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Detecting Bovine Tuberculosis (bTB) at an Exporter Slaughterhouse: Improving Food Security and Preserving Sovereignty

Jose Amoril [1+], Ervaldo Sena [2] and Laerte Baldani [3]

[1] Ministry of Agriculture, Livestock and Food Supply, Goiania, GO, Brazil

[2] Laboratory of Molecular Biology, Food Research Center - CPA at the Veterinary School of Goias Federal University, Goiania, GO, Brazil

[3] Laboratory of Veterinary Pathology - Lapavet, Santo Andre, SP, Brazil

Abstract. Bovine tuberculosis (bTB) persists as a serious public health problem in a world where food security is decisive as a global issue. We detected five bTB suspect carcasses during routine meat inspection procedures at an exporter Brazilian abattoir located in the State of Goias, Central-Western part of the country. We collected samples and sent them to be processed by a quantitative polymerase chain reaction (qPCR) at the laboratory of Molecular Biology of the Food Research Center (CPA) of Goias Federal University, Goiania, and by histopathology and Ziehl-Neelsen staining (ZNS) at Lapavet - Laboratory of Veterinary Pathology, Santo Andre, to confirm the suspicion. After laboratory processing, all five samples were positive to bTB and in two of them ZNS detected acid-alcohol resistant bacillus (BAAR). We conclude that laboratory confirmation of bTB is very important and that food security will not work properly without food safety.

Keywords: beef, federal inspection, food hazards, meat hygiene, zoonosis

1. Introduction

Animal disease is a serious impairment in the meat trade and bovine tuberculosis (bTB), an ancient disease [1], persists as one of the main public health problem worldwide [2] [3] .

According to FAO "food security is a situation that exists when all people, at all times, have physical, social and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life" [4]. In this concern, if animal diseases are not correctly detected, we can not guarantee food security for any individual and this is critical because food demand can be the major problem within the next 30 years [5].

As the biggest world beef exporter, it is imperative to Brazil the availability of a reliable and sound official veterinary service so the country can succeed selling meat to 142 different countries around the world, a six billion dollars year business as recently reported [6] [7].

Here we describe findings of tuberculosis in five animals during routine post mortem procedures including laboratory analysis, and we try to highlight the importance of these procedures to keep food security moving in the right direction.

2. Experiment

One of us (ja) had inspected by ante and post mortem exams 17,803 bovines from January 11 through November 18, 2013. Most of the animals were zebu crossed breed comprising 11,546 males and 6,247 females ages between 2 and 8 years old.

[+] Corresponding author. Tel.: + 5562 32591906; fax: +5562 32290499.
E-mail address: jgamoril@terra.com.br/jose.amoril@agricultura.gov.br.

2.1. Sampling and Methodology

During post mortem exam, we sampled granulomatous lesions resembling bTB from five suspect bovines.

2.2. Molecular Biology

Sterile swabs from lesions were transferred onto FTA®-Elute cards from WHATMAN FTA® Elute Technology (Piscataway, NJ, USA) and sent to the Laboratory of Molecular Biology at the Food Research Center from the School of Veterinary of Goias Federal University, Goiania, GO. After its elution, DNA was analysed in Nanophotometer (IMPLEN®, Westlake Village, CA, USA) for integrity and concentration. Then, a quantitative polymerase chain reaction (qPCR) with specific primers previously made at Food Research Center (CPA), targeting *Mycobacterium tuberculosis* Complex and *M. bovis*, was run in a 7500 Fast Real-Time PCR System and Step-One Plus Real-Time PCR System (Life Technologies, Grand Island, NY, USA).

2.3. Histopathology

Tissue fragments 20-30mm thick were excised from suspect lesions, kept for at least 48 hours for fixation in a 10% formaldehyde solution and sent to Lapavet-Laboratory of Veterinary Pathology, Santo Andre, State of São Paulo. Once in the lab, samples were dehydrated by ethanol, clearing by a xylene solution, infiltrated in paraffin wax followed by embedding in paraffin, maximum 4-5µm sectioned and staining by Hematoxylin & Eosin followed by observation of slides under optical microscopy.

2.4. Ziehl-Neelsen Staining

Additionally slides were screening for acid-alcohol resistant bacillus (BAAR) by Ziehl-Neelsen staining method.

3. Results and Discussion

Laboratory exams showed in the Table 1 below, confirmed bTB in all five suspect samples.

Table 1. Epidemiological, microbiological and biological patterns of five bTB positive samples (qPCR and histopathology) of bovines slaughtered in Goias, Brazil, from January 11th to November 18th, 2013.

# of the sample and date of slaughter	Breed	Sex	Age (years)	County of Herd	BAAR present	Type of DNA amplified	
						M. tuberculosis complex	*M. bovis* only
15-0762 01/21/13	Zebu crossed	Female	6	Montes Claros	Yes	Yes	No
01-0220 03/26/13	Zebu crossed	Male	3	Itabera í	No	Yes	No
16-1052 09/24/13	Zebu crossed	Male	5	Avelinopolis	Yes	Yes	No
09-0967 09/30/13	Zebu crossed	Male	3	Heitora í	No	Yes	Yes
05-0924 10/02/13	Holstein crossed	Male	3	Ivolandia	No	Yes	Yes

Our five suspect samples were confirmed as bTB. This is in disagreement with some authors when they claimed that "bTB diagnosis based on gross appearance is unreliable" [8]. After 25 years dealing with ante and post mortem inspection of cattle we can say that so far most of gross findings of bTB can be confirmed during post mortem examination prior to laboratory exams but of course the previous experience of the meat inspector must be take into account. Because sometimes we can be wrong, the better way is just submit all suspect samples for laboratory confirmation no matter the amount of experience a vet officer has [9].

Although it is not shown, our qPCR results had a 100% agreement with histopathology. These results are different from those obtained by Taylor et. al. [10] when PCR sensitivity was 91% while histopathology had a sensitivity of 78% when both were compared to microbiological culture, their "gold standard". Our "gold standard" was the qPCR that along with our small number of samples might explain the difference.

Traditional meat inspection methods are 50% sensitive, and that is the reason we can hardly agree with any proposition of just visual examinations of lymph nodes for routine post mortem inspection [11]. Certainly this procedure will narrow still more chances to detect unfit conditions, of course including bTB, during post mortem exams.

4. Conclusions

Undoubtedly globalization plays a pivotal role in the increasing of food trade but ambiguously while this scenario brings more food and more profit it also introduces more food hazards. So efficient veterinary services are necessary, and the outcome is that food security will not work properly without food safety.

Using qPCR results as "gold standard" is more efficient than microbiological culture because PCR, even the traditional non quantitative one, is less time consuming than Mycobacterium culture which can take many days before acceptable results come out.

According to these findings we also conclude that submitting suspect biological material for bTB laboratory confirmation is a "must", under a food safety point of view, but bTB diagnosis based on gross appearance is not unreliable and the professional experience of the veterinary officer counts.

Further similar studies using a larger number of bTB suspect carcasses are welcome.

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