



UPDATES ON BOVINE TUBERCULOSIS IN CATTLE AND BUFFALOES, SPECULATED FUTURE OF DISEASE AND CONTROL PROGRAM FOR PAKISTAN

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UPDATES ON BOVINE TUBERCULOSIS IN CATTLE AND BUFFALOES, SPECULATED FUTURE OF DISEASE AND CONTROL PROGRAM FOR PAKISTAN

FIRST EDITION
A Guide Manual

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UPDATES ON BOVINE TUBERCULOSIS IN CATTLE AND BUFFALOES, SPECULATED FUTURE OF DISEASE AND CONTROL PROGRAM FOR PAKISTAN

ABSTRACT

The published data reveal varied prevalence of disease in different regions of Pakistan. The prevalence is lower than 10% in most places, higher than that, but lower than 20% in some places. Most of the studies were carried out by skin testing and very few involved the slaughterhouses those were restricted to Lahore and Faisalabad. There is no regular screening of animals in the country except at public owned livestock experiment stations. A report published in 1969 indicates prevalence of 6.72% in dairy animals in Faisalabad. In 1972, a prevalence of 8.3% in Sahiwal cattle and 2.9% in buffaloes was reported. In early 1970s, 0.53% prevalence in Rohri area and 5.31% in Quetta has been reported in buffaloes. In 1979, an overall prevalence of 9.1% was reported. A report published in 1989 indicated 7.3% prevalence in cattle and buffaloes slaughtered at Lahore abattoir. In 1992, an abattoir-based study in Lahore reported 7% prevalence in buffaloes. In 2001, a prevalence of 1.76% in buffaloes and 5.1% in cattle was observed in the Faisalabad area. In the same year in Lahore area a prevalence of 1.3% was observed in cattle. In year 2003, a report indicated 6.91% prevalence in buffaloes and 8.64% in cattle in the Lahore area. In 2006, the prevalence in buffaloes at two experimental livestock farms in Punjab was 2.45 and 8.48%, respectively. In 2009, a prevalence of 2.2% in buffaloes around two cities of Punjab was reported. In 2010, 3% of buffaloes were found positive with a herd prevalence of 14%. In 2011, 7.6% prevalence was recorded in cattle with 100% herd prevalence, although the animal level prevalence varied from 2.0 to 19.3% of these farms. In 2012, 11.3% prevalence was recorded in buffaloes with 86% herd prevalence. The future of disease looks to be graved as there is no knowledge about the disease to the local farmer and animals are being purchased without testing. As the dairy farming is rapidly turning into one of the profitable future industry. The farms are rapidly expanding so the disease situation is expected to become worse. Therefore, there is need to have a control program in place to keep the disease in animals to the minimum which require involvement of the Government, stakeholders and the community in large. The control program can be multifaceted which also require necessary legislation, surveillance and proper disease control setup.

UPDATES ON BOVINE TUBERCULOSIS IN CATTLE AND BUFFALOES

The published reports on bovine tuberculosis reveal varied prevalence of disease at different times and in different localities of the country. The prevalence of disease in most of the published reports is below 10%, but in some others, it is higher than 10%. However, none of the reports indicated higher than 20% prevalence before the year 2013, while now the prevalence beyond 20% has also been recorded at some of the farms. All the published reports are based on the results of tuberculin testing with very few being undertaken at slaughterhouses in the country. The isolation of the organism were the methods used to indicate the prevalence at slaughterhouses. Very recently, some reports are based on the use of the latest diagnostic methods like PCR. However, there is no published report available on the use of Gamma interferon or other sero-diagnostic methods in the diagnosis of bovine

tuberculosis in the country. It has also been observed that there is no regular screening of the animals in the country, neither at sale/purchase points (Mowaishi Mandeas) nor at slaughterhouses, except for Government Livestock Farms where an annual tuberculin testing is a regular feature carried out by people of Veterinary Research Institute at least in Punjab Pakistan.

Bovine tuberculosis is a disease prevalent all over the world, but the magnitude of the disease does vary in different countries, in different animal population and obviously in different regions within any country. Therefore, Pakistan is no exception to that and tuberculosis is widely prevalent in the country. The published reports accessed through various sources including internet indicate that the first publication about bovine tuberculosis dates back to 1969 where in a prevalence of 6.72% in dairy animals in Faisalabad area reported (Barya, 1969).

The second report available was published in 1972, where a prevalence of 8.3% in Sahiwal cattle and 2.9% in buffaloes was reported (Akhtar et al., 1972). Two years later in 1974, 0.53% prevalence in Rohri area and 5.31% in Quetta was reported in buffaloes (Khilji, 1974). The method used in these reports to declare the prevalence was tuberculin testing. In 1979, culture isolation method was used in an abattoir-based study in Lahore in cattle and buffaloes on morbid tissue samples. On the basis of positive cultures an overall prevalence of 9.1% was reported (Niaz and Siddiqi, 1979). With a gap of thirteen years, a slaughterhouse-based study was carried out in Lahore wherein a prevalence of 7% in buffaloes was reported. This study involved pre-slaughter tuberculin test and later on some of these animals were confirmed by isolation of the organisms after slaughter (Amin et al., 1992). Nine years down a prevalence of 1.76% in buffaloes and 5.1% in cattle was reported from Faisalabad based in skin testing (Ibrahim, 2001). In the same year prevalence of 1.3% was reported in cattle from Lahore based on skin testing (Rehman, 2001). Two years later a report indicated 6.91% prevalence in buffaloes and 8.64% in cattle in the Lahore based on skin testing (Jalil et al., 2003). They detected acid fast bacilli in milk samples and were also able to isolate *Mycobacterium* in 8.07% of milk and 12.28% of nasal secretion samples from buffaloes. In cattle, AFB were detected in 25% of milk samples and 12.3% of nasal secretions, while *Mycobacterium bovis* was isolated from 27.4% of milk and 12.33% of nasal secretions among skin test positive cattle. In 2006, the prevalence in buffaloes at two experimental livestock farms in Punjab was 2.45 and 8.48 percent (Javed et al., 2006). In 2009, a prevalence of 2.2% in buffaloes (Fig 1) around two cities of Punjab, while in 2010, 3% prevalence at animal level and 14% at herd level was reported. The study was carried out in two cities of Punjab, Pakistan, including Faisalabad and Okara (Javed et al., 2010). In 2011, 7.6% prevalence was recorded in cattle (Fig 2) at 11 livestock experiment stations of Punjab with 100% herd prevalence, although the animal level prevalence varied from 2.0 to 19.3% (Javed et al., 2011). In 2012, 11.3%



Fig 1: A buffalo showing positive tuberculin reaction.

prevalence was recorded in buffaloes at seven livestock experiment stations of Punjab with 86% herd prevalence (Javed et al., 2010).

These studies indicate that the prevalence in a private setting is lower as compared with public owned farms. Similarly, the prevalence is lower in studies carried out at slaughterhouses of two big cities, i.e., Faisalabad and Lahore. Most of the data on bovine tuberculosis comes from Faisalabad, Okara or Punjab public livestock farms, while data from other cities and provinces is lacking seriously, thus there have to be studies carried out in other regions of the country so that the current status of the disease can be ascertained in different localities of the country. Recently, some studies also highlighted the risk factors associated with the prevalence of diseases in both private and public settings. The interested persons are directed to read the papers published by Javed et al. from 2009 onward, which are accessible through internet. These later studies used logistic regression procedures by controlling the confounding factors to ascertain the better association of risk factors with bovine tuberculosis in cattle and buffaloes in selected areas of Punjab Pakistan.

SPECULATED FUTURE OF DISEASE

If we look through the published reports on bovine tuberculosis from different areas of Pakistan at different times, there is a clear indication that the prevalence is on the increase in the Punjab and may be in other provinces of the country as well. The reports before 2001 indicated less than 10% prevalence. However, later on increase in animal and herd level prevalence of the disease has been reported which at



Fig 2: A tuberculin positive Sahiwal Cattle

some farms was beyond 10% (at animal level), with 100% herd prevalence. The herd prevalence varied between private and public farms, as at the private farms herd prevalence was very low and was around 14% (Javed et al., 2010), while on public farms (Fig 3) herd prevalence was almost 100% (Javed et al., 2011). Thus, it might be concluded that the future of disease in the country is going to be worse. In recent times, the dairy farming in private sector is on the increase and large-scale farming is developing very rapidly. Five to ten years before, dairy animals were kept at home in cities and villages in a group of less than ten and in rare cases they were more than ten. But recently, this situation is changing rapidly, and now dairy farming is developing at a fast pace, especially in the villages. Now there are dairy farms in the country where thousands of animals are being kept and most of the practices at those farms are also mechanized. Now, there are about 10 dairy farms with animals beyond 2000 and there are about 15-20 where animals are beyond 300. These days dairy farms having animals between 20 and 100 are more numerous in the country (Fig 4). Two of the important reasons for a rapid development of the dairy industry are 1) the rebate on income tax given by the Government and 2) government is extending soft loans to people to establish dairy farms. These reasons along with availability of fodder, agriculture land and availability of better animal resources are contributing to this increasing trend in dairy farming.

The rapid progression of dairy farming in Pakistan will bring many challenges to the sector. Apart from nutritional and economic challenges, the diseases will be the threat to this rapidly developing industry. The two kinds of diseases are, the more important factor in this fast-developing dairy industry, one kind is that which cause acute morbidity and mortality, like FMD and HS, but interestingly a new disease named as three-day sickness (bovine



Fig 3: A public Livestock Experiment Station.

ephemeral fever) has caused heavy mortalities at some farms. The second kind of disease is that which is transmissible from animals to human, like tuberculosis. Tuberculosis is an important disease of animals and humans which is known to mankind since ancient times. The prevalence of this disease is on the increase in the country and the Government has not given proper attention to this disease. There is no regular surveillance program for this disease or any other in any province of the country, nor there a policy to control this disease in the country. As the dairy farming is developing quite rapidly and is without proper planning, therefore, the disease like tuberculosis is speculated to increase in animals in Pakistan. What we are also experiencing is that large scale dairy farmers are keeping exotic animals (100% or cross bred). These animals are not previously exposed to the environmental conditions of Pakistan and thus had not developed the genetics to face the environmental challenges of Pakistan. Similarly, the disease-causing agents are also somewhat different from those for which these animals have faced before or their ancestor and thus have developed the genetics accordingly. Owing to difference in genetic makeup and potential of the disease agents, including that of tuberculosis, such exotic animals easily fall prey to these disease agents. What our personal experience in Department of Pathology, University of Agriculture, Faisalabad is that we have witnessed severe disease lesions in the lungs and regional lymph nodes in the dead exotic animals while conducting postmortems. Therefore, it is not beyond sound speculation that the tuberculosis will be an important animal disease, scientist would be talking in future in Pakistan. However, by that time a lot of loss in a different way would already have been done. With a widespread presence of disease agent in the future, it will become difficult to control this disease in animals and also in humans. There is also a fear of the development of some more notorious and dangerous strains of this organism under such harsh environmental circumstances which may become a challenge.

HOW TO CONTROL TUBERCULOSIS IN ANIMALS IN PAKISTAN

There is a simple way to control a disease at farm level and that is keeping a good hygiene and Biosecurity. The other ways are by the use of vaccines or by stamping-out policy, the later means depopulation (slaughter) of the infected animals so as to dispose of the potentially infectious material and then quarantine the remaining stock. Thereafter, continue the surveillance of the area where disease was observed. Vaccination programs as preventive measures are among the most often practiced methods for controlling disease, particularly of high-risk (Delgado et al., 2003). To control tuberculosis, stamping out

method is being used all over the world. Stamping-out method involve the eradication of tuberculosis by the killing/slaughtering of all the infected animals. Where infected animals are found, around that farm, surveillance zones are established. The level of surveillance in the area is increased and the movement of animals from surveillance zone is restricted. Stamping-out programs are used when there is no vaccine available, or disease has low incidence, or when a country wants to maintain disease free status of animals without vaccination as such certificate is required by the export markets. Stamping-out method requires compensation to the farmer. If there is no compensation, then the farmer will be reluctant to participate and if they do so, then they will have to leave this business on economic grounds. The other way of controlling a disease is by vaccination, but it does not help to achieve eradication of a disease on its own due to various reasons. As not everyone comply for the program to be effective? Furthermore, that the program must be maintained for a long period of time and animal's movement should be checked at all times along with other ways of keeping infection at check. However, the government should in principle intervene to address the animal health risk associated with the production of goods that create a human health risk. The Government interest is required without wasting time in reaching TB-free-status to enable better trade. The best way to move forward in Pakistan is now, when we have low disease incidence and least number of animals have to be sacrificed. Further that the dairy or beef farming is just in its infancy stage. The farmers should be educated right now rather than later when their farm premises are already heavily loaded with the disease-causing organism, i.e., Mycobacterium.

Eradication Program in USA and EU

The bovine tuberculosis eradication program started in the early 1900's in the U.S. because of the human and animal health issues. Officially, it began in 1917. At that time, it was estimated that 1 out of every 20 cattle slaughtered had bovine tuberculosis (Bruning-Fann, 1998). Currently, all States except California and Michigan are recognized as officially free from bovine tuberculosis (Clifford and Álvarez, 2013). To declare officially tuberculosis free status (OTF), all the animals must



Fig 4: A private livestock Farm at Faisalabad.

be free of clinical signs of tuberculosis and all animals over six weeks of age must have passed two tuberculin tests, carried out by an official veterinarian at six-month intervals (Caffrey, 1994). The herd must be tested subsequently at one-year intervals to maintain its status. OIE further states that on regular and periodic testing of all cattle, water buffalo, and wood bison herds must demonstrate that *M. bovis* was not present in at least 99.8% of the herds and 99.9% of the cattle, water buffalo and wood bison for 3 consecutive years (OIE, 2010).

In Europe, the tuberculosis eradication program started between 1953 and 1980. In some countries in 1953, in others in 1960s, 1970s up to 1980. An eradication scheme in Britain was started in 1935 and about 50% of the herds were certified clear of the disease

by 1954 (Watchorn, 1965). The Ireland started this campaign in 1954 when £1,200,000 were available from the American Grant Counterpart Funds and the Irish Government further added £653,000 from the National Development Fund. At that time an estimated 80% of the cattle herds were infected in Ireland. The main features of the eradication program were free tuberculin testing of animals by the Veterinarian of the herd-owner's choice and full payment to the owners to purchase the reactor animals on the market price. In 1954, there were about 250,000 herds-owners in Ireland with a total cattle population of about 4,500,000. During the first 10 years of the program, about 800,000 TB reactors were slaughtered. Animal disease incidence fell rapidly from 17% overall to less than 0.5% in 1965. During that time all the herds individually achieved Officially Tuberculosis Free (OTF) status in accordance with the Directive 64/432/EEC and no herds of unknown status remained in the Ireland (Good, 2006). In Spain, the bTB eradication program was progressively reinforced with pre-movement testing and inspections by the field teams, etc., but the OTF status could not be achieved. However, the herd prevalence in 2012 was around 1.3%, but later on declined just marginally (Anonymus, 2012). Among European countries, Officially Bovine tuberculosis free (OTF) declared in 2003 are Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Luxembourg, Netherlands, Slovakia, Sweden, Norway and six provinces of Italy (Anonymous, 2003). New Zealand and Scotland also are declared officially bTB free. Australia was also declared bovine TB free in 2002 (Anonymus, 2007). Some of the OTF countries are currently observing rising bTB prevalence, although the increases are only slight (Schiller et al., 2011).

Eradication/Control Program for Pakistan

Keeping in view the above facts Pakistan has to **make a decision** and need to answer these questions.

1. Is bovine tuberculosis a serious problem?
2. Does Pakistan need to control it or eradicate it?
3. Can each Province do it independently?
4. How Pakistan should proceed?

The answers are simple but need to be well understood. Yes, bovine tuberculosis is a serious disease problem not only for animals but for humans as well. The disease exists and is as old as the history of mankind, so it did not vanish on its own by the time. However, the disease was controlled to a great extent in the developed world, but it re-emerged. There is a long treatment time and the drugs used for the treatment of disease can also be toxic to some humans and such a long treatment is economically not feasible for animals. The disease in animals is also responsible for the loss in production and reproduction, thus making animal economically unsuitable. The development of drug resistant strains of the organism is another threatening reality and many more? Therefore, it is important to control or eradicate this disease from animals for which the goals have to be set and a comprehensive control or eradication program has to be developed. To eradicate or control this disease, Pakistan needs to have a collaborative and concerted effort by all the provinces as hypothetical borders means nothing and this disease can cross these hypothetical borders by various ways. One of the ways is the animal movement from one province to another that include domestic animals and wild animals. Therefore, control or eradication in one province can never be achieved effectively unless a collaborative and concerted approach is adopted. In the succeeding text the whole program is given as how Pakistan should

approach this disease and same program can be used for the control of other diseases in the country.

We need to set certain **strategic goals** to achieve which will help us to move forward. The goals have to be very clear with greater objectivity. These goals can be:

1. Minimal risk to public health
2. Slow down and stop the spread of disease
3. Understand the disease and the agent

These are the important goals to be achieved in connection with bovine tuberculosis. One of the important goals is to minimize the source of infection of human coming from animals. To achieve this goal, the surveillance of the disease at home and farms in animals is important so as to know where the disease is present in high magnitude, so that effective measures can be taken to minimize the spread of disease to humans. This surveillance will have to be a continuous process in the future, covering all animals. Second, the slaughterhouse surveillance is also important, this will not only help to control the spread of disease from animals to the consumers but also to the slaughterhouse workers. Therefore, again the disease situation in slaughtered animals will also help to trace back the source of infection or area from where the animals were brought to slaughter. The third way of minimizing the infection to human would be through the supply of only heat treated or pasteurized milk. Then apart from slaughterhouse worker, which are exposed to the disease agent owing to direct contact with the blood and viscera of the slaughtered animals, the milk seller who is selling fresh milk or meat seller should also be saved by adopting necessary control measures. There is also a need to have a better understanding of the disease in human as how many cases are caused by animal pathogen which will help us to make more stringent actions if needed by understanding the fact that how these humans are contracting the disease of animal origin.

The second important goal to achieve is to slow down or stop the spread of the disease in animals which can be brought about by reducing the prevalence of disease in high incidence areas and further minimizing in low incidence areas. This can only be achieved when an effective surveillance program is in place and necessary measures to reduce the infection are taken and the infected animals are removed from the farms, houses and other places and slaughtered and properly disposed off if needed.

The third important goal is to understand the disease in different animal species, and what kind of disease-causing agent is circulating in different geographic localities of the country. We must understand the disease in native animals and exotic animals. The genetic of animal is an important player in rejecting or accepting certain kind of pathogens. This factor is important to understand, and we must make all efforts to understand that, do we have domestic animals or livestock species and breeds which are genetically resistant to bovine tuberculosis or the disease in what specie or breed is not severe. This will help us to make strategies and plan accordingly to keep animal species or breeds which can fight well with this disease. It is important to have better diagnostic tests in place and better tools to characterize and strain type the organisms to understand the evolution occurring in the organism and also to have an early diagnosis of disease in the animals so that the infected animals are culled much earlier. It is imperative to understand the molecular epidemiology as by that the understanding about the strain distribution of the organism in different geographic localities will be better understood. Furthermore, the molecular epidemiology will also help to understand the evolutionary scenario of the organism and which of the

environmental factors are influencing the change in the genetics of the organism. The latter can be speculated, and thus remedial measures can be adopted. It is also important to understand the genetics of the organism in broader perspective involving different genes, especially those involved in the pathogenicity of the organism and development of drug resistance in the organism. To understand the disease in wild animals and birds is also of significant importance as it will help us to understand the evolution of the organism and also that how the organism is reaching from wild animals and birds to the domestic animals or dairy animals. If we will be able to better understand the disease scenario in animals and evolution in the organism, we may be able to better work on to have a better vaccine for animals. The vaccination provides the other option if the killing and removal of the infected animals from the farm fail to eradicate the disease.

It is important to mention here that if epidemiology of the disease is poorly understood then it will be impossible to have a better control program in place. The epidemiological tools help us to understand that how the organism is being maintained and transmitted. Also, what are the hot spots of the disease and what are vulnerable points to attack. The epidemiological investigations also help to establish effective clinical diagnosis and laboratory confirmation. Further epidemiological investigations need a better laboratory support and there should be a continuous monitoring of the disease.

What should be the components of the disease **control plan** and how it should be implemented is very important to garnish the goals set to achieve. The control plan should have the following components:

1. Making a Task force for initial work.
2. Formulating an organization or body.
3. Developing necessary infrastructure, including
 - a. Laboratory setup
 - b. Internet and data management resource
 - c. Special Slaughterhouse(s) and incineration facilities
4. Devising a plan of action
5. Executing and implementing the action plan.
6. Reviewing the progress of the action plan from time to time
7. Reassessing the situation after 5 or 10 years.

There is a need to have a task force made which should do the preliminary paperwork, setting up the goals and making initial legislation/regulations to set up the necessary organization, laboratory and other infrastructure. Then there is need to have a comprehensive body or organizational setup in place which should have a centre at the country level, nested with provincial, divisional, district and tehsil level setups of various kinds. The TORs and SOPs at each level have to be framed. Then there is a need to develop infrastructure pertaining to the official setup at the centre, provincial and other levels. The laboratory infrastructure, including the central reference lab, provincial labs and divisional labs. The internet and data management resource centre is also being needed. This has to be centrally managed and operated and can be used to collect and store various kinds of activity and information data, future activity data, job portal and public awareness portal. The central organization, then needs to setup an actual plan of action through consultation of the experts from all over the country and provinces, involving provincial and central governments and setting up a target. Then this central body should review the progress made at different times, which has to be a regular feature. This can be done through

meetings, conferences, seminars about how the problem is addressed, what is the success made and future plan based on previous results. These review plans should be sent to the regional centres and necessary documents and informative material has to be placed on the internet portal also for public awareness. Then the central and regional bodies should join heads and review the progress made after five and ten years. One should look whether the plan given is implemented everywhere with the same level of enthusiasm and work force. Based on results, future plan should be chalked out and a coordination with human health workers and stakeholders should be in place.

Now we are going to present you the **actual plan for Pakistan** through:

1. Country Body or organization
2. Provincial setup
3. The Role of Government
4. The role of stakeholders

The country organization should be headed by a director or coordinator, a secretary, member from each province in the central body and a Central Reference lab, Special slaughterhouse and other infrastructure development. This central organization should be working to frame out necessary legislation or regulations, prepare a comprehensive plan of work, should monitor and run central and regional labs, should also make arrangements to train the manpower and should control the overall activity. This should also maintain the net and data management centre.

The provincial setup should have a provincial organization or body, provincial lab setup, divisional lab setup, field activity setup and special slaughterhouse in each province. These should also frame meat and milk regulations in the province, should execute the plan of action given by the Center, monitor the plan at tertiary level and should make necessary arrangements and work in public and stakeholder awareness and feedback.

The role of federal and provincial governments is important, and their interest is required to take this disease seriously and show its full commitment to make Pakistan a responsible state/country in the world. The Government after realization should do the necessary legislation in the control of this disease and should allocate necessary funds to take this program. The Government should also establish reference and other associated labs in the country along with other infrastructure required in this connection. The legislation should be on the supply of pasteurized milk, processed meat, the animal numbering system, genetic profiling/genotyping/genetic coding of each animal, movement of the animals, incentives to dairymen and research. It may further be explained here that along with animal numbering, the corresponding genetic identity should also be made of each animal. This will also help in preventing theft of animal and animals can be traced back easily for any reason.

The role of stakeholders is also important in the control program of bovine tuberculosis in the country. There is need to have a stakeholder's regulatory body, an association and there should be a registration system of stakeholders in place. Then there is need to have an accreditation system in place in collaboration with stakeholders. All this is needed for cooperation, feedback, policy planning, remuneration and annual appraisal system.

Now we are going to address that **how the actual plan of action should be executed** as that should be the component addressed in execution of the control program.

1. Field work
2. Laboratory work

3. Regulations

4. Awareness

To carry out the **field work**, the field staff is required. There should be some separate fully trained field staff and then the provincial livestock Veterinary setup can also be used in the implementation of the program, so it can be a mix. Definitely, there would be a proper infrastructure in place, including head office, divisional offices linked with Provincial Veterinary setup. The field staff should be properly trained to carry out the tuberculin testing, its interpretation and recording data including results of tuberculin testing, about the animal, its surrounding and farm as a whole. For Pakistan, comparative cervical intradermal tuberculin test is recommended to be carried out on all the animals of more than six months of age. All animals found positive by this tuberculin testing should be removed from the farms and moved to a special slaughterhouse for slaughter. This tuberculin testing should be done initially after every six months and later every year. However, in high disease incidence areas tuberculin testing should be done after every six months till the incidence is drastically reduced. In case the prevalence in the area in the animal is less than five percent, then the yearly program may be used otherwise six month program should be used. The herd prevalence is also important, but in villages, animals are still kept in homes in small groups so at the moment the herd prevalence should not be given importance. However, the data should be viewed in context with the number of animals at each farm if more than 50 should be considered a herd and prevalence at herd level may then be worked out and looked into for interpretation purposes. In such situations, the herd prevalence of more than 5% must be considered serious. Efforts should be made to reduce it to less than one percent. There should be a regular training centre which should train the field staff, lab staff and office staff. The data collection, management and analysis staff should also be properly trained. There are different types of data, epidemiological data, about samples from specific animals, kind of sample, culture, ZN staining, PCR and finger printing. All kinds of data should be properly handled and managed. There are separate software packages to analyse each kind of data. Then there would also be data about sequencing of certain genes, especially those related to DNA fingerprinting and drug resistance that require a specific kind of analysis. So, there is need to have a proper training of people engaged in data management and analysis. There should be regular meetings with field staff for feedback and about the success of the control program. The field staff should also be encouraged through promotions, appraisal and other monetary allowances on the basis of performance. This is required for the program to be more vigorously followed. The role of field staff is to carry out the field work. This involves the surveillance of disease in food animals at the first stage and later on in wild animals as well. The slaughterhouse inspection is another work allocated to field staff. The slaughterhouse inspection should include pre-slaughter tuberculin testing and positive animals should immediately be removed from the slaughterhouse and transported to special slaughterhouses allocated for the slaughter of tuberculin positive animals. The animals found negative by tuberculin testing at slaughterhouses should be monitored for lesions of tuberculosis in the lungs and lymph nodes. The special slaughterhouses should also have the facility of an incinerator to properly dispose off the infected tissues/material or whole animal if required. The field staff should also be responsible for data collection and sample collection along with feedback activities. After carrying out the field work in relation to the tuberculin testing, disease zones can be arbitrarily made, i.e., low disease and high disease zones and of course in

between disease zones. The animal movement should not be allowed from high disease zones to low disease zones. However, the animal movement from low disease zones to high disease zones should be made under vigilance and all the record of the animal including its genetic identity should also be moved along with the animal. It should also be mentioned here that each animal should be properly registered and numbered. The data about each animal has to be digitized, including its production record, a disease suffered, its reproduction record etc.

To carry out **lab work** a comprehensive and environmentally controlled BSL-III and BSL-II level laboratory setup is required. This should include a country reference lab (BSL-II), which may be named as Jinnah Tuberculosis Reference Lab, Pakistan. This reference lab should be linked with the provincial lab system which should include a well-furnished provincial lab (BSL-II) and relatively less so divisional or district labs. The personnel's engaged in these labs should be properly trained for the job they will be doing. The lab worker should know how to receive the samples from the field, where to store them initially, how to be taken to culture units, how to open those for culture purpose, how to culture, what culture media should be used. Further, how to monitor the culture, how to obtain DNA from culture and carry out PCR for confirmation. What kind of primers should be used for confirmation of *Mycobacterium*, and what kind of primers to be used to find out whether they belong to *M. tuberculosis* complex or not. Thereafter, spoligotyping and VNTR is recommended to be carried out for fingerprinting of isolates. This will not only help to understand that what kind of strains are circulating in different geographic localities, but also that how the organism is evolving. Sequencing of selected genes, especially those related to drug resistance should also be carried out. For this purpose, initially, PCR-RFLP may be carried out and then strains may be selected for sequencing rather than going directly to sequencing of each strain that will be a very costly procedure. There should be regular meetings between all the heads of the labs in the central lab premises. The staff of these labs should also be treated in the same way as field staff in the form of promotions, appraisal and other monetary allowances on the basis of their performance. The work activity of the lab staff should be isolation, identification, strain typing based on PCR methods, research on the organism and host, to keep the isolates as DNA banks. These labs should also act as reporting agency to the central head office and to the Government.

The **regulations** framed by the Government's special cell and monitoring protocol should be fully implemented in letter and spirit. The legislation on pasteurization of milk, processed meat, nutrition, remuneration, incentives, genetic identity system of the animal and animal movement from one place to another should be framed and subsequently implemented. For the first five years, full price of the animal should be paid to the owner and later on half of the market price of animal may be paid to the owner for the tuberculosis positive animals. There should be ban imposed on sale of raw milk and only pasteurized milk should be sold in the market. The need from time to time should be assessed for the sale of only processed and packaged meat rather the authors of this article encourage to allow the sale of properly packaged and processed meat, thus the regulations should be in place. The people violating these sales rules must be heavily fined and may be imprisoned. The rules and laws should also be framed for the use of nutrition to the animals. There is likelihood that the nutritional supplements and concentrate or feed is being imported. The foreign feeds used for animals also include a protein source of animal's origin, which also include the pork meat. Therefore, there should be laws pertaining to this also and

punishment in the form of heavy fines and imprisonment must be done. The fine on unauthorized movement of animals from one place to another should also be decided and it should be a heavy one if dairy or meat type animals are being transported or moved, while may be slightly soft when pet animals are moved. An annual cash award may be given to farmers who are keeping their animals free from bovine tuberculosis, which could be of value of two animals if the farmer is keeping 100 animals and likewise.

The **awareness** of the stakeholders and the general public is required. For this purpose, a Government cell is required and the internet resources along with print and electronic media for the awareness campaign should be utilized. Regular seminars, conferences and promotional programs are needed in this connection. The awareness about milk and meat issues relating to tuberculosis, the disease situation of bovine tuberculosis and its spillover in the human population is needed. This can be done by various means and a bi-annual disease reporting, of each area is also required to keep the public and Government functionaries aware about the disease. The awareness from time to time about surveillance strategies, results, impact and precautionary measures is also needed.

A BULLETID BRIEF

- A resolution that bovine tuberculosis control program is the need of the time and it is never late.
- Setting up a task force which should help to put up suggestions for necessary early regulations, framework, goals and objectives.
- Making a setup/body at country level with its wings in each province and going down to the District or Tehsil level, and setting up the necessary infrastructure, including reference lab, provincial, divisional and district labs, internet resource setup, printing unit etc.
- Setting up control program by the country body with further necessary legislation, TORs and SOPs at different levels/bodies, etc.
- Setting up the infrastructure of stakeholders with necessary legislation, TORs and SOPs, etc.
- Creating an awareness among stakeholders and general public from time to time through electronic and print media.
- Revisiting the control program after five or ten years and making changes if necessary.

The corresponding author is in contact with Coordinator, GF-TADs Global Secretariat, Food and Agriculture Organization, Animal Health Service, Animal Production and Health Division, Agriculture and Consumer Protection Department, through Monica Cagiola of Italy, who want to have a collaboration in the control program for tuberculosis in Pakistan. The scope of the envisaged collaboration may include:

- Identification of Mycobacteria, including the causal agents of bovine tuberculosis (and paratuberculosis).
- Capacity building on tuberculosis diagnosis and screening with a view on differential diagnosis with paratuberculosis.
- Provision of avian and bovine tuberculin for skin testing, and advice on the appropriate use of tuberculin.
- Support for implementation of laboratory proficiency testing.
- Training activity (in collaboration with FAO)

- Development and implementation of control programmes.
- Establishment of a programme on the epidemiological investigation to study prevalence/incidence/occurrence of TB in different species.
- Assessment of the socioeconomic impact of the disease in poorest community levels (in collaboration with FAO).

The corresponding author is already working in close collaboration with Monica Cagiola, a scientist from UODOF Officina Farmaceutica, Istituto Zooprofilattico dell'Umbria e delle Marche, Perugia (Italy). We are signatory of MoU with the Institute, wherein I have been designated as coordinator for Faculty of Veterinary Science, and Monica Cagiola as coordinator from Institutur Zooprofilattico dell Umbria e Delle Mache, Italy. We have published together dozens of research papers, most of which in impact factor journals, book chapters and presented papers in national and international conferences, seminars and workshops. The corresponding author is also in close contact with VISAVET Health Surveillance Centre, Universidad Complutense de Madrid, Spain where I worked for my Posdoc program for one year.

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Research Publications / Chapters in Books On Bovine Tuberculosis

VETERINARSKI ARHIV 76 (3), 193-206, 2006

A study on tuberculosis in buffaloes: some epidemiological aspects, along with haematological and serum protein changes

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Tuberculosis (2007) 87, 437-445



Available at www.sciencedirect.com



journal homepage: <http://intl.elsevierhealth.com/journals/tube>



Improvement of spoligotyping with additional spacer sequences for characterization of *Mycobacterium bovis* and *M. caprae* isolates from Spain

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Tuberculosis (2007) 87, 565-571



Available at www.sciencedirect.com



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Drug susceptibility of Spanish *Mycobacterium tuberculosis* complex isolates from animals

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Epidemiological Basis of Bovine Tuberculosis in Buffaloes

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Risk factors associated with the presence of positive reactions in the SCCIT test in water buffalo around two cities in Punjab, Pakistan

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Haematological and Serum Protein Values in Tuberculin Reactor and Non-Reactor Water Buffaloes, Cattle, Sheep and Goats

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Risk factors identified associated with tuberculosis in cattle at 11 livestock experiment stations of Punjab Pakistan

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***Mycobacterium caprae* Infection in Livestock and Wildlife, Spain**

Sabrina Rodríguez, Javier Bezos,
Beatriz Romero, Lucía de Juan, Julio Álvarez,
Elena Castellanos, Nuria Moya,
Francisco Lozano, M. Tariq Javed,
José L. Sáez-Llorente, Ernesto Liébana,
Ana Mateos, Lucas Domínguez, Alicia Aranaz,
and The Spanish Network on Surveillance and
Monitoring of Animal Tuberculosis¹

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Original article

Analysis of some of the epidemiological risk factors affecting the prevalence of tuberculosis in buffalo at seven livestock farms in Punjab Pakistan

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Short Paper

Prevalence of bovine tuberculosis in zoo animals in Pakistan

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Brief communication (Original)

Certain risk factors associated with positive SCCIT test for tuberculosis in cattle at two cities in Pakistan

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An Abattoir Based Study on Brucellosis, Bovine Tuberculosis and Paratuberculosis in Buffaloes and Cattle at Faisalabad, Pakistan

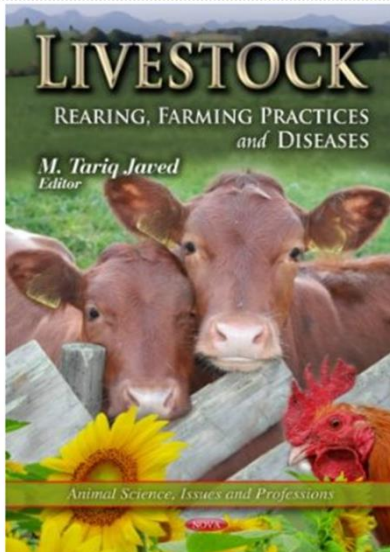
Research Article

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Chapter 10

BOVINE TUBERCULOSIS AT THE HUMAN-ANIMAL INTERFACE, SITUATION AND POSSIBLE RISK FACTORS OF DISEASE IN ANIMALS IN PAKISTAN, FUTURE OF DISEASE AND ACTION PLAN

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ABSTRACT

The situation of bovine tuberculosis in Pakistan and the role of *M. bovis* as cause of human tuberculosis have been presented. The study in 1969 indicated a prevalence of 6.72percent in animals in Faisalabad. In 1972, it was 2.9, 1.6 and 8.6percent in buffaloes, Australian and Sahawal cattle, respectively; in 1974 in Quetta, it was 0.53percent in buffaloes. In 1989, it was 7.3percent in cattle and buffaloes being slaughtered at Lahore Abattoir. In 2001, a study reported 1.76 percent prevalence in buffaloes in Faisalabad. In 2003, the respective prevalence of bovine tuberculosis in buffaloes and cattle was 6.91 and 8.64percent, respectively in Lahore. In 2006, the prevalence in buffaloes at two farms was 2.45 and 8.48percent. A study during 2006 and 2007 reported 2.2percent prevalence of tuberculosis in buffaloes at 11 farms. Another study reported a prevalence of 2.4percent in goats and 0.9percent in sheep at 3 and 7 livestock farms, respectively. A

Chapter 15

Status and control of tuberculosis in animals in Pakistan

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Pakistan's neighboring countries include India, China, Afghanistan, and Iran. The economic survey report of 2010-11 indicates that livestock accounts for 55.1% of Pakistan's agriculture, and it contributes to 11.5% in the national gross domestic product. Among dairy animals, the same report indicates that 35.6 million head of cattle, 31.7 million head of buffalo, 28.1 million head of sheep, and 61.5 million head of goats are present in the country. Bovine tuberculosis is an important zoonotic disease of animals present all over the world, but its prevalence is higher in developing countries. Some studies in Pakistan on bovine tuberculosis in cattle, buffaloes, sheep, goats, and zoo animals have been carried out. Here is presented an account of the disease at different times in Pakistan and what control measures have been adopted at different levels.

Prevalence in large ruminants and risk association

Prevalence

These data reveal varied prevalence of bovine tuberculosis in different localities of Pakistan. The prevalence remained below 10% in most places, with a few exceptions where it was higher than 10% but less than 20%. The data also indicate that most of the studies were based on skin testing; very few studies used skin testing along with the isolation of organisms. There were few other studies carried out in abattoirs, which are mainly restricted to Lahore and Faisalabad. There is no regular screening of animals anywhere in any city in the country except at public-owned livestock experiment stations. There is also no regular screening of animals at abattoirs, as observed personally during visits to various abattoirs and as learned through interaction with various concerned people. A three-year study was carried out by our laboratory which involved tuberculin testing (Figure 15.1), and we were also able to get a few isolates from milk

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