Export of Bovine Paratuberculosis Vaccine to Nepal

Ashleigh Ahrens

Bovine Paratuberculosis

Bovine paratuberculosis, or Johne's Disease, is caused by the organism *Mycobacterium paratuberculosis* or *Mycobacterium avium subsp. paratuberculosis* (Raymond W. Sweeney, 1996). It causes economic losses due to decreased milk production of infected cattle (Stabel, 1998).

Symptoms

The symptoms of this disease are decreased milk production, diarrhea, internal lesions, and weight loss (A. P. Koets et al., 2001). The symptoms do not appear until later in life which is a major problem in controlling this disease (Rosseels & Huygen, 2008). No management can be taken until symptoms appear and by then other cattle would be infected.

Transmission

Sweeney (1996) has studied the transmission of the disease. A large cause of transmission is from ingestion of infected fecal matter. Calves can also be infected in the uterus of the infected mother. Milk and semen also have the potential to contain the bacteria. Infected milk is most likely a large source of transmission to young calves feeding from their mothers.

Management and Prevention

Proper sanitation of bottles used to feed calves is essential in order to limit the amount of bacteria ingested by the calf. Limiting interaction with older cattle who may have the disease can decrease infection in a susceptible young calf (Raymond W. Sweeney, 1996). There is no cure for this disease so prevention and management are key (R. W. Sweeney, Collins, Koets, Mcguirk, & Roussel, 2012).

Link to Crohn's Disease

There is great controversy on whether M. avium subsp. Paratuberculosis can cause Crohn's disease in humans (Timms, Daskalopoulos, Mitchell, & Neilan, 2016). Crohn's disease is an inflammatory syndrome or disease of the bowel (Chamberlin et al., 2001). There is no definite answer on whether the bacteria causes this problem in humans but it is thought to be caused by contaminated milk (Chamberlin et al., 2001).

Vaccine Development

Multiple vaccines exist on the market for bovine paratuberculosis. Whole-cell vaccines have been shown to interfere with bovine tuberculosis diagnosis and therefore cannot be used in countries with strict management plans in place (Santema, Hensen, Rutten, & Koets, 2009). They can also cause tissue damage where injected (Santema et al., 2009).

Subunit vaccines can eliminate interference with the diagnosis of bovine tuberculosis and potentially reduce tissue damage (Santema et al., 2009).

<u>Hsp70 Vaccine</u>

The vaccine has been developed by Koets and colleagues (2001) at Utrecht University. It is a subunit vaccine using recombinant heat shock protein 70 from *Mycobacterium avium subsp. paratuberculosis*. It works by increasing the antibody levels in the vaccinated animal to combat infection (Santema, 2011).

Bovine Tuberculosis (Bovine TB) Impact

The pathogens of bovine tuberculosis are related to human tuberculosis and could infect humans, although rare (Katale et al., 2012). Pasteurization of dairy products has eliminated the risk in developed countries (CFIA, 2015). The subunit vaccine using heat shock protein 70 (hsp70) has been proven to not interfere with the diagnosis for bovine tuberculosis (Santema et al., 2009). This is important as the vaccine can not impact the diagnosis of bovine TB due to the severity of the disease. The common management practice is to cull any sick cattle, so if the vaccine creates false negatives or false positives it cannot be used (A. Koets et al., 2006). If a false test is created, a sick animal could be left to infect the rest of the herd or a clean animal could accidentally be slaughtered, leading to unnecessary farmer economic loss.

Benefits

This vaccine has been shown to reduce fecal shedding of the disease and increase the lifespan of the sick cow (Santema et al., 2013). This reduces transmission of the disease. It also increases productivity as the cow lives longer and is not experiencing decreased milk production. This means more milk production per cow in its lifetime, which is economically desired. It reduces clinical symptoms such as diarrhea, which will increase animal welfare as the animal is not suffering from symptoms. It could also reduce the potential to contaminate milk with the bacteria, thought to be a cause of Crohn’s disease (Chamberlin et al., 2001).

Manufacture

The manufacture of this vaccine is not produced commercially yet. It is produced by creating recombinant hsp70 proteins as discussed in a study done by A. P. Koets et al. (2001). This process is too complex to go into detail here, but the protein needed could be obtained by a biological supplier such as StressMarq Biosciences Inc.

Companies

StressMarq Biosciences is based out of Victoria, BC in Canada. Biogenuix is based out of New Delhi, India. Biogenuix is already a distributor of StressMarq BioSciences. The products could then be shipped to Nepal from India. This may pose challenges as the infrastructure of roads in Nepal is poor.

Cost

The pricing for this product can be quite expensive once all the required materials are gathered. This report will only focus on the main protein required, the hsp70 protein. The approximate cost for this from StressMarq BioSciences Inc. (2016) is around 300$.

Benefits to Canada

The benefits to Canada would be that if this proves successful, it could also benefit our cattle herds and provide a new vaccine for our use. Canada has strict bovine tuberculosis management practices that cannot allow interference with the diagnosis process (CFIA, 2015).

It would not be a large source of income from Nepal as the product is expensive to manufacture and the Nepal society is quite poor. The vaccine shows promise in helping cattle with paratuberculosis, but may not be cost effective enough to launch more research and use in the future. However, research could be put in to work on making it cost effective for future use.

This could create jobs or even a new company that manufactures the vaccine in its final ready to use form rather than selling products needed to create the vaccine. It could also create further research opportunities for Canada scientists.

<u>Nepal</u>

The CIA World Factbook (2016) information on Nepal covers all aspects of the Nepalese life. Nepal is a landlocked country between China and India. The population is approximately 28 million. In 2013, only 72% of the rural population had electricity. There is 25% of the population below the poverty line, making Nepal one of the poorest countries worldwide. There is a total of 10,844 km of roadways, with only half being paved.

Agriculture

The CIA (2016) indicates that 69% of Nepal is involved in agriculture. Most of this agriculture is subsistence farming. Their livestock is therefore important to them for providing food and potential income, so it is in their best interest to keep them productive and healthy. A common stressor in livestock agriculture is mortality from disease (Pradhanang et al., 2015).

Religion

In Nepal, 81% of the population practices Hinduism (CIA, 2016). This means that cattle are considered sacred (Korom, 2000).

Cattle

The cow is the national animal of Nepal (Nepal Law Commission, 2015). This demonstrates that the Nepalese people respect the cow. Keeping the cattle healthy by preventing bovine paratuberculosis would be a benefit to Nepal, not only for religious and political reasons but also for productivity. Bovine paratuberculosis can lead to a decrease in milk production (A. P. Koets et al., 1999). Cows and other livestock provide manure, milk, and labour (Redding et al., 2012). Nepalese families rely on self production of milk as an excellent source of food (MOAD, CBS, & FAO, 2016). Milk contains a wide variety of nutrients as it is produced to support a growing calf. One of these important nutrients is calcium (Drewnowski, Tang, & Brazeilles, 2015). Calcium has many important benefits such as reducing osteoporosis risk and having many important functions such as muscle contraction (Miller, Jarvis, & McBean, 2001). The importance of milk in a country where poverty is a problem is high as it provides a cheap way to get essential nutrients required for growth and development.

Transport

Transport Chain

The products would be shipped from Vancouver in British Columbia, Canada to Kathmandu, the capital of Nepal. This could be done by a combination of methods: air, boat, and on-land vehicle such as a truck.

Transport Costs

The exact size of box the products are shipped in could not be found. The shipping costs are based on a box of approximately 30x30x30 cm. This was estimated as the box would most likely contain ice inside to keep it cool and could contain many vials of product based on a packaging description of a similar product on Alibaba.com (2016). The weight was estimated as 1 lb. Shipping by ocean would require extra transportation from the dock in India to Nepal as there is no water directly leading to Nepal.

Table 1: Estimated Shipping Costs with Various Methods based on an estimated box size of 30cm x 30cm x 30cm (A1 Freight Forwarding, 2016)

Method	CAD	NPR
Air	317	25,900
Ocean	337	27,537
Courier	218	17,813

The costs of transport are very high, with a courier being the cheapest (Table 1).

Storage

An issue with this product is the issue of storage and transportation. The materials need to be stored at cool temperatures as they are live biological reagents. This would require a refrigerated vehicle to transport the materials to an airport or dock, to get the product overseas by boat or plane. That boat or plane would also need to have some method of insulation and refrigeration to keep the product cold. The product would be fragile, most likely stored in containers and unable to handle rough shipping, so fragility would need to be taken into consideration. Storing the products once they have reached Nepal is also an issue. Electricity is not in every home, so the individual farmer couldn't store the vaccine in a refrigerator at home to use on their cattle. The vaccine could be stored at a veterinarian office with proper facilities and picked up when required.

Licenses

In order to get this vaccine to Nepal, a license to trade pharmaceuticals will be required (Nepal Business, 2012). This license will cost 205 Nepalese rupees, which converts to about 2.51 Canadian dollars (XE, 2016). It is valid for 2 years, so this cost will not affect the cost of the product significantly.

Total Cost

The estimated cost of the vaccine from Canada to Nepal is around 50,000 Nepalese rupees or 614 Canadian dollars.

Table 2: Estimated Total Costs of Export to Nepal (XE, 2016)		
Product	CAD	NPR
Vaccine	312	25500
License	2.51	205
Transport	300	24500
Total	600	49000

Benefit to Nepal

The vaccine improves productivity and longevity of cattle, which can increase the income or food supply for Nepalese families. It reduces fecal shedding, thereby reducing transmission of the disease to other animals. This will help control the disease in the long run as there is less chance of other animals becoming infected. This will help farmers prevent the disease in their cattle which is essential as there is no cure. Benefits of vaccination have been shown in a study by Groenendaal, Zagmutt, Patton, & Wells (2015) to be positive due to reduction of shedders of the bacteria in their feces. This shows promise that vaccination will be a worthwhile investment.

This could also benefit Nepal by providing science-based jobs to research the vaccine further and to manufacture the vaccine. It could provide more veterinary positions as they would be the most reasonable target consumers.

It could encourage more people to invest in cattle as the disease would not be such a threat to farmers.

Target Consumers

Nepalese farmers would use this vaccine on their cattle to increase productivity and longevity. It could be distributed to local veterinarians who then provide access to the farmers to ensure proper training and use.

Problems

Manufacture

This vaccine is not yet manufactured to sell as an individual product. This vaccine would need to be put together when the products arrive in Nepal. This would require a team of scientists who know, or who could learn, how to create the vaccine using recombinant techniques. It would require a laboratory and proper equipment, along with a sterilized environment to ensure no contamination. It would also require further access to other chemicals potentially needed.

Access

Access to veterinarians in rural Nepal could be quite difficult, with the minimal road infrastructure. A lot of Nepalese may not have vehicles as well and it could be a long journey to access a veterinarian, or a large cost to have the veterinarian visit them.

Cost

Affordability of this vaccine would be quite low as the vaccine would cost upwards of 600$. This converts to 49,000 Nepalese rupees (XE, 2016). With Nepal being a very poor country, this will not be affordable for most farmers.

Competition

China sells the same types of products for around the same price ($200-500/box) according to Alibaba.com (2016). This could be a source of competition as China is closer than Canada, so transportation costs would be lower. However, Biogenuix is already a distributor of StressMarq products and therefore must already have a transportation system in place in India that could reduce costs associated with transportation. They could also group the products with other products they are currently shipping to India to reduce the individual costs.

Another source of competition is the different types of vaccines on the market. With no strict regulatory practices in Nepal, the interference with diagnostic testing for bovine tuberculosis may not matter if other benefits are present at a cheaper rate.

Future Research

Further research will need to be conducted to determine if it is as an efficient vaccine as it is claimed to be and if it should be mass produced as an individual product available commercially. There should also be research devoted to finding a way to lower the cost of the vaccine so that it is affordable to all farmers. This could be done by changing which products are used to create the vaccine, or potentially reducing the amount of vaccine needed to be given.

Conclusion

Overall, this product is not incredibly cost effective to the Nepalese, and they may not consider this disease as much of a problem or have much information about the significance of bovine paratuberculosis. They would however want to increase their productivity and keep their cattle for longer, but they may push this aside in favour of saving money upfront, or just not be able to afford the vaccine. This product would not work to be exported to Nepal as there are significant challenges with poverty, accessibility, and storage.

References

A1 Freight Forwarding. (2016). Free Instant Quote. A1 Freight Forwarding. Retrieved from: http://www.a1freightforwarding.com/

Alibaba.com. (2016). Monoclonal Mouse HSP70 antibody. Retrieved from: https://www.alibaba.com/product-detail/Monoclonal-Mouse-HSP70-antibody_60318295564.html?spm=a2700.7724838.0.0.64XE6N

Canadian Food Inspection Agency. (2015). Bovine Tuberculosis – Fact Sheet. Retrieved from: http://www.inspection.gc.ca/animals/terrestrial-animals/diseases/reportable/tuberculosis/fact-sheet/eng/1330208938232/1330209051950

CIA. (2016). Nepal. *The CIA World Factbook.* Retrieved from: https://www.cia.gov/library/publications/resources/the-world-factbook/geos/np.html

Drewnowski, A., Tang, W., & Brazeilles, R. (2015). Calcium requirements from dairy foods in France can be met at low energy and monetary cost. *The British Journal of Nutrition*, *114*(11), 1920–8. https://doi.org/10.1017/S0007114515003669

Katale, B. Z., Mbugi, E. V, Kendal, S., Fyumagwa, R. D., Kibiki, G. S., Godfrey-Faussett, P., … Matee, M. I. (2012). Bovine tuberculosis at the human-livestock-wildlife interface: is it a public health problem in Tanzania? A review. *The Onderstepoort Journal of Veterinary Research*, *79*(2), 463. https://doi.org/10.4102/ojvr.v79i2.463

Koets, A., Hoek, A., Langelaar, M., Overdijk, M., Santema, W., Franken, P., … Rutten, V. (2006). Mycobacterial 70 kD heat-shock protein is an effective subunit vaccine against bovine paratuberculosis. *Vaccine*, *24*(14), 2550–2559. https://doi.org/10.1016/j.vaccine.2005.12.019

Koets, A. P., Rutten, V. P. M. G., De Boer, M., Bakker, D., Valentin-Weigand, P., & Van Eden, W. (2001). Differential changes in heat shock protein-, lipoarabinomannan-, and purified protein derivative-specific immunoglobulin G1 and G2 isotype responses during bovine Mycobacterium avium subsp. paratuberculosis infection. *Infection and Immunity*, *69*(3), 1492–1498. https://doi.org/10.1128/IAI.69.3.1492-1498.2001

Koets, A. P., Rutten, V. P. M. G., Hoek, A., Bakker, D., Van Zijderveld, F., Müller, K. E., & Van Eden, W. (1999). Heat-shock protein-specific T-cell responses in various stages of bovine paratuberculosis. *Veterinary Immunology and Immunopathology*, *70*(1–2), 105–115. https://doi.org/10.1016/S0165-2427(99)00062-8

Korom, F. J. (2000). Holy cow! The apotheosis of zebu, or why the cow is sacred in Hinduism. *Asian Folklore Studies*, *59*(2), 181–203. https://doi.org/10.2307/1178915

Miller, G. D., Jarvis, J. K., & McBean, L. D. (2001). The importance of meeting calcium needs with foods. *Journal of the American College of Nutrition*, *20*(2 Suppl), 168S–185S. https://doi.org/10.1080/07315724.2001.10719029

MOAD, M. of A. D., CBS, C. B. of S., & FAO, F. and A. O. (2016). *Food and Nutrition Security in Nepal: A Status Report*.

Nepal Business. (2012). License to import pharmaceutical products. *Nepal Business: License e-Portal.* Retrieved from: http://licenseportal.gov.np/index.php/browse_license/license_details/35

Nepal Law Commission. (2015). Constitution of Nepal. Retrieved from: http://www.lawcommission.gov.np/en/?workflow_state=prevailing-laws-constitution

Pradhanang, U. B., Pradhanang, S. M., Sthapit, A., Krakauer, N. Y., Jha, A., & Lakhankar, T. (2015). National Livestock Policy of Nepal: Needs and Opportunities. *Agriculture*, *5*(1),

103–131. https://doi.org/10.3390/agriculture5010103
Redding, L., Chetri, D. K., Lamichhane, D. K., Chay, Y., Aldinger, L., & Ferguson, J. (2012). Animal production systems of small farms in the Kaski district of Nepal. *Tropical Animal Health and Production*, *44*(7), 1605–1613. https://doi.org/10.1007/s11250-012-0114-4
Santema, W. (2011). *Hsp70 as a candidate subunit vaccine for paratuberculosis*.
Santema, W., Hensen, S., Rutten, V., & Koets, A. (2009). Heat shock protein 70 subunit vaccination against bovine paratuberculosis does not interfere with current immunodiagnostic assays for bovine tuberculosis. *Vaccine*, *27*(17), 2312–2319. https://doi.org/10.1016/j.vaccine.2009.02.032
Santema, W., Rutten, V., Segers, R., Poot, J., Hensen, S., Heesterbeek, H., & Koetsa, A. (2013). Postexposure subunit vaccination against chronic enteric mycobacterial infection in a natural host. *Infection and Immunity*, *81*(6), 1990–1995. https://doi.org/10.1128/IAI.01121-12
StressMarq BioSciences Inc. (2016). Hsp70 Protein. StressMarq BioSciences Inc. Retrieved from: http://www.stressmarq.com/products/protein/hsp70-protein-spr-103/?v=3e8d115eb4b3
Sweeney, R. W. (1996). Transmission of Paratuberculosis. *Veterinary Clinics of North America: Food Animal Practice*, *12*(2), 305–312. https://doi.org/10.1016/S0749-0720(15)30408-4
XE. (2016). XE Currency Converter: NPR to CAD. Retrieved from: http://www.xe.com/currencyconverter/convert/?Amount=205&From=NPR&To=CAD