BELIZE:

STATUTORY INSTRUMENT

NO. OF 2011

BELIZE AGRICULTURAL HEALTH AUTHORITY (PREVENTION, CONTROL AND ERADICATION OF BOVINE TUBERCULOSIS) REGULATIONS, 2011

ARRANGEMENT OF REGULATIONS

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REGULATIONS made by the Minister, after consultation with the Belize Agricultural Health Authority, in exercise of the powers conferred upon him by section 86 of the Belize Agricultural Health Authority Act, Chapter 211 of the Substantive Laws of Belize, Revised Edition 2000-2003; and all other powers thereunto it enabling.

(*Gazetted* ……., 2011).

Citation.

1. These Regulations may be cited as the

BELIZE AGRICULTURAL HEALTH AUTHORITY (PREVENTION, CONTROL AND ERADICATION OF BOVINE TUBERCULOSIS) REGULATIONS, 2011.

Interpretation.

2. In these Regulations

"accreditation" means the act through which the Authority recognizes veterinarians and laboratories to perform sanitary activities referred to in these Regulations;

"accredited veterinarian" means a veterinarian recognized and approved by the Authority to conduct sanitary activities pertinent to these Regulations;

"Act" means the Belize Agricultural Health Authority Act;

"animal" means a bovine animal;

"animal positive to the tuberculin test" means an animal that has been subjected to one or more official intradermal tuberculin diagnostic tests with positive results;

"approved fattening unit with restricted pasture" means installations authorized by the Authority according to the protocols established in "Appendix G", given prior request by the district veterinarian and producers;

"approved laboratory" means laboratory recognized and authorized by the Authority to perform sanitary activities referred to in these Regulations;

"area under quarantine" means a geographical area, production unit or farm declared under quarantine by provisions of the Act;

"Authority" means the Belize Agricultural Health Authority established under section 3 of the Act;

"authorization" means the act through which the Authority recognizes organizations coordinating movement controls to perform the sanitary activities referred to in these Regulations;

"beef cattle" means the type of animal in a production unit specialized for beef production;

"BNLR" means the Belize National Livestock Registry;

"bovine" means cattle;

"bovine tuberculosis" means an infectious and contagious zoonotic disease caused by mycobacterium bovis characterized by a progressive and chronic course;

"castrated animal" means an animal whose testicles or ovaries have been surgically removed;

"cattle" means *bos indicus*, *bos taurus* and water buffalo;

"certification of free herds" means the procedures through which the official documents are granted by the Authority to the owner of a herd for demonstrating that the animals are free of bovine tuberculosis through the application of procedures described in these Regulations;

"Committee" means the Committee established to support the implementation of the Programme;

"compartment" means an animal subpopulation contained in one or more establishments under a common biosecurity management system with a distinct health status with respect to a specific disease or specific diseases for which required surveillance, control and biosecurity measures have been applied for the purpose of international trade;

"confinement" means the isolation, observation and movement restriction of animals due to suspicion or existence of bovine tuberculosis until the final destination of the amimals is determined;

"control" means the group of sanitary measures the objective of which is to reduce the incidence or prevalence of bovine tuberculosis in a prescribed geographical area;

"controlled dairy replacement unit" means a production unit recognized by the Authority for the housing and rearing of dairy heifer calves and heifers;

"country" means Belize;

"dairy cattle" means the type of animal in a production unit specialized for milk production;

"designated fattening unit" means the installations authorized by the Authority in accordance with the protocol established in "Appendix C";

"diagnostic test" means a test authorized by the Athority for the Programme;

"district veterinarian" means the official veterinarian designated to supervise and coordinate Programme activities in the northern, central, western or southern region of Belize;

"dual purpose animal" means the type of animal in a production unit which produces cattle for beef or milk;

"entire animal" means an animal that has its reproductive organs intact;

"epidemiological surveillance" means the the systematic ongoing collection, collation, and analysis of information related to tuberculosis and the timely dissemination of information so that action can be taken;
"eradication" means the group of sanitary measures the objective of which is to eliminate bovine tuberculosis in a prescribed geographical area;

"establishment" means the premises in which animals are kept;

"exposed animal" means a bovine that has had contact with one or more infected animals;

"free herd" means a herd that has a valid Free Herd Certificate issued by the Authority;

"Free Herd Certificate" means the official document granted by the Authority to the owner of a herd for compliance with the requirements established for the recognition of herds free of bovine tuberculosis;

"herd" means a number of animals of one kind kept together under human control or a congregation of gregarious wild animals; and for the purposes of these Regulations a herd shall be regarded as an epidemiological unit;;

"herd of origin" means a herd in which the origin of one or more animals is known and in which is found the breeding animals that gave birth to the animals in question;

"herd test" means the application of the tuberculin test to a group of animals in a herd older than six weeks of age;

"herd tuberculosis status report" means an official document issued by official or accredited veterinarians in which the results of a diagnostic test are reported;

"identification of positive animals" means the application of a permanent mark with the letter "T" on the left masseter muscle;

"infected animal" means an animal in which the presence of *M. bovis* has been laboratory confirmed or in which epidemiological studies, including field and laboratory tests, show evidence of the presence of *M. bovis*;

"infected herd" means a herd in which there is strong and substancial evidence of the existence of *M. bovis*;

"*M. bovis"* means *Mycobacterium bovis;*

"Ministry " means the Ministy responsible for Agriculture;

"negative animal" means an animal that has been subjected to one or more official tuberculosis diagnostic tests producing negative results;

"OIE" means The World Organization for Animal Health;

"OIE Terrestrial Code" the most up-to-date version of the Terrestrial Animal Health Code as approved and published by the OIE from time to time;

"official programme ear tag" means the ear tag used for individual animal identification by the Belize National Livestock Registry;

"official veterinarian" means a professional employee of the Authority who performs functions under these Regulations;

"officially free herd" means the assurance of free herds in control areas conducted by official veterinarians for the purpose of moving cattle to areas in more advanced phases;

"PCR" means Polymerase Chain Reaction;

"permanent mark" means any indelible mark on the body of an animal for identification purposes;

"population" means the total number of bovine in a given area;

"PPD" means Purified Protein Derivative;

"prevalence" means the number of cases or outbreaks of tuberculosis in an animal population in a defined geographical area during a specific period;

"producer" means the owner of cattle whose activities include breeding, cow-calf operations, fattening or milk production;

"production unit" means a ranch, farm, stable, corral or other similar place that houses cattle;

"Programme" means the National Bovine Tuberculosis Programme;

"Programme coordinator" means the veterinarian designated by the Authority to coordinate programme activities and the district veterinarians in the different regions of the country;

"Programme phases" means the sanitary classification of the stages in which the region, district or community is found, according to the advances of the Programme as recognized by the Authority;

"quarantine" means the sanitary measure based on the isolation, observation and movement restriction of animals due to suspicion or existence of bovine tuberculosis;

"ranching" means the livestock activity the purpose of which is the feeding of cattle on pasture;

"registered disinfectants" means chemical products registered by the Authority for use in the installations and vehicles for the elimination of *M. bovis*;

"routine slaughter" means cattle for consumption that enters the slaughter plant without any reference to bovine tuberculosis;

"sample" means blood, sera, fluids, tissues, organs, secretions or excretions on which laboratory diagnostic tests are to be conducted to identify the presence of bovine tuberculosis;

"seal" means an official provision installed on doors or compartments of vehicles transporting animals for the purpose of guaranteeing that during transportation animals are neither added nor removed;

"slaughterhouse" means premises, including facilities for moving or lairaging animals used for the slaughter of animals to produce animal products and approved by veterinary services or other competent authority;

"subpopulation" means a distinct part of a population identifiable according to specific common animal health characteristics;

"suspect herd" means the herd in which infection has not been confirmed but is under epidemiological investigation;

"transporter" means the person licensed by the Authority to transport animals;

"tuberculin" means the antigen used for the diagnosis of Bovine tuberculosis;

"tuberculosis field control forms" means basic working forms on which are entered the initial results of field diagnostic tests;

"unit" means an individually identifiable element used to describe, for example, the members of a population or the elements selected when sampling;

"zoonosis" means a disease or infection which is naturally transmissible from animals to humans or vice versa;

"zone" means the northern, central, western and southern part of the country.

Competent authority.

3. (1) The Authority is– designated as the competent authority responsible for the implementation of these Regulations.

(2) In implementing these Regulations as prescribed in sub-regulation (1), the Authority may collaborate with auxiliary organizations, the government and the cattle industry.

Notifiable animal disease.

4. (1) Bovine Tuberculosis is a notifiable animal disease in Belize.

(2) A person who suspects or has diagnostic evidence of the presence of Bovine Tuberculosis in an animal whether the animal is alive or dead, shall notify the Authority within twenty-four hours of the suspicion or evidence of Bovine Tuberculosis.

General provisions of the National Bovine Tuberculosis Programme.

5. (1) The Programme being implemented through these Regulations shall be implemented in a general, permanent and mandatory manner.

(2) The Programme consists in the establishment of the diagnosis, prevention, control and eradication of tuberculosis in cattle and Water Buffalo regardless of breed and production activity.

(3) The protection of regions, districts, communities, herds free of the disease or in advanced programme phases shall be effected through the strict control of animal movement, coordinated among all stakeholders.

(4) The accredited veterinarians must coordinate activities with the Programme Coordinator and the official veterinarians of the Authority.

Determination of health status and free herd certification.

6.(1) The Authority may declare the country of Belize or a zone within the country of Belize as free from bovine tuberculosis where the country or a zone complies with the following requirements

(a) that *M. bovis* infection in domestic (permanently captive and owned free-range) bovines including cattle and water buffalo has been declared as as a notifiable disease in the country or zone;

(b) that an on-going awareness programme is in place in the country or zone to encourage reporting of all cases suggestive of bovine tuberculosis;

(c) that regular and periodic testing of all cattle and water buffalo herds demonstrates that *M. bovis* infection was not present in at least ninety-nine percent (99.9%) of the herds and ninety-nine percent (99.9%) of the cattle and water buffalo in the country or zone for three (3) consecutive years;

(d) that a surveillance programme is in place to detect bovine tuberculosis in the country or zone through ante-mortem and post-mortem inspection as described in Chapter 6.2 of the OIE Terrestrial Code;

(e) if the surveillance programme in place in the country or zone described in paragraphs (c) and (d) has demonstrated that *M. bovis* infection was not present in at least ninety-nine percent (99.9%) of the herds and ninety-nine percent (99.9%) of the cattle and buffalo in the country or zone for 5 consecutive years, surveillance may be maintained through ante-mortem and post-mortem inspection as stipulated in paragraph (d);

(f) that cattle and water buffalo introduced into the country or a zone free from bovine tuberculosis is accompanied by a certificate from an Official Veterinarian attesting that the cattle or water buffalo come from a country, zone, compartment or herd free from bovine tuberculosis or comply with the relevant provisions in Article 11.6.5 or in Article 11.6.6 of the OIE Terrestrial Code.

(2) A bovine producer or a group of bovine producers may apply in writing to the Authority for a certification that a compartment in which bovine animals are reared by that bovine producer or group of bovine producers qualifies as a compartment free from bovine tuberculosis.

(3) A bovine producer or group of bovine producers applying under sub-regulation (2) shall satisfy the Authority

(a) that the cattle and water buffalo in the compartment

(i) have shown no signs of bovine tuberculosis or lesions at ante-mortem or post-mortem inspection for at least three (3) consecutive years;

(ii) were over six (6) weeks of age at the time of the first tuberculin test and have shown a negative result to at least two tuberculin tests carried out at a minimal interval of six (6) months, the first test being performed at least six (6) months following the slaughter of the last affected animal;

(iii)have met one of the following conditions

(aa) shown a negative result to twice yearly tuberculin tests to ensure the continuing absence of bovine tuberculosis if the annual percentage of herds confirmed as infected with tuberculosis is more than one percent (1%) of all herds in the country or zone during the last two (2) years; or

(bb) shown a negative result to an annual tuberculin test to ensure the continuing absence of bovine tuberculosis if the annual percentage of herds confirmed as infected with tuberculosis is more than zero point one percent (0.1%) but not more than one percent (1%) of all herds in the country or zone during the last two (2) years; or

(cc) shown a negative result to a tuberculin test every three (3) years to ensure the continuing absence of bovine tuberculosis if the annual percentage of herds confirmed as infected with tuberculosis is not more than zero point one percent (0.1%) of all herds in the country or zone during the last four (4) years; or

(dd) shown a negative result to a tuberculin test every four (4) years to ensure the continuing absence of bovine tuberculosis if the annual percentage of herds confirmed as infected with tuberculosis is not more than zero point one percent (0.1%) of all herds in the country or zone during the last six (6) years,

(b) that the bovine introduced into the compartment come from a herd free from bovine tuberculosis

provided that this condition may be waived for animals which have been isolated for at least ninety (90) days and which, prior to entry into the compartment, were subjected to at least two (2) tuberculin tests carried out at a six (6) month intervals with negative results and with the second tuberculin test performed during the thirty (30) days prior to entry into the compartment;

(c) that the cattle and water buffalo in a compartment free from bovine tuberculosis are protected from contact with wildlife reservoirs of bovine tuberculosis and are managed under a common biosecurity plan protecting the bovine from contamination with *M. bovis*, and the compartment has been approved by the Authority in accordance with Chapters 4.3 and 4.4 of the OIE Terrestrial Code.

(4) A bovine producer or a group of bovine producers may apply in writing to the Authority for a certification that a herd of bovine is free from bovine tuberculosis.

(5) A bovine producer or a group of bovine producers applying under subregulation (4) shall satisfy the Authority

(a) that the entire country, zone or compartment is free from bovine tuberculosis and is certified free by the Authority; or

(b) that the cattle and water buffalo in the herd

(i) have shown no signs of bovine tuberculosis or lesions at ante-mortem or post-mortem inspection for at least one (1) year;

(ii) were over six (6) weeks of age at the time of the first test and have shown a negative result to at least two (2) tuberculin tests carried out at a minimal interval of 6 months

Provided that in the case of regaining a designation of free status after an outbreak, the first test should be performed at least six (6) months following the slaughter of the last affected animal;

(iii) to maintain the free status, met one of the following conditions

(aa) showed a negative result to an annual tuberculin test to ensure the continuing absence of bovine tuberculosis; or

(bb) showed a negative result to a tuberculin test every two (2) years to ensure the continuing absence of bovine tuberculosis if the annual percentage of herds confirmed as infected with tuberculosis is not more than one percent (1%) of all herds in the country or zone during the last two (2) years; or

(cc) showed a negative result to a tuberculin test every three (3) years to ensure the continuing absence of bovine tuberculosis if the annual percentage of herds confirmed as infected with tuberculosis is not more than zero point one percent (0.1%) of all herds in the country or zone during the last four (4) years; or

(dd) showed a negative result to a tuberculin test every four (4) years to ensure the continuing absence of bovine tuberculosis if the annual percentage of herds confirmed as infected with tuberculosis is not more than zero point one percent (0.1%) of all herds in the country or zone during the last six (6) years;

(c) that the bovine introduced into the herd come from a herd free from bovine tuberculosis. This condition may be waived for animals which have been isolated for at least 90 days and which, prior to entry into the herd, were subjected to at least two tuberculin tests carried out at a 6-month interval with negative results with the second tuberculin test performed during the 30 days prior to entry into the herd ;

(d) that the females should not be tested if more than seven months pregnant or less than 45 days post partum. This should be recorded on thetuberculosis field form;

(e) that the free recognition of the country, zone or herd shall be published in the Gazette.

(6) Where the Authority is satisfied that the conditions in subregulation (5) are met, it shall issue a free herd certificate which shall be valid for a period of one year from the date of issue.

Cancellation and revalidation of free herd certification.

7. (1) The Authority shall temporarily suspend a free herd certificate when the Authority detects that animals tested positive to the tuberculin test.

(2) The Authority may restore the validity of a free herdcertificate when the epidemiological investigation is concluded and it is determined that the herd is not infected.

(3) The Authority shall cancel a free herd certificate by notice in writing to the holder of the certificate in any of the following cases

(a) when tuberculosis infected animals originating from a free herd are detected by the Authority at a slaughter plant and are confirmed by laboratory tests;

(b) when animals introduced into the herd do not originate from free herds;

(c) when there is epidemiological evidence that the herd is infected;

(d) when there is failure to meet the specifications under these Regulations.

(4) A bovine producer or group of bovine producers may apply to the Authority for a re-certification of a free herd certificate within thirty days of expiration.

(5) Every bovine producer or group of bovine producers shall meet the following criteria when applying under subregulation (4) for the revalidation of a free herd certificate

(a) that all animals that entered the herd in the last twelve (12) months originated from a free herd;

(b) that a caudal fold tuberculin test conducted by the Authority or by an accredited veterinarian yielded negative results in all animals from six (6) months of age, not more than thirty (30) days prior to the expiration of the certification. Where positive results are obtained to the caudal fold test the comparative cervical test must be conducted according to the Regulation 11 of these Regulations;

(c) the revalidation certificate must have a validity period of 12 months;

(d) the owner of the herd must show the corresponding records;

(3) The Authority shall issue a certificate of officially free herd with a validity period of twelve months when the following conditions are met

(a) that the tuberculin test has been conducted by the Authority's veterinarians and shall be valid for one year. re-certification tests shall also be conducted by BAHA veterinarians;

(b) the owner must have free herd certification for two years and not have any history of tuberculosis infection; the owner of herds that were infected and released from quarantine must have maintained free herd certification for the last five consecutive years;

(c) adjacent herds must have tested negative within the 12 months prior to certification. These adjacent herds must be tested by official veterinarians or accredited veterinarians under official supervision.

(4) The certification of officially free herd shall be valid for one year.

(5) An epidemiological investigation of the free herd shall be conducted by the Authority or an accredited veterinarian. "Appendix E".

Programme evaluation.

8. (1) The Authority, through the Animal Health Department, on an annual basis shall evaluate the Programme operation in the regions and districts through an annual report prepared by the Programme Coordinator and validated by the Director of Animal Health.

(2) The Authority may conduct visits to verify compliance with the requirements for each health status and shall issue a statement on the change of status according to the findings of the visits.

Identification. S.I. of 2011.

9. (1) For the purpose of the Programme, all animals must be fully identified in accordance with the Belize Agricultural Health Authority (Animal Identification) Regulations, 2011.

(2) Notwithstanding subregulation (1), for the purpose of the Programme the following specifications apply

(a) an animal shall bear a permanent "T" mark on the left masseter muscle when the animal tests positive to the tuberculin test;

(b) a bovine animal shall bear a yellow flagged and button ear tags with black laser impriniting on its left and right ear respectively, and that shall be the official identification of the bovine for the tuberculosis Programme;

(c) the individual number that corresponds to each animal must be registered in the official documents of diagnostic tests;

S.I. of 2011.

(d) an animal identified under the Belize Agricultural Health Authority (Animal Identification) Regulations, 2011 shall be exempt from any other identification mark.

Diagnosis and tuberculinization.

10. (1) For the purpose of the Programme and these Regulations, the diagnosis of tuberculosis shall be conducted through tuberculinization.

(2) The following tuberculin tests authorized by the Authority shall be administered by an official or accredited veterinarian on cattle six (6) weeks of age and older, as necessary

(a) caudal fold test,
(b) comparative cervical test,
(c) simple cervical test.

(3) The tuberculin registered and authorized by the Authority for the Programme are

(a) Bovine PPD - prepared with *Mycobacterium bovis strain* AN5, containing 3,250 IU per 0.1 ml dose, and to be used for the caudal, comparative cervical and simple cervical tests;

(b) Avian PPD - prepared with *Mycobacterium avium* strain D4, containing 5,000 IU per 0.1 ml dose and to be used in the comparative cervical test. The avian PPD tuberculin should contain Ponceau red dye to distinguish it from the bovine PPD that has no dye;

(c) the tuberculin must be transported maintaining the cold chain at a temperature between 4°C - 8°C and must be protected from direct sunlight during use in the field; the lot number and the expiry date of the product need to be verified; and once the vial is opened, the remaining contents must be discarded if not used on the same day.

(4) The Authority shall ensure that in conducting the tuberculin test the equipment conforms to the following specifications

(a) graduated disposable 1ml sterile syringes, with 0.1 ml graduations;

(b) hypodermic, disposable, sterile needles (24-29 gauge x 0.5 to 1.5 cm in length). Asterile needle should be used per animal;

(c) a metallic or plastic calibrated caliper (in mm) shall be used for the comparative cervical test.

(5) The Authority shall ensure that for the administration of any type of test, other management activities including but not limited to branding, castration, deworming, vaccination, treatments shall not be performed, 48 hours before and 72 ±6 hours after the administration of tuberculin so that the results are not affected.

Caudal fold test.

11. (1) The caudal fold test shall be administered by an official or accredited veterinarian when the tuberculosis status of the herd or lot of animals is unknown or as a substitute for the simple cervical test and the results of the test shall be recorded and shared with the interested party.

(2) The management techniques for the administration of tuberculin in the caudal fold test shall consist of

(a) restraint of the animal,

(b) cleaning of site where the biologic will be applied,

(c) intradermal insertion of the needle administering 0.1 ml of tuberculin (a small bleb shall appear at the site of injection),

(d) in the case that no bleb appears, a repeated injection.

(3) The caudal fold test shall be interpreted by the official or accredited veterinarian as follows

(a) the same veterinarian that applied the tuberculin must read the results, through observation and palpation of the site of injection, 72 hours (± 6 hours) after the injection is administered;

(b) the veterinarian shall also ensure that he is reading the same animals tested and recorded on the field form.

(4) The reactions to the caudal fold test are classified as follows

(a) negative - when no change is observed or palpated at the injection site;

(b) positive - when any thickening, redness, warmth, pain or necrosis is visible or palpable or any change however minimal, is noted at the site of injection.

Comparative cervical test.

12. (1) The comparative cervical test is the test authorized to confirm or rule out animals positive to the caudal fold test.

(2) The comparative cervical test

(a) may be used once within ten (10) days or sixty (60) days after the caudal test injection;

(b) must be applied by an official or accredited veterinarian;

(c) shall not be used in herds in which the presence of tuberculosis has been confirmed through isolation of *M. bovis* from samples obtained from animals slaughtered;

(d) shall only be conducted where the veterinarian is in possession of documentation of the previous test(s).

(3) For the application of tuberculin for the comparative cervical test, the following practices shall apply

(a) two quadrangular areas of at least 5 cm per side of the animal shall be shaved;

(b) the tuberculin shall be injected on the medial third part of the neck;

(c) the dorsal site shall be 10 cm beneath the crest;

(d) the ventral site shall be approximately 10 cm below the dorsal site;

(e) the test shall be administered using the intradermal injection of 0.1 ml of avian PPD in the shaved dorsal site and 0.1 ml of bovine PPD on the ventral site;

(f) prior to injection, a fold of skin shall be lifted at the centre of the shaved site and the thickness of this fold shall be measured using the calipers;

(g) the readings obtained shall be recorded on the comparative cervical test forms;

(h) the test shall be read 72 hours (± 6 hours), measuring the thickness with the calipers at the site of injection and the measurements shall be written down on the field form for the comparative cervical test according to 'Appendix A" subtracting the value of the first reading from the value of the second reading and rounding the final result as follows

(i) from 6.2 it decreases to 6.0,
(ii) from 6.3 it increases to 6.5;
(iii) from 6.7 it decreases to 6.5 and
(iv) from 6.8 it increases to 7,

(i) when the measurements are finalized the results obtained for avian PD as well as bovine PPD shall be graphed and the intersection point shall give the result of the test;

(j) using the official graph, the results shall be interpreted according to "Appendix A";

(k) where the reaction of an animal is classified according to the graph as suspect in two consecutive tests, it shall be classified as positive to the test.

Simple cervical test.

13. (1) The simple cervical test shall be used to test infected, exposed animals or as a screening test.

(2) For the application of tuberculin for the simple cervical test, the following practices shall apply

(a) a quadrangular area should be shaved at least 5 cm per side;

(b) the tuberculin shall be injected on the medial third part of the neck approximately 10 cm below the crest;

(c) the test is administered through the intradermal injection of 0.1 ml bovine PPD;

(d) the same veterinarian that applies the injection shall conduct the reading;

(e) the reading is done by observation and palpation of the site of injection, 72 hours (± 6 hours) after injection.

(3) The reactions to the simple cervical test are classified as

(a) negative - when no change is observed or palpable on the skin at the site of injection;.

(b) positive - when any thickening, redness, warmth, pain or necrosis or any change, however minimal, is visible or palpable at the site of injection.

(4) The simple cervical test may be substituted by a caudal fold test after

(a) obtaining a negative simple cervical test in all the animals tested in the herd, or
(b) obtaining no positive laboratory result in animals that test positive,

but the animals that test positive to this caudal fold test shall not be subjected to the comparative cervical test and any reaction shall be considered positive.

Bacteriology, histopathology and molecular biology analysis.

14. (1) The sampling methods for bacteriology, histopathology and molecular analysis shall be carried out as follows

(a) specimens for tuberculosis lesions (caseous or calcified) may be taken from any organ showing these typical lesions;

(b) lymph node samples shall be taken preferably from the head region (retropharyngeal, mandibular and parotid), cervical, mediastinum and tracheobronchial nodes;

(c) other organs subject to sampling are lungs, spleen, liver, kidney, bone marrow, ovaries, uterus, testicles and mammary glands;

(d) If the animal is positive for the tuberculin test but on post mortem does not show granulomatous lesions, suggestive of infection, any of the following may be sent to the laboratory

(i) lymph nodes from the headregion(retropharyngeal, mandibular or parotid),

(ii) tracheobronchial lymph nodes, and

(iii) mediastinum lymph nodes.

(2) The samples for histopathology must be fixed in 10% formalin and the size of tissues should be approximately 2cmx2cm in a ratio of one part tissue to nine parts of formalin.

(3) All samples taken under this Regulation shall be submitted to the laboratory and be accompanied with a completed prescribed requisition form.

(4) In the laboratory, samples shall be tested using Bacteriology, Histopathology or Molecular Diagnostic Assays or any combination of tests, as applicable.

Diagnosis.

15. (1) In conducting histopathologic diagnosis by the Authority

(a) staining shall be performed with Haematoxylin-Eosin stain to identify any morphologic changes as well as granulomatous lesions;

(b) Ziehl Nielsen staining shall be performed to identify the presence of acid fast bacilli;

(c) Carbol Fuchsin stain may be utilized to stain suspicious smears.

(2) The histopathology results shall be interpreted by the Authority as follows

(a) suggestive of tuberculosis - when granulomatous lesions of tuberculosis are observed; this is characterized by necrotic lesions or calcified by mineralization, multinucleated epithelial cells and ganglial cells (Longhans and Macrophages);

(b) compatible with tuberculosis - when acid fast bacilli are present intra or extra cellularly, in addition to the granulomatous lesions characteristic to tuberculosis;

(c) negative - when no characteristic lesions of tuberculosis are observed, no acid fast bacilli are seen, when a differential diagnosis confirms another disease or when no lesions are observed indicative of any disease. Differential diagnosis should be indicated.

(3) (a) Bacteriologic diagnosis may be conducted by the Authority as follows

(i) direct exam - Ziehl Nielsen or Carbol Fuchsin stains shall be used to identify the presence of acid fast organisms; if specimen is positive, the bacilli appear red in colour;

(ii) indirect exam - culture, isolation and identification of *Mycoplasma bovis* spp. is performed by inoculation of suspicious samples to cellular cultures of Middlebrock 7H10, Middlebroke 7H11, Stonebrink with sodium pyruvate, and Lowenstein Jensen; typing must be performed using biochemical methods.

(b)an official or accredited veterinarian conducting a bacteriologic diagnosis shall

(i) collect specimens of not more than 2cm x 2cm for bacteriologic studies
(ii) place the specimensin a saturated borate solution in a 1:1 ratio and
(iii) send the specimens to the within 7 days of collection.

(4) The laboratory technician shall immediately process specimens submitted under subregulation (3) above. (See Appendix B).

(5) The techniques applicable in conducting molecular biology diagnosis shall be performed by the Authority only on post mortem samples as follows

(a) PCR

(i) shall be performed within 72 hours of collection, on fresh tissue samples that show typical tuberculosis lesions;ee Appendix B)

(ii) shall be used as a preliminary diagnostic tool prior to bacterial isolation in accordance with Appendix B;

(b) Oligonucleotide typing (spoligotyping)

(i) may be utilized on fresh tissue compatible or suggestive to tuberculosis or with microbial isolates; or

(ii) may be used as an alternative diagnostic tool for the typing of tuberculosis. (See Appendix B)

Gamma Interferon Assay.

16. (1) The Authority may utilize the Gamma Interferon Assay as a complementary test and for the purposes of these Regulations is considered as the "in vitro comparative assay" since it quickly distinguishes positive animals due to the production of γIFN specific to *M.bovis* and *M.avium* in blood plasma.

(2) The Gamma Interferon Assay shall be used to detect the presence of gamma antibodies in plasma present in average concentration by reading the optical density of the γIFN by an immuno-enzymatic method when cattle are sensitized by the tuberculin test and in the laboratory when blood is sensitized with avian and bovine PPD.

(3) The results of Gamma Interferon Assay test are reported as either positive or negative to *M.bovis* or *M.avium* depending on the established cutoff value where any value equal or greater than 0.500 isconsidered positive.

Management of unit infected with bovine tuberculosis.

17. (1) Every owner of a farm with a dairy production unit infected with bovine tuberculosis shall ensure that the dairy production unit is managed by an accredited veterinarian for the purpose of reducing the prevalence of tuberculosis in dairy units through the replacement of animals from a calf rearing unit until the disease is eradicated.

(2) The accredited veterinarian of a dairy production unit under subregulation (1) is responsible for the management of the herd, including the activities within the calf rearing unit.

(3) For the purposes of this Regulation, the animals in the herd shall be subjected to an initial test to determine prevalence and to identify infected animals, provided that the test shall not be conducted in a period greater than 6 months.

(4) When it is known which are the infected animals, the accredited veterinarian shall identify the animalwith a red ear tag on the left ear or a permanent brand with the letter "T" on the left masseter muscle in accordance with the provisions of these Regulations .

(5) The elimination of positive animals shall be carried out in a programmed manner, through an agreement with the owner, taking into consideration age, level of production and the genetic quality of the animals and in accordance with Regulation 19.

(6) The Authority shall send the animals selected for slaughter to a BAHA inspected slaughterhouse to provide follow-up to the monitoring process.

(7) The accredited veterinarian shall segregate, by lot within the same installations, all positive animals that remain in the herd and the negative animals shall be tested every year and must be segregated by lots.

(8) Every owner of a farm having a dairy production which has been infected with bovine tuberculosis shall not move animals to other farms except where movement is to a controlled calf rearing unit operated by the same owner with the same sanitary status which has beenauthorized by the Authority, in which case the movement must be in compliance with a movement permit.

(9) Every owner of a farm which has been infected with bovine tuberculosis in its dairy unity shall

(a) have a calendar of cleaning and disinfection for all the installations, using approved disnfectants.

(b) apply sanitary measures for the control of rodents and harmful wildlife.

(10) In managing a calf rearing unit in herds affected by tuberculosis in specialized dairy production units, an accredited veterninarian shall not test a Female animal if that animal is more than seven months pregnant or less than 45 days post partum.

(11) Where a calf tests negative to the tuberculin test, an accredited veterinarian shall send the calf to a controlled rearing unit.

(12) The owner of a farm shall separate calfves from the dam immediately after parturition, and shall house the calves in individual pens. The farmer shall feed the calves shall be fed with colostrum from cows negative to the tuberculin test or with colostrum substitute or pasteurized colostrum.

(13) The Authority shall test two to three month old female calves with tuberculin and the calves that are negative may be moved to a communal pen within the same installation, but isolated from the rest of the animals.

(14) The Authority shall test two to three month old female calves with tuberculin and the calves that are positive may be moved to a communal corral for segregation and elimination within the same installation, but the communal corral must be isolated from the rest of the animals.

(15) The Authority shall again test the female calves sixty (60) days later and may move the female calves to a controlled calf rearing unit where the animals are to be placed in the "receiving corral" (occupied only by the lot of female calves that are entering with the same health status.

(16) The female calves up to 6 month of age test positive to any of the tests must be identified with a permanent brand with the letter "T" on the left masseter muscle or with the red ear tag on the left ear and these female calves may only be moved to slaughter at an Authority-inspected slaughterhouse with the corresponding health certificate in a period not more than 10 days from the day of reading of the test and to a slaughterhousewith the Authority's inspection. In the case of animals older than 6 months of age these shall be slaughtered or may be returned to the segregation unit of the herd of origin.

(17) In the establishments that have a controlled calf rearing unit, the accredited veterinarian shall submit a monthly report of activities conducted and work plan.

(18) The owner of a farm must implement a calendar of cleaning and disinfection in all the installations of the farm and the controlled calf rearing unit. The hygiene must be approved by an accredited veterinarian with special emphasis between each lot of heifer calves that enter the receiving pen.

Management of controlled calf rearing unit.

18. (1) A person keeping a controlled rearing calf unit shall

(a) ensure that the animals that enter the units exclusively originate from production units that implement the sanitary measures for the control of bovine tuberculosis in their dairy herds;

(b) ensure that the units as well as the herds are established within the same district and in the same phase of the Programme;

(c) in the case of location of calf rearing units in regions or districts at a higher or lesser phase of the programme or at a different district, the units must have the authorization of Authority following a favourable risk analysis;

(d) ensure that the calves are isolated without any possibility of contact with any bovine, caprine, ovine, avian and swine production unit that and the production of other species in the area of the controlled calf rearing unit is not allowed;

(e) install a double perimeter fence with at least 6 m between them the calf rearing unit and the unit of other production species;

(f) have an accredited veterinarian at each calf rearing unit kept;

(g) have a loading chute, corrals, feed storeroom, water, chutes for the tuberculin testing, and an exclusive corral for the reception of heifer calves or heifers;

(h) ensure that a rearing calf unit kept in pursuance of these Regulations does not exceed the capacity of the installation;

(i) implement a calendar of cleaning and disinfection of installations and equipment in the reception pens upon entry and exit of each lot;

(j) identify all animals in a controlled calf rearing unit with an official ear tag;

(k) conduct a tuberculin test on animals which have attained sexual maturity and can be used as replacements, and where the test is negative, may proceed to move it to its herd;

(j) ensure that animals within a calf rearing unit that test positive to the tuberculin test are identified with a red ear tag on the left ear or with a permanent brand with the letter "T" on the left masseter muscle and that such animal be sent to slaughter in accordance with these Regulations;

(k) ensure that access by people and vehicles to a calf rearing unit be restricted and that the entry of personnel and vehicles is subjected to cleaning and disinfection before entering the controlled calf rearing unit.

(2) A person who desires to move a herd for re-entry into the herd of origin shall possess the corresponding sanitary certificate to proceed with the movement and shall ensure that the vehicle is sealed.

(3) The accredited veterinarian keeps the control of the movements referred to under subregulation (2).

(4) A person who is moving animals for re-entry into the herd of origin shall house the animals in such a manner that there is no contact with the segregated animals of the infected herd with the aim of developing a new herd that is negative.

(5) A person who keeps a calf rearing unit shall ensure that the programming in the milking room is such that animals of different sanitary status do not coincide.

Slaughter.

19. (1) Every owner of exposed animal in an infected herd shall keep the infected herd at the farm where it is kept under quarantine and be tested on day sixty (60) and again on day ninety (90) and a third test shall be conducted one hundred and eighty (180) days after the first test.

(2) Every owner of suspect or positive animals shall have the animals slaughtered at an Authority-designated slaughterhouse immediately or within thirty (10) calendar days of the reading of the tuberculin test.

(3) The circumstances leading to the slaughter of an animal under this Regulation shall be assessed for the purposes of compensation payable to the owner of the animal.

(4) A slaughter to be conducted under subregulation (2) shall require inspection by the Authority.

(5) Notwithstanding subregulation (2), the Authority may authorize the slaughter of exposed or positive animals in another district provided that proper notice is given to the District Veterinarian in the district where the animal will be slaughtered.

(6) The owner of the herd of origin of the exposed or positive animals should ascertain the slaughter of the anmals and collate the verification certificate from the veterinarian in charge at the slaughter plant. The owner must keep this certificate for one year after slaughter.

(7) When the movement of an animal or herd is for slaughter, the District Veterinarian of the district of origin must submit a monthly reports of movement for slaughter to the District Veterinarian where the slaughterhouse is located.

(8) The District Veterinarian where the slaughterhouse is located must submit monthly reports on inspection results to the Authority.

Movement.

20. A person who desires to move an animal to another district shall apply to the Authority for a Movement Permit and shall comply with the Belize Agricultural Health Authority (Animal Identification) Regulations, 2011.

S.I. of Importation.

21. (1) A person desiring to import an animal into the country shall have among the importation documentation an official certificate that attests to the origin of the animal from a country or zone with Bovine Tuberculosis status recognition by the Authority.

(2) Every importer of an animals shall have the relevant official animal identification informationfrom the exporting country.

(3) In accordance with subregulation (3) above, the Animal Health Department, may establish with international scientific support, the sanitary requirements for regionalization or compartmentalization in country or zones in which a prior quantitative or qualitative risk analysis showed an insignificant risk, provided that it does not contravene the provisions ofese Reguations.

(4) Where it is known that the animals originating from a country or zone officially recognized by Belize come from a herd infected with tuberculosis, the animals in transit to Belize or that are under retention at an entry point into the country as well as the animals that are already in the country, shall be quarantined in specific authorized sites

and the corresponding epidemiological investigation shall be conducted by the Authority. When the Authority determines that the importation should be denied the animals shall return to the country of origin or be slaughtered at the expense of the owner or importer.

(6) The procedures to evaluate the regionalization of exporting country or zones by the Authority are

(a) expressed request from the exporting country or from the country's official veterinary services;

(b) respond to "Appendix F" and provide the additional information requested, as the case may be, with documentary evidence;

(c) epidemiological analysis conducted by the Authroity of the information submitted by the exporting country, butthe Authority may request additional information or information for technical support;

(d) personnel from the Animal Health Department shall conduct a technical visit to evaluate and verify the information submitted;

(e) once the corresponding risk analysis has been conducted, a decision shall be made and where it is favorable, the import requirements shall be established in accordance with these Regulations and where the recognition is of free status, the administrative procedures shall be carried out to have the information published in the *Gazette*.

(f) where there is a change in sanitary status or non-compliance with these Regulations, the Authority shall cancel the recognition of free status or the equivalency granted;

(g) the Animal Heath Department may re-test live animals to verify the official test results of the exporting country through the diagnostic tests established in these Regulations.

Epidemiological surveillance.

22. (1) As tuberculosis is a notifiable animal disease, all cases of bovine tuberculosis must be reported by livestock producers, veterinarians, investigators, slaughter plant inspectors, laboratory personnel and all those who are involved with the production and commercialization of cattle.

(2) The Authority shall implement epidemiological surveillance through the analysis of information generated by Programme activities.

(3) In the Programme, epidemiological surveillance of tuberculosis shall be carried out as follows

(a) by official and accredited veterinarians who shall submit all the reports of results obtained in the tuberculin testing of cattle. Such information must be submitted monthly or within 24 hours of a positive finding to the district veterinarians and the Director of Animal Health;

(b) by at least one veterinarian responsible for the inspection of cattle at slaughter plants to detect lesions suggestive of tuberculosis;

(c) the veterinarians responsible for the inspection of cattle at slaughter plants shall be responsible for the collection and submission of samples of granulomas of animals during routine slaughter as well as of animals identified as positive or suspect. The veterinarians shall maintain records of the suspect cases and shall inform the district veterinarian in accordance with these Regulations for reporting;

(d) the laboratory technicians in official and accredited laboratories shall conduct diagnostic assays on the samples from slaughterhouses and they shall inform the Director of Animal Health within twenty-four hours of test results;

(e) an investigation that implicates the use of *M. bovis* must be previously evaluated and authorized by the Authority.

(4) A complete epidemiological investigation, following Programme guidelines, shall be conducted by the accredited veterinarian on all animals diagnosed with bovine tuberculosis.

Quarantine measure.

23. (1) When quarantine is to be implemented, the owner of the herd or lot of animals shall be duly informed in writing by the Authority and the reason for the qurantine, , legal base and the procedure for its removal shall be given by the Authority.

(2) Quarantine may be precautionary or definitive and precautionary quarantine must change to definitive quarantine in the cases mentioned in sub-regulation (4) (a) and (b).

(3) Precautionary quarantine shall be applied by the Authority in any of the following cases

(a) in the herd or lot of animals where the official or accredited veterinarian detects animals positive to the caudal fold tuberculin test and the comparative cervical test was not conducted within 10 days and a release from quarantine shall be upon a negative result to the comparative cervical test of the animals that tested positive to the caudal fold test;

(b) in the herd or lot of animals where the official or accredited veterinarian detects animals positive or suspect to the comparative cervical test and in the case of positive or suspect anmals sent to slaughter, quarantine shall be lifted once these animals are slaughtered and no suggestive lesions are found, a negative laboratory result and all the animals in the herd or lot test negative to the caudal fold test;

(c) in the herd or lot of origin of the animals which are found by the accredited veterinarian with lesions at routine slaughter and the histopathology result is suggestive of or compatible with tuberculosis. The Authority shall lift quarantine when all the animals in the herd or lot test negative to the caudal fold test;

(d) in the herd or lot of animals that is the origin of the animals of an infected herd or lot. The Authority shall lift quarantine when all the animals in the herd or lot test negative to the caudal fold test;

(e) in the herd or lot of animals determined to have had contact with an infected herd or lot. The Authority shall lift quarantine when all the animals in the herd or lot test negative to the caudal fold test;

(f) in the herd or lot of animals adjacent to an infected herd or lot of animals. The Authority shall lift quarantine when all the animals in the herd or lot test negative to the caudal fold test;

(g) in the herd where the owner does not have the caudal fold test performed in his entire herd within one hundred and twenty (120) days of test notification. The Authority shall lift quarantine when all the animals in the herd or lot test negative to the caudal fold test.

(4) The Authority shall implement definitive quarantine in any of the following cases

(a) in the herd or lot of animals where infection with *M. bovis* is confirmed by culture and typing;

(b) when the Authority determines that the presence of *M. bovis* is indicated through epidemiological studies which include field tests, post mortem lesions, histopathology results compatible or suggestive of infection or by PCR or Gamma interferon;

(5) The owner of a farm with exposed animals in herds under definitive quarantine must keep the exposed animals in the herd until depopulation or completion of tests for the lifting of quarantine.

(6) The owner of a farm of Exposed animals may move the animals to quarantined fattening units within the same region or district, complying with a current negative test, officially identified and with authorization from the Authority.

(7) To lift a definitive quarantine, four consecutive tuberculin tests with negatve results need to be conducted on all the animals in the herd six weeks of age and older and there must be compliance with all other quarantine measures stipulated in these Regulations. The second test may be conducted at least 60 days after the first test, the third test after at least 180 days after the second test is conducted and the fourth test at least 180 days after the third test.

(8) The lifting of quarantine shall be conducted through a written letter from the Authority when the production unit has complied with all the sanitary measures stipulated in these Regulations.

(9) It is mandatory to conduct another tuberculin test for epidemiological surveillance purposes 12 months after the last test to lift quarantine. A person intending to move a herd shall notify the Authority of the movement of animals out of the herd and the Authority shall record movement during this period, specifying their destination.

(10) In the case of dairy cattle registered in the Programme for the management of a dairy production unit affected by bovine tuberculosis, an accredited veterinarian may place the herd under containment according to the results obtained in the tuberculin tests.

Disinfection.

24. (1) When a reactor animal is detected and eliminated in an intensive system, the owner of the slaughterhouse shall disinfect the slaughterhouse, including the areas where the animal was housed, through a vigorous wash with soap and water followed by mechanical cleaning and the application of approved disinfectants.

(2) The owners or operators of slaughterhouses shall register, for approval by the Authority all disinfectants used in the Programme.

(3) For the purposes of these Regulations, approved disinfectants include all phenols.

Contingency funds.

25. The Authority may make agreements with other parties for the creation of contingency funds to quickly implement the necessary sanitary measures to decrease the prevalence or eradicate bovine tuberculosis in a specified region.

Evaluation of compliance.

26. The Authority shall conduct periodic evaluations of the Programme according to the procedures developed for this purpose.

Fees.

27. (1) The Authority may charge fees for testing and services related to testing for tuberculosis in bovine.

(2) Fees imposed by the Authority under subregulation (1) shall be borne by the producer.

(3) The amount of fees to be charged under this Regulation shall be published by Notice in the *Gazette*.

Offence and penalty.

28. A person who contravenes these Regulations commits an offence and is liable on summary conviction to a fine not exceeding five thousand dollars or to imprisonment for a period not exceeding three years or to both fine and imprisonment.

Commencement.

29. These Regulations shall come into effect on the _____ day of _____, 2011.

MADE by the Minister responsible for Agriculture, after consultation with the Belize Agricultural Health Authority, this day of , 2011.

_____

(RENE MONTERO)
Minister responsible for Agriculture