCTION

TB is an infectious disease caused by *Mycobacterium* species that has been a major health risk to man and animals for more than a century. It is widely distributed throughout the world affecting all age group of human and animals. It is being responsible for more deaths than any other bacterial disease ever today [1]. The varies forms of the disease have many features in common but the exact pattern according to the species of Mycobacterium involved species of the animal affected [2].

Mycobacterial diseases have been difficult to study and data on bovine TB is always under estimated due to lack of diagnostic facilities and epidemiological investigations. A presumptive diagnosis of TB in cattle and other susceptible species is done based on history, clinical findings, tuberculin skin tests and necropsy findings [3]. In addition to these modern diagnostic techniques such as *in vitro* lymphocyte proliferation assay, Gamma interferon assay [4] and Enzyme linked immuno sorbent assay (ELISA) have been developed for the detection of Mycobacterial infection.

Mycobacterial disease have been difficult to study and data on bovine TB is always under estimated due to lack of investigation and A presumptive diagnosis of TB in cattle and other susceptible species is often made based on history, clinical findings, tuberculin skin tests and necropsy findings [3]. Similarly in case of routine work diagnosis of TB in human is usually based on

history, clinical findings (Sputum, Ultrasound, Erythrocyte sedimentation rate, etc). Direct microscopy of sputum and Histopathological examination of affected lymph nodes, Microscopy of direct smear for acid fast bacilli is the most commonly used methods for diagnosis of TB but its major disadvantage is discouragingly low sensitivity. Based on study under gone in several African laboratories indicated that sensitivity of direct microscopy ranged from 8.8 to 46.4 % [5].

The first step in diagnosing TB is to include the disease in differential diagnosis, followed by demonstration of the causative agent in clinical specimens. Provisional detection of acid fast- bacilli (AFB) in pulmonary and non pulmonary specimens. TB diagnosis differentiates between active and inactive TB and treatment differs. Active TB is a series and contagious condition that requires isolation of the patient and aggressive treatment with several drugs over several months [6]).The world health organization estimated that approximately 8.8 million persons develop TB and 1.8 people died of this disease. Although most cases are caused by *Mycobacterium tuberculosis*, reliable information on *Mycobacterium bovis* (*M. bovis*) is not generally available on the incidence in developing Countries, where diagnosis is based on microscopic examination and techniques for differentiating the two organisms are not widely accessible [7].

Bovine TB can be eliminated from a country or region by applying a test and slaughter policy, if other reservoirs of do not exist. In developing Countries, however, this method is not socially and economically accepted. So, alternative strategies have to be used. these are slaughter house surveillance and trace back of tuberculous animals to herds origin [8] and varying forms of a test and segregation with the test and slaughter programme applied on the final stage of eradication [4].

Objective of this paper is to review updated information on diagnosis of TB.

2. DIAGNOSIS OF TB IN ANIMALS

2.1 Clinical examination

Because of chronic nature of the disease and multiplicity of signs caused by the variable localization of infection, TB is difficult to diagnose based on clinical examination [1].The main clinical findings observed in animals in advanced cases are a capricious appetite and fluctuating temperatures, Pulmonary blow characterized by a chronic cough, which is stimulated by squeezing the pharynx or exercise and is common in the morning or during cold weather. Affected animals tend to become more docile and sluggish [3].

Uterine TB is characterized by drops of yellow fluids, which may have high influence on reproductive performance of the cow. Tuberculous mastitis can be recognized as agranulomatous inflammation of the mammary gland with hard swelling on the bottom of the udder. This is a major from public health point of view and as means of transmission to the Calf [9].

2.2. Tuberculin skin test

A general form of any superficial skin or intracutaneous test used to diagnose TB. The test is based on hypersensitivity to tuberculin, a concentrated preparation of TB antigen, the standard preparation of which is purified protein derivatives [10]. Since Robert Koch used it for the first time in 1891 in his effort develop a treatment to TB, tuberculin test have successfully been worldwide for the diagnosis of TB in cattle. Over the years, different reagents and techniques have been used, ranging from subcutaneous injection of Koch's old tuberculin prepared from *Mycobacterium tuberculosis* (*M. tuberculosis*), with assessment by determining the animal's temperature on repeated occasions, to measuring the degrees of tissue reaction in millimeter at injection site. The most important types tuberculin used for this purpose is purified protein derivatives (PPD).Whose major antigenic component is probably a heat shock protein [11]. When PPD is injected intradermally into normal animals, there is an allergic skin reaction, which is typically delayed type hypersensitivity reaction. There are two types of tuberculin skin tests, the single intradermal skin test (SIDT) and comparative intradermal test (CIDT). The choice between the two tests generally depends on the actual prevalence of TB and on the environmental level of infection with other sensitizing organisms [3].

2.2.1. Single intradermal tuberculin test

It is applied by intradermal injection of bovine tuberculin purified protein derivatives into skin fold and subsequent detection of swelling as a result of delayed hypersensitivity [3]. The singe intradermal test involves the inoculation of mycobacterial antigen, prepared from a filtrate of culture of either *M. bovis* or *M. tuberculosis* [2].

2.2.2. Comparative intradermal tuberculin test

The comparative test depends on the greater sensitivity to homologous tuberculin. Avian and bovine tuberculin are injected simultaneously into two separate sites on the same side of the neck, 12cm apart and one above the other and the test read 72 hours later. The greater of the two reactions indicates the organism responsible for the sensitization (Figure 1). The comparative test is adequate to differentiate between vaccination against John's disease and TB and the distinction is either the longer the time between vaccination and testing [3].

Figure 1: Comparative intradermal tuberculin skin test
(http://tacklingbovinetb.tumblr.com/post/53187886605/testing-for-tb-the-testers-share-their)

Interpretation of tuberculin skin test as indicated by Allan, (2008) is as follows.

A. If the reaction of bovine PPD is >4mm greater than to avian PPD the test is considered Positive.
B. If the reaction of bovine PPD is between 1and 4mm greater than to avian PPD the test is considered Inclusive.
C. If the reaction of bovine PPD is <1mm less than to avian PPD the test is considered Negative.

2.3. Post mortem examination

Necropsy finding of the classic tuberculous granuloma are often suggestive of the disease and tuberculous granuloma usually be found in any of the lymph node, but particularly in bronchial, retropharyngeal and mediastinal lymph nodes [2]. On gross necropsy examination TB may be provisionally diagnosed when caseous or calcified foci are involved in various tissues of the body, but this is difficult in the initial stage of the disease. Early lesion consists of small caseous and calcified granulomatous nodules mainly in the retropharyngeal, broncho-mediastinal and occasionally the mesenteric lymph nodes (Figure 2). In advanced and generalized type TB which is known as military TB, lesions are the size of millet seeds distributed all over the visceral organs of the body mainly in the lungs, liver, spleen, midiastinal and retropharyngeal lymph nodes, mammary glands and intestine [12].

Postmortem examination should be supported by histological examination of samples stained with hematoxylin and eosin [4]. Typical lesions caused by *M. bovis* in cattle are described as having a center of caseous necrosis with some calcification and a boundary of lymphocytes, neutrophils and epitheloid cells. An outer border of fibrous connective tissue is always present, giving a lesion a focal appearance and providing encapsulation to some extent, which may limit the spread of infection since the lesion, are not conclusive, it is necessary to demonstrate the etiological agent using Ziehl-Neelsen stain [13].

2.4. Laboratory techniques

2.4.1. Sputum /FNA/Ascitic fluid processing and isolation of Mycobacteria

Sputum samples are collected and decontaminated by adding 2% sodium hydroxide (1:3 ratios), agitated in a vortex mixer for 15 minutes at room temperature and centrifuged at 3,500rpm for 15 minutes at 4 degree centigrade. The supernatant are suspended in 2ml of sterile physiological saline. One to two drops of 0.05%phenol red indicator is added to indicate the PH change and the neutralized using concentrated Hydro chloric acid until the color changed to yellow. The sediment are inoculated into two slants of Lowenstein-Jensen media one with pyruvate and the other with glycerol. Fine needle aspiration and ascitic fluid samples are collected from TB lymphadenitis and TB peritonitis cases, respectively and processed as sputum samples and inoculated to the media [4].

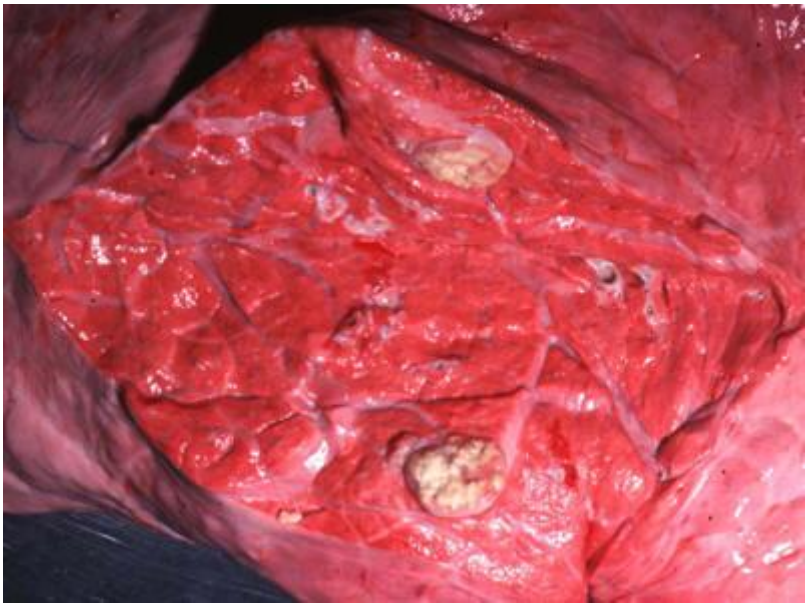

Figure 2: Tuberculous lung.
(http://www.uwyo.edu/vetsci/undergraduates/courses/patb_4110/2009_lectures/7_resp_cattle/html/class_6.jpg)

2.4.2. Milk samples processing and inoculation of Mycobacteria

Milk samples are centrifuged at 3000rpm for 15 minutes and the supernatant are discarded. The sediments are suspended in 2ml of sterile physiological saline solution and decontaminated with equal volume of sterilized 4% sodium hydroxide solution. One to two drops of 0.05%phenol red indicator is added and the mixture is incubated for 30 minutes at 37degree centigrade and then neutralized using concentrated hydro chloric acid. The suspensions are centrifuged at 3000rpm for 15 minutes at 4% and the sediments are used for microscopic and cultural examination [14].

2.4.3. Histopathological examination

In case of histopathological examination, tissue samples are formalin fixed, embedded in paraffin cut into 6micrometer sections and slide is prepared using standard hematoxylin. The collected tissue specimens from the tuberculin positive cattle or to be purchased and slaughtered are transported to the laboratories and subjected to histopathological examination as follows the finding of grey or yellow nodules with caseous centers are considered as positive for microscopic TB lesions. On the basis of this definition the collected specimen are fixed in neutral buffered 10% formalin and transported to the laboratory [8]. In the laboratory the fixed tissue are trimmed, dehydrated in series concentrations of alcohol. Embedded in paraffin, sectioned at 2 micrometer deparaffinized in xylazine. Two smears are prepared from each samples and stained with haematoxylin-eosin and Ziehl-Neelsen. The result is interpreted based on the presence or absence of histological lesions having a center of necrosis usually with some calcification, boundary of epitheloid cells giant cells and a few to numerous lymphocytes by haematoxylin eosin stain and demonstration of AFB by Ziel-Neelsen staining [15].

2.4.4. Culturing of the specimen collected from human and cattle.

All specimens collected from human TB patients (Sputum, FNA, asitis) and from tuberculin cattle positive cattle (milk, nasal secretion, and tissue specimens) are processed and prepared for Mycobacterial culture [12]. Primary isolation is done onto two Lowenstein-Jensen media favoring growth of *M. bovis* with the addition of 1%sodium pyruvate. Thick inoculums of sediment are smeared on the surface of medium slopes and cultured tubes are inoculated at 37% degree centigrade and 5% Carbon dioxide for about 8-12 weeks. Growths for Mycobacteria are checked every week. Positive culture are sub-cultured onto another set of media and incubated for 3-4 weeks for further identification [1]. The colonies are subjected to the biochemical tests for further identification and characterization [16].

2.5 Bacteriology

2.5.1. Direct Microscopy

Examination of Smears prepared from sputum, tissue, milk, urine and other samples taken from suspected individuals are stained with the Ziel-Neelsen staining method and organisms appear as red due to their acid-fast property. However direct microscopic examination of nasal discharge and tissue sections from animals have limited diagnostic value [9]. The Ziel-neelsen acid-fast stain is used to stain smears from lesions and other smears stained by fluorescent dyes such as auramine, acridine orange or flourochrome can be examined under ultraviolet microscope [13].

2.5.2. Culture

The definitive diagnosis of TB depends on the isolation and identification of the Mycobacteria in specimens taken from suspected individuals [17]. The main factors that influence the success of primary isolation of *M. bovis* from clinical specimen are the culture media, the

decontamination procedure and incubation condition [12]. Lymph nodes are trimmed of excess fat, macerated in phenol red nutrient broth (Becton-Dickinson, Cockeysville, Maryland, USA) decontaminated in 2% NaOH for 10 min, centrifuged for 20 min and the supernatant fluid is decanted. Stonebrickmedia were inoculated with the sediment. The inoculated media are incubated at 37°C for 12 weeks and examined for colony formation every week. The contaminated samples are discarded and inoculated once again. The growing colonies are classified to species using standard growth and confirmed by PCR [16]. Before inoculation the samples are digested, decontaminated and concentrated. The commonly used decontaminants for *M. bovis* preparation are Hexadeculphridinium chloride at both 0.075%W/V, Benzalkonium chloride (Zepharin) 0.25%W/V, Oxalic acid 5%W/V and 100% sodium hypo chlorate [4].

The growth on the agar base media is much faster than on the egg based media with mean time to the first appearance of colonies being 27 days and 28 days on B83 and 7H11, respectively compared to 36 days on stonebrincks medium [12]. It is done by using modified 7H11 agar medium, *M. bovis* from infected tissue can be isolated within three weeks. However, the agar medium is highly liable to contamination even after decontamination of the specimen, so egg base media is preferable for Mycobacterial growth [1]. Identification is done by observation of the growth on Lowenstein-Jensen pyruvate media based on the criteria for distinguishing *M. TB* from *M. bovis* on primary culture, *M. TB* and *M. bovis* show difference in growth characteristics. On egg media, the human type produces a dry wrinkled, warty growth; luxuriant colonies are yellowish roughened surface. The bovine type grows much less luxuriantly and cohesive than that of human strain [1].This type of growth is referred to as dysgonic in contrast to the more profuse growth of *M. TB*, which is described as eugenic. *M. avium* is moist, slimy, glistering, luxuriant, and frequently yellow to gray [18].

2.5.3. Biochemical and drug sensitivity tests

Identification of Mycobacterial isolates depends upon colony morphology. Staining characteristics as well as biochemical tests such as niacin production, nitrate reduction, urease test and pyrazinamidase activity (deamination of pyrazinamide to pyrazinoic acid in 4-7 days) and drug sensitivity test [19]. *M. bovis* grows slowly and subculture requires a minimum of 14 days for colonies to become visible on media [12]. The most useful biochemical tests for an *M. bovis* are susceptible to Thiopene-2-Carboxylic acid hydrozide and the biochemical test takes 3 to 4 weeks [4].

2.5.3.1. Niacin production test

The niacin test is conducted only on 3-4 weeks on old pure sub-culture growth on Lowenstein-Jensen media which showed heavy growth, in order to avoid false negative result [6]. A niacin positive result on Mycobacteria are considered strongly indicative for *M. TB* and an attempt are done to classify the niacin negative isolates as *M. bovis* and atypical Mycobacterium according to the morphology of their colonies, growth rate and pigment production [17].

2.5.3.2. Nitrate reduction test

M. TB produces the enzyme nitro reductase, which catalyze the reduction of nitrate to nitrite [17]. Some of the other atypical Mycobacterial species that reduce nitrate could be differentiated from *M. TB* by the pyrazinamidase test. The development of red color up on addition of the reagents indicates the presence of nitrite and positive result for *M. TB*. Most Mycobacterial cultures to be tested for nitrate reduction are examined 3 to 4 weeks after inoculation into the sub-culture medium that is after visible colonies appear [20].

2.5.3.3. Pyrazinamidase deamination test

One of the most useful biochemical tests in the classification scheme for an *M. bovis* is susceptible to pyrazinamidase [9]. The diamination of pyrazinamidase to pyrazinoic acid and ammonia is helpful in separating the weakly niacin positive strains of *M. bovis* from member of the *M. avium* complex. The enzyme acts to split pyrazinamide to pyrazinoic acid in 4-7 days. *Mycobacterium bovis* is pyrazinamidase negative even after 7 days, whereas both *M. TB* and *M. avium* complex are positive within 4 days [17].

2.5.3.4. Drug sensitivity test

The most useful biochemical tests in the classification scheme for a *M. bovis* are susceptibility to Thiophene-2-Carboxylic acid hydrozide sensitivity test is used to distinguish *M. bovis* from *M. TB* other and other non-chromogenic slow growing Mycobacteria [16]. *M. bovis* is sensitive to low concentration of Thiophene-2-Carboxylic acid hydrozide. *M. TB* and other species of Mycobacteria generally resist to this compound [17].

2.6. Animal inoculation

It is historical method of distinguishing between the tubercle bacilli that is based on the variation in the pathogenicity of each for different laboratory animals and animal inoculation is now rarely performed because of aesthetic and economical reasons as well as the risk infection to laboratory staff [13]. Inoculation in guinea pig was earlier widely used for diagnosis of TB but now regarded as obsolete because it is cumbersome, costly and less sensitive than culture. Guinea pig inoculation is however, superior to culture for isolation of *M. bovis* from clinical samples [20].

2.7. Blood-based laboratory test

2.7.1. Lymphocyte proliferation assay

The invitro assay detects cellular reactivity to tuberculin antigen in whole blood samples. The assay has scientific value, but it is not used for diagnosis because the test is time consuming and the logistics and laboratory exclusion is complicated. It requires long incubation times and the use of radioactive nucleotides [4]. The method involves incubating lymphocytes in the whole blood diluted with tissue culture media for 3 to 5

days and then using radioactive nucleotides to detect the level of cell proliferation [14].

2.7.2. Gamma interferon assay

Gamma interferon assay is used to conduct an invitro blood based assay of cell mediated immunity to *M. bovis* PPD tuberculin to detect bovine TB infection. Production of gamma interferon from cell is then detected using amino clonal sandwich ELISA. Lymphocytes of animals which are free from *M. bovis* infection will not be stimulated to reduce gamma interferon by Mycobacterium PPD tuberculin [11]. In this test the release of lymphokine in whole blood culture system is measured. The assay is based on the release of gamma interferon from sensitized lymphocytes during a 16-24 hours inoculation period with specific antigen (PPD tuberculin). A sandwich ELISA which utilizes two monoclonal antibodies to bovine gamma interferon is used for the detection of the interferon gamma released by sensitized T-lymphocytes [18]. The sensitivity and specificity varies from 76.8% to 93.6% depending on interpretation method [21].The advantage of this assay over tuberculin test is that animals need only captured once, but series disadvantage is the relatively high cost and the fact that the examination of blood sample has to be started within 8 hours after collection. More over, this test only works with the family bovidae and hence should only be used on cattle, buffalo etc [20].

2.7.3. Enzyme linked Imuno sorbent assay

The ELISA appears to be the best choice sero-diagnostic test for TB and can be a complement, rather than an alternative to test based on cellular immunity [17]. Improvement may be possible by using different antigens, including proteins and peptide glycolipids. More over, in *M. bovis* infected animals an anamnestic rise has been described, resulting in better ELISA results 2-8 weeks after a routine tuberculin skin test. ELISA can be used to differentiate *M. bovis* from other mycobacteria and related microorganisms. The indirect ELISA detects antibodies to *M. bovis* in the serum, its specificity is relatively high but its sensitivity can be very low. It is useful for detecting infectious cases in the short term because it yields good results in advanced cases of pulmonary and disseminated TB [4].

2.8. Molecular techniques

Molecular techniques help the molecular differentiation of isolates, to determine the origin of the out breaks, to understand the link between different out breaks, to show the relationships between domestic TB and wild TB and identify the source of human infection, It is particularly important to emphasize that before the development of molecular typing techniques, the available techniques of strain determination did not allow the differentiation of *M. bovis* isolates [18]. These include the insertion sequence IS6110, the direct repeats region, the poly guanine-cytosine rich sequence and the variable number tandem repeats [1].Because of the slow growth of the causative agent, its isolation, identification and drug susceptibility of the organism and other clinically important bacteria can take several weeks. These methods can potentially reduce the diagnostic time from weeks to days [16].

2.8.1. The insertion sequence IS6110

The insertion sequence IS6110 is considered as specific to the members of the *M. TB* complex and the difference in the location and number of copies of this insertion sequence is a source of polymorphism between isolates. The classical technique used to observe such polymorphism is RFLP-IS6110, still considered as the reference typing technique for *M. TB* for the majority of isolates of *M. bovis*, which harbor only one or a few copies of this insertion sequence. Other techniques based on other genetic areas such as the direct repeats are preferred [22].The differentiation of isolates is necessary if the spread of *M. bovis* infection within and/or between cattle and other animal species, the route of transmission and common source of TB occurrence is to be appreciated. The RFLPs and spoligotypes detected in isolates from different geographical areas in different cattle herds will be analyzed and the DNA types related to particular geographical regions are observed [20].The base pairs used to probe the IS6110 sequence, will be obtained by polymerase chain reaction [16].

2.8.2. The direct repeats region

It is a monolocus area, virtually specific to the *M. TB* complex. Each direct repeats region corresponds to the regular repeats of two types of short sequence; sequences which are all identical called the direct repeats sequence and sequences which are all different called the spacers. The polymorphism between two isolates resides in the fact that one or more spacers can present or absent in the direct repeats region of one isolate and not of the other. It is also related to the number of direct repeats region in different isolates [6]. The major technique used is a reverse line blot hybridization technique called spoligotyping. Spoligotyping is a method a genetic finger printing that will be used to distinguish between different strains of *M. bovis* and will enable patterns of origin, transmission and spread of *M. bovis* to be described. It will also be used to differentiate strains inside each species belonging to the *M. TB* complex, including *M. bovis* and also distinguish *M. bovis* from *M. TB* [4].

The spoligotyping technique is performed by application of the direct repeats region with two primers specific for the direct repeats locus. the polymerase chain product will be hybridized to a spoligo-membrane to which synthetic oligo nucleotides of the direct repeats region of *M. TB* H37 RV and *M. bovis* BCG are covalently bound and detected using a septavidin-peroxidase conjugate [16]. Using mini blotter, up to 45 isolates can be compared at the same time. In addition, in the classical techniques, 43 different oligo spacers are linked to the membrane and theoretically 43 different characters are available for any spacers. But, for each sub-species, some spacers are constantly absent, allowing the differential

identification at the level of sub species [18].This is particularly interesting as this technique can potentially be applied directly to pathological samples, allowing simultaneous bacterial identification and typing without previous primary isolation. In the case of *M. bovis*, spacers3.9, 16 and 39-43 are always lacking [19].

2.8.3. Variable number tandem repeats

In Mycobacteria, the majority of the variable number tandem repeats corresponds to the so called Mycobacterial interspersed repetitive units that are minisatellites structures composed of the bacterial chromosome [23]. The variable number tandem repeats to be analyzed will first amplified using different pairs of primers one for each variable number tandem repeats [19]. Two different techniques are used to characterize the variable number tandem repeats allele present in isolates. The first applies as the simple electrophoresis technique, the amplified products corresponding to each variable number tandem repeats at each loci. In the second case, an automated technique based on the use of fluorescent based DNA analyzer with computerized automation of the genotype, allows simultaneous analysis of multiple PCRs, In this case, one primer of each oligo nucleotide pair of primers is preliminary tagged with different fluorescent dye for each pair [23].

2.8.4. Poly Guanine-Cytosine rich sequence

These sequence, which present guanine-cytosine content of approximately 80% are genetic domains belonging to the so called PE-multi gene family. More than 65 repeats of these highly homogenous genes, which are only present in Mycobacteria, have been found [6].These genes encode the PE-PGRS proteins which are characterized by the presence of abundant glysine and alanine residue, in the terminal poly guanine-cytosine sequence domain. There is evidence that these glysine-alanine repeats act as inhibitors of antigen processing and of subsequent antigen presentation. These poly guanine-cytosine domains could be involved in the generation of immune diversity [8]. This is generated by the detection and insertions which occur most commonly in the glycine rich repetitive regions of the poly guanine –cytosine rich sequence domain. For typing, polymorphism is based on the number and location of the poly guanine-cytosine domain [22].

3. DIAGNOSIS OF TB IN HUMAN

3.1. Medical history

The medical history includes obtaining history of pulmonary TB, productive prolonged cough of three or more weeks, chest pain and hemoptysis. Systemic symptoms include low grade remittent fever, chills, night sweet, appetite loss, weight loss, easy fatiguability and production of sputum that start as mucoid but changes to purulent [10]. Other parts of medical history include prior TB exposure, infection or disease, past TB treatment, demographic risk factors for TB and medical conditions that increase the risk for TB disease such as HIV infection. TB should be suspected when persistent respiratory illness or when healthy individuals does not respond to regular antibiotics [24].

3.2. Microbiological studies

A diagnosis made other than by culture may only be classified as probable or presumed. For diagnosis negating the possibility of TB infection, most protocols requires that two separate culture both test negative [10]. Sputum smears and cultures should be done for acid fast bacilli if patient is producing sputum. The preferred method for this fluorescent Microscopy (aura mine, rhodememine staining), which is more sensitive than conventional, Ziehl-Neelsen [25]. In case where not spontaneous sputum production is a sample is induced, usually by nebulized inhalation of saline or saline with broncho dilator solution. A comparative study found that finding three sputum samples is more sensitive than three gastric washing [26]. In patient incapable of producing sputum sample, common alternative sample source for diagnosing TB include gastric washing, laryngeal swabs, bronchoscopy, (with broncho alveolar lavage, bronchial washing and/or trans bronchial biopsy). FNA (trans tracheal or trans bronchial). In some cases, a more invasive technique is necessary including tissue biopsy during mediastinoscopy or thoracoscopy [26].

As other Mycobacteria are also acid fast, polymerase chain reactions is used to distinguish *M. TB* from other Mycobacteria, if the smear is positive; even if sputum smear is negative, TB must be considered and is only excluded after negative culture. Frozen tissue can also be tested by polymerase chain reaction. Extraction of DNA from tissue was done using Qlamp inc or Quegen inc following the manufacturer's instruction [16]. Many types of culture are available. Traditionally cultures have used the Lowenstein-Jensen, Kitchner, or Middle brook media (7H9, 7H10 and 7H11). A culture of the acid-fast bacilli can distinguish a varies forms of Mycobacteria, although results from this may take 4-8 weeks for a conclusive answer. New automated systems that are faster include MB/BACT, NACTEC9000, and Microbial Growth Indicator Tube (MGIT) [27]. Microscopic observation drug susceptibility assay culture may be faster and more accurate method [28].

3.3. Chest X-ray

In active pulmonary TB infiltrates or consolidation and/or cavities are often seen in the upper lungs with or without mediastinal or liver mediastinal lymphadenopathy (tuberculous pleurisy). However, lesion may appear any where in the lung. In disseminated TB a pattern of many thiny nodules through out the lung field is common the so called military TB. In HIV and other immuno suppressed peoples any abnormality may indicate TB or chest X-ray may even appear entirely normal. Abnormalities on chest radiography may be suggestive but are never diagnostic of TB; however, chest radiography may be used to rule out the possibility of pulmonary TB in a

person who has a positive reaction to the tuberculin skin test and no symptom of disease. Cavitations or consolidation of the apexes of the upper lobe of the lung may be descrambled by chest X-ray [10](Figure 3).

Old healed TB usually present as pulmonary nodules in the hilar area or upper lobes, with or without fibrotic scars and volume loss. Bronchiectasis and pleural carrying may be present. Nodules fibrotic scars may contain slowly multiplying tubercle bacilli with potential for the future progression to active TB [24]. People with these findings, if they have a positive tuberculin skin test reaction. Should be considered high priority candidates for treatment of latent infection regardless of age [25].

3.4. Tuberculin skin test

Two tests are available; Mantoux and Heaf test [10]. The Mantoux test for TB involves intradermal injection of purified protein derivatives and measures the size of induration 48-72 hours later (Figure 2). Erythema or redness should not be measured. Mantoux test injection without chronic condition or in high risk group clinically diagnosed as negative at 50 hours [26]. Heaf test is a diagnostic skin test performed in order to determine whether or not child has been exposed to TB, the test is also known as sterneedle test [10], and is administered by Heaf gun [25], which is spring loaded instrument with six needles changed in circular formation [26]. The test is named after F.R.G. Heaf until 2005, the test was used in United Kingdom to determine if the BCG vaccine was needed, and the Mantoux is used instead. The Heaf test was withdrawn because the manufacturer could not found for tuberculin or for Heaf gun. Heaf test is used to test for tuberculin in adolescent aged around 13-14 [27]. An indurations (palpable raised skin) of more than 5-15mm depending up on the persons risk factor to 10 Mantoux test is considered positive results, indicate TB infection [10].

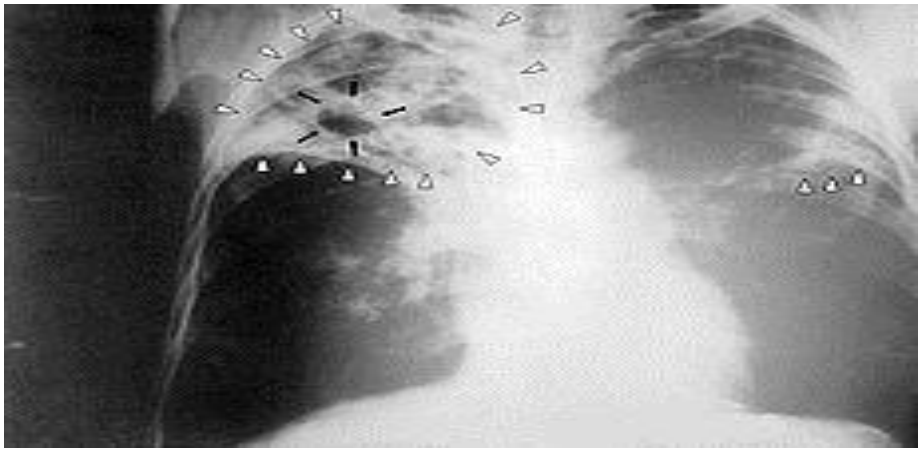

Figure 3: Chest X-ray of patient with far advanced TB
(https://upload.wikimedia.org/wikipedia/commons/9/9c/Tuberculosis-x-ray-1.jpg)

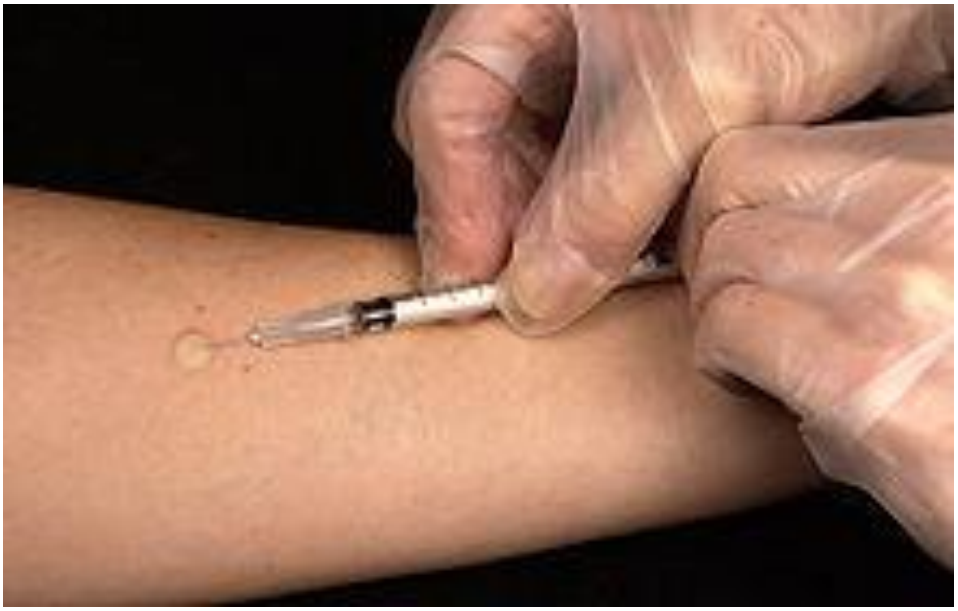

Figure 4: Mantoux tuberculin skin test
(http://www.clinicalcorrelations.org/?p=178)

3.5. Interferon-gamma release assay

Interferon gamma release assay are existing new development in TB infection testing. Interferon gamma release assays are based on the ability of the *M. TB* antigen for early secretary antigen target (ESAT-6), and culture filtrate protein 10 (CFP-10) to stimulate host production of interferon gamma. Because, these antigens are not present in non-tuberculous Mycobacteria or in any BCG vaccine variant, this test can distinguish latent TB infection. The blood test QuantiFERON-Gold Tube and T-SPOT-TB use these antigens to detect people with TB, lymphocytes from patient blood are incubated with antigen, these tests are called Interferon gamma test [21]. If the patient T-lymphocytes produces interferon gamma response the QuantiFERON-TB Gold In Tube uses and ELISA format to detect whole blood production of interferon gamma with great sensitivity of 89%. The distinction between the test is that QuantiFERON-TB Gold quantifies the total amount of interferon when whole blood is exposed to the antigen (ESAT-6, CFP-10), whereas guide lines for use of this test approved Quantiferon for diagnosing latent TB, three systemic review of Interferon gamma release assays concluded that the tests noted excellent specificity for the test to distinguish latent TB from vaccination [24].

4. CONCLUSION AND RECOMMENDATIONS

Diagnosis of TB is difficult due to lack of well improved diagnostic facilities in developing Countries. Diagnosis in animals is based on history, clinical findings, tuberculin skin test, postmortem examination and modern techniques such as lymphocyte-proliferation assay, gamma interferon assay and ELISA. Similarly in case routine diagnosis of TB in human is based on history, microbiological studies of Sputum examination, Chest X-ray, Gamma interferon release assay and tuberculin skin test. There are also Biochemical tests and Molecular techniques that are used for diagnosis of TB. The chronic nature of the disease make the diagnosis difficult based on clinical examination. Direct microscopy of smear for diagnosis of TB is the most commonly used method but its disadvantage is its low sensitivity. The diagnosis is confirmed by culture. Growth for Mycobacteria will be checked every week. Positive culture will be sub cultured onto another set of media and incubated for another 3to4 weeks for further identification. The colonies are subjected to the biochemical tests for further identification and characterization.

Based on the above conclusion the following recommendations are drawn:

- Veterinarians and human health professionals should have to work together for the improvement of diagnostic facilities to decrease the economic as well as public health impact of TB.
- There is lack of diagnostic facilities for diagnosis of TB in developing Countries which increase its zoonotic impact and this problem have to be solved.
- Most of the techniques available for diagnosis of TB take long time to show the result. So, the techniques for diagnosis of TB have to be improved.
- Government should have to give attention for the improvement and adequate availability of diagnostic facilities of TB.

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