Scrapie Blood Test as a Solution for Neurodegenerative Disease for Small Ruminant in Nepal

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Nepal is a small country in Asia that is rich in culture with its many ethnic groups such as Limbuwan, Sherpa, Newa, Awadh, Magarat Khas Arya to name only a few (Bhattarai, 2015). Nepal's topography of mountains, hills and terai which are much like plains, create a diversified landscape within the country complete with microclimates and as well as an abundance of natural resources (Paudel, Chan, Halrendt et al., 2016). The country is categorised as an underdeveloped nation 28% of land in Nepal currently dedicated to agriculture (Chapagain, 2016) with much arable land on which to expand upon. It is the objective of this study to aid the Nepalese in stabilising the nations economy and raising the GDP. This can be done by improving land and or livestock management at a local level within an achieveable budget and through Canadian companies or products. For the purposes of research small ruminants in Nepal affected by scrapie are examined in the production system in an effort to increase overall profitability.

PART ONE

Nepal

Population

In 2015 the population of Nepal was over twenty-eight and a half million and the nations GDP was around 20 billion US dollars (World Bank, 2016). The Majority of the population can be found in the terai with 32.9 percent of the population and the hill regions with 32.3, areas in which agriculture in most prominent (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004). The mountains house far fewer citizens with only 12.1 percent of the population while the remaining citizens reside in the urban and developed areas of Nepal (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004).

Due to the high levels of poverty over 80% of the population of Nepal relies on the agricultural industry for employment (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004). The mean total annual income in Rural Regions of Nepal in 1996 was 30 980 Nepalese Rupees and the average income

for non-livestock owners when compared to livestock owners was almost triple the income (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004). This is the area in which it is necessary to perform research so as to improve livestock management practices.

Agriculture

Nepal is a small country in Asia located between India and China. The country itself has an area of just over 147 thousand square kilometres (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004) and as such is much smaller compared to its neighboring countries. Of the total land area in the country currently 28% of total land area is used agriculturally (Chapagain, 2016), this land is deemed arable. Terai are the primary location of agricultural land, the plains are planted with rice paddies, maize, wheat, millet, barley, pulses, oil crops and vegetables (Shrestha, S., Pandey, V.P., Chanamai, C. et al., 2013).

Hilly regions of Nepal are also used for plant agriculture, growing maize, millet and legumes such as cowpeas and black gram (Paudel, Chan, Halrendt et al., 2016). Hills are also an area of animal agriculture. Hill regions can be grazed upon where crops do not yield well. Large animals are also not taking up potential fertile cropping field space in the terai. The mountainous area of Nepal can be used for agricultural however it is more limited in its functionality. Mountains are used mainly for nomad like farming (Chapagain, 2016). Animals as well as farmers travel to grazing land. 50 percent of agricultural land in Nepal is found in the hills, 42 in the terai and 8 in the mountains (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004). This corresponds to citizen populations with the different regions, population density is low in lesser agricultural areas (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004).

Goats and Sheep

The average lot size per farmer owned and operated by said farmer is less than two hectic acres. Over 50 percent of the farms in Nepal own small ruminant livestock primarily goats and sheep (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004).

Figure 1- Ownership of livestock per farm

Region	Livestock owners	Herd size (TLU)		Own LR	Own SR	Own Poultry	Own Pigs
	%*	Mean**	sd	%***	%***	%***	%***
Mountains	95.1	3.3	2.7	94.6	52.4	47.0	11.3
Rural hills	93.9	2.9	2.3	94.7	58.5	60.0	13.2
Rural terai	86.2	2.3	2.5	84.7	55.1	44.7	12.2
Other urban	37.1	0.6	1.1	69.8	44.0	44.7	2.5
Katmandu	13.7	0.1	0.6	54.3	23.9	50.0	2.2
Total	76.3	2.2	2.4	88.7	54.8	51.2	11.7

* Percentages refer to whole subsample
** Means refer to livestock owners only
*** Percentages refer to the regional livestock owners subsample
Note: LR=Large Ruminants, including cows, buffaloes and yaks.
SR=Small Ruminants, including sheep and goats.
Source: NLSS (World Bank, 1996), calculations by the author.

Source: Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004

This data is only slightly variant through the three main geographical regions of Nepal mountains, hills and terai are respectively 52.4, 58.5 and 55.1 percent (Maltsoglou, Irini, Taniguchi, Kiyoshi, 2004). While small ruminants contribute to the economy in 2000 managing goats and sheep only contributed about 4% of Nepal's gross domestic product (Bain et al, 2000). Goats and sheep are raised in small herds and flocks this accounts for about 29 percent of goat production and 14 percent of sheep production (McLeod, 2001). Large scale goat production accounts for the rest of the population and is concentrated in the mid hill region of Nepal (McLeod, 2001). There are about 9 and a half million goats in Nepal (Chapagain, 2016) with the population continuing to increase, this can be attributed to an increase in the value of goat meat (McLeod, 2001). Sheep production has declined significantly due to reduced value in the market from between the years of 1990 and 2000 the FAO estimated that sheep production had been reduced by 40 000 individuals. When this is compared to the total population of sheep in Nepal which currently stands at approximately 800 000 (Chapagain, 2016).

Figure 2- The comparative populations of goats and sheep in Nepal

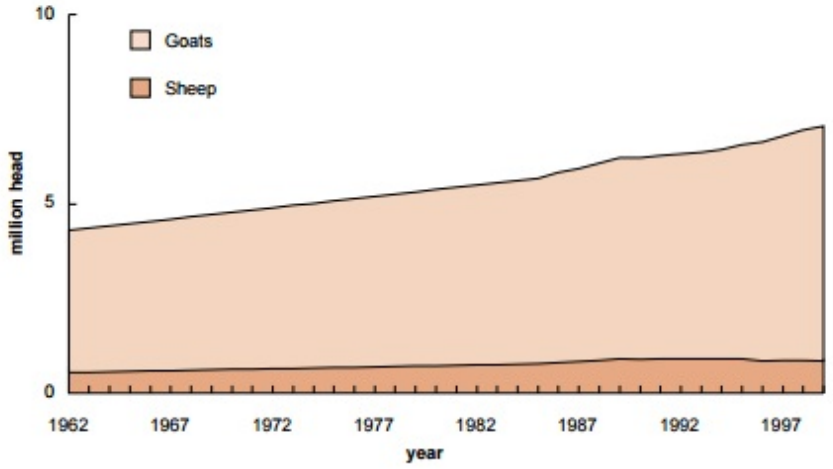

Figure 2. Small ruminant population in Nepal (1962–1999) (source: FAO 2000)

Sheep are raised in the Himalayan region of Nepal this is the mountainous area in which cropping is quite difficult (McLeod, 2001). Small ruminants are important to production in the conversion of cellulose into useable products. Large ruminants in Nepal such as yaks have a diet that consists of mainly graminoids such as leafy grasses that are found mainly in the terai and hill regions, sheep and goats even more so have a diet that is made up of forbs and browses which are less leafy and more woody plants found in the mountains (Shrestha, Wegge, 2008). Small ruminants can be raised for innumerable purposes some main reasons for production include milk, meat, wool, hair, leather, medicine and fertilizer (Son, 1999).

Issues with Small Ruminant Production in Nepal

Veterinary Services

With low funds and little income attributed to farmers there are many limitations in the management of goats and sheep. Constraints to this form of livestock production include poor animal health and breeding, a lack of genetic material as well as few treatment options for sick and diseased animals such as medication and vaccinations (McLeod, 2001). After the earthquake on April 25th 2015 veterinary efforts were increased however much of the veterinary resources in Nepal are volunteer based and do not have permanent residence in the country, the IWAF and

FAO often lend resources to countries in need (Veterinary Record, 2015). Nepalese farmers must have serious reason to call in veterinary resources as treatment is expensive, rather than veterinarian visiting the farms in Nepal farmers prefer to use the services of Village Animal Health Workers (VAHW) (Lamichhane, Shrestha, 2012). The main factor affecting the decision to use VAHW over trained and certified veterinarians was however the proximity of the service. Farmers are in need of affordable, simple and rapid animal care.

Box 1
Diseases affecting sheep and goats included on the list of notifiable diseases of the World Organisation for Animal Health, 2010
Listed alphabetically, not by importance

Diseases listed specifically for sheep and goats
Caprine arthritis/encephalitis
Contagious agalactia
Contagious caprine pleuropneumonia
Enzootic abortion of ewes (ovine chlamydiosis)
Maedi-visna
Nairobi sheep disease
Ovine epididymitis (*Brucella ovis*)
Peste des petits ruminants
Salmonellosis (*S. abortusovis*)
Scrapie
Sheep pox and goat pox

Other significant diseases of sheep and goats listed in the multiple species category
Anthrax
Bluetongue
Brucellosis (*Brucella melitensis*)
Foot and mouth disease
Heartwater
Paratuberculosis
Q fever
Rabies
Rift Valley fever
Rinderpest

Source: World Organisation for Animal Health (17)

Figure 1- Diseases contracted and spread by small ruminants

Source: Sherman, 2011

Scrapie

How the Disease Works

Scrapie is a neuro-degenerative disease meaning it affects the brain and is classed as one form of transmissible spongiform encephalitis (TSE). (Scrapie Canada, 2016). Another instance of TSE is bovine spongiform encephalopathy often referred to as mad cow (Scrapie Canada, 2016). This particular form of TSE appears in small ruminants specifically sheep and goats. The disease is transmitted through fluid and tissues of female's placenta; males can contract the disease but are unable to transmit it (Scrapie Canada, 2016). The symptoms of Scrapie may not present themselves for months or years the disease can lie dormant for long periods of time (Sherman, 2011). After the extreme appearance of mad cow in the Canadian beef industry the government dedicated funds to research scrapie so as to limit and prevent its appearance in the goat and sheep industry (Canadian Food Inspection Agency, 2014). Scrapie is caused by misfolded proteins called prion proteins (PrP) this is caused by a change in the conformation of the amino acid chain (Jackman, Everest, Schmerr, Khawaja, Keep, Docherty, 2006). The peptide changes shaped by unravelling or unfolding itself and as such is found in a different form and may no longer perform tasks and produce the amino acid chains the previous protein was coded for (Jackman, Everest, Schmerr, Khawaja, Keep, Docherty, 2006). When the abnormally folded prion protein comes in contact with correctly structured proteins the proper proteins unravel and this creates a build up of malformed prion proteins (Tsunoda, K., Namikawa, T., Sato, K., et al, 2010).

Prevalence

In a study conducted on sheep in Asia many of which appear in Nepal the frequency of alleles indicating the risk of developing scrapie where examined, the risk levels where ranked from lowest (R1) to highest (R5) (Tsunoda, K., Namikawa, T., Sato, K., et al, 2010)

	Middle Eastern Sheep	British Sheep Raised in Japan	East Asian Sheep
High Risk Frequency of Genes (%) *Above R3 (R4 and R5)	19 and 52 when examining different genes	74 Corriedale Specifically 67.5	90

Table 1- Sheep as ranked by frequency of allele in relation to risk of Scrapie

Source: Tsunoda, K., Namikawa, T., Sato, K., et al, 2010

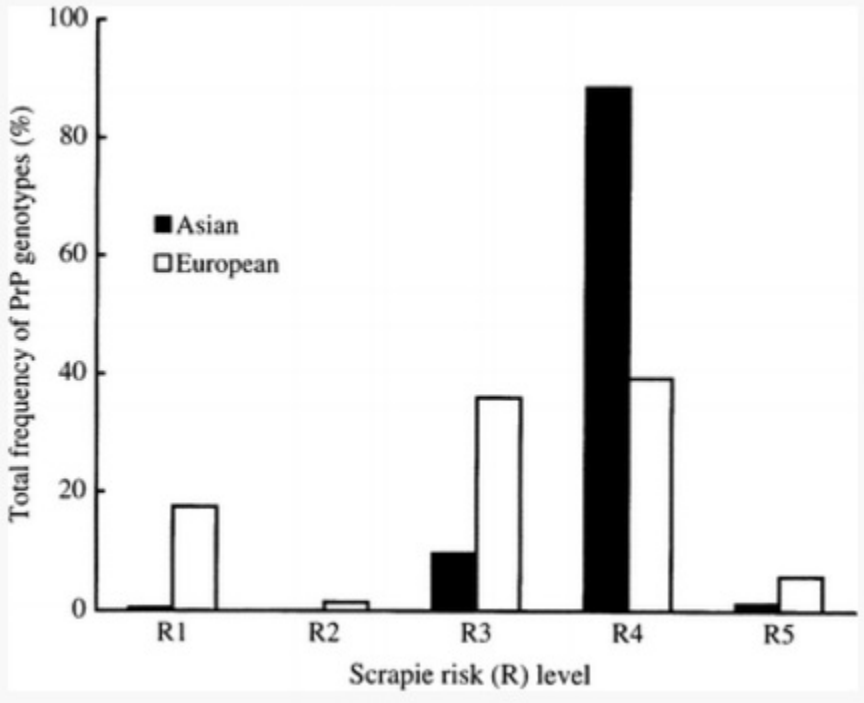

Figure 1- Visual representing scrapie risk as measured by alleles and frequency of alleles comparing the total European and East Asian sheep breeds. Source: Tsunoda, K., Namikawa, T., Sato, K., et al, 2010

It is estimated that the prevalence of scrapie in a goat herd would be between 0.042 and 0.37 making it a 4.2 or 37 percent chance the animal is infected (Ortiz-Pelaez, Kelly, Adkin, 2012)

Detriment to Agriculture

Both Canada and the United States have experienced the aftermath of instances of TSE particularly BSE. As a precautionary measure companies now offer livestock insurance in an

attempt to prevent loss (Hoag, Thilmany, Koontz, 2006). The greatest risk of loss in the livestock industry is that of disease. Scrapie is a disease that affects livestock and as such lowers the profit farmers could potentially bring in. Scrapie is fatal and contagious making the diseased animal as well as surrounding animals by products virtually useless. Livestock insurance cannot be found in Nepal, so the Nepalese must be determine a method in which there is a constant income despite scrapie.

Treatment

The protein is resistant to the normal process of cell degradation (Tsunoda, K., Namikawa, T., Sato, K., et al, 2010). Nerve cells are especially at risk as the are not in a state of constant regeneration, and as the proteins become misfolded there are no proper proteins replacing the corrupted prions (Tsunoda, K., Namikawa, T., Sato, K., et al, 2010). Because the degeneration is caused by proteins and not cells the disease can not be killed by heat. As of yet there has been no course of treatment for the disease (Canadian Food Inspection Agency, 2014).

Solution

Amorfix Lifesciences

A Canadian Company that furthered the research into the disease scrapie was Amorfix Lifesciences. Established in 2004 by researchers from the University of Toronto Amor fix was a company that aims to detect and cure Alzheimer's (ProMIS Neurosciences). In an effort to expand the company's knowledge of human neurodegenerative diseases research was expanded to the field of animal degenerative diseases. This company is very unique in the combined research of humans and animals (CNW, 2007). On June 30th 2015 Amorfix changed its name to ProMis Neurosciences (CNW, 2015). The company is now focused on antibody therapeutics and Disease Specific Epitopes (DSEs) the technology developed is an ultra-sensitive dual-bead immunoassay, Epitope Protection and AMFIA (CNW 2015). This technology allows the company to identify miniscule levels of misfolded proteins in animal body fluids (ProMIS Neurosciences, 2016). Amorfix was working on a test to identify the prions using blood samples from small ruminant livestock (Properzi, Pocchiari, 2013).

Product

Amorfix conducted in neurodegenerative disease research into small ruminants, which ceased when ProMIS took over. Research into prion detecting tests was ended. As such the proposed product is purely conceptual.

This product includes a needle and syringe, required to sample blood. The needle would require replacing with each test subject unless more expensive needles are purchased and that specifically stated reusability, equipment must be cleaned thoroughly or else farmer's risks contaminating samples, the same parameters apply for syringes (BioChemed Services, 2016). Prices for reusable syringes range from eight to seventy US dollars (Valley Vet Supply, 2016) and can be purchased from and vet or livestock supplier. Needles can be purchased in the same locations and prices begin at around twenty-five cents US for disposable needles (Valley Vet Supply, 2016). The farmers would require training in the proper method of blood sample extraction this service however could be provided as a one-time volunteer session by the FAO or IWAF that would include all small ruminant producers in the village.

A test kit is also included much like those of the ELISA tests. This test involves an assay that allows the tester to witness the method in which antibodies react to antigens within the blood (Jackman, Everest, Schmerr, Khawaja, Keep, Docherty, 2006). In this case the antigen would be the prion proteins and the way in which these proteins would be identified is through binding patterns of antigens and antibodies (Jackman, Everest, Schmerr, Khawaja, Keep, Docherty, 2006). The accessibility or inaccessibility to the epitope is assessed (Jackman, Everest, Schmerr, Khawaja, Keep, Docherty, 2006). The epitope being the binding portion of the antigen it presents the area for the antibody to bind to the protein, misfolded or unfolded proteins limit this epitope access (Jackman, Everest, Schmerr, Khawaja, Keep, Docherty, 2006). ELISA test kits indicate when there is an accumulation of binding antigens and antibodies (Mena Report, 2016). This is how scrapie occurs and can also be detected. Amorfix's research would have to be analysed and incorporated into ELISA technology in order to create this product. ELISA tests have a self life of one year when stored at four degrees Celsius in a dark environment (Guomin, 2010). ELISA kits cost 495 US dollars and are discounted at rates of 10 percent when purchased in bulk for example when ten kits are purchased the discount is 10 percent, when twenty kits are purchased at a discount of 20 percent (Ayres, 2016).

PART TWO

Demand in Nepal

This product would act as a type of insurance to the farmers. It would allow the farmers to look into the animal's health in an attempt to create the best financial outcome. Diseased livestock can be culled from the herd to as the rest to the herd or flock do not become infected. This aids in ensuring that the flock remains Animals are tested around 17 months of age and need only be tested once as the disease is detectable at this age however symptoms may nor become apparent until three to five years of age. (CNW 2007). This product would be aimed at all goat and sheep producers in Nepal.

Export

This product would be developed and exported out of Canada as the Amorfix laboratory was located in Mississauga. Air Canada offers an AC Pharmacair service that insures that pharmaceuticals remain at constant cool temperature and are handled with care. This service is available at the Toronto location and service costs to ship cargo to India are 718.00 Canadian dollars while cost to China is only 110.00 Canadian dollars, correction fee may be applied (Air Canada, 2016). The product would then have to been transported by vehicle across China and into Nepal.

Documentation

Any pharmaceutical exports from Canada must abide by the following documents and policies, (Health Canada, 2016)

- Guidance Document on the Application for a Certificate of a Pharmaceutical Product
- Import and Export Policy for Health Products under the Food and Drugs Act and its Regulations
- Guidance Document on the Import Requirements for Health Products under the Food and Drugs Act and its Regulations
- Guidance on Evidence to Demonstrate Drug GMP Compliance of Foreign Sites

- Policy for the Importation or Sale of Active Pharmaceutical Ingredients for Veterinary Use

The exporting company must also complete the "Intention to Invoke Section 37 of the Canada Food and Drugs Act for Products being Exported" form (Health Canada, 2016).

The actual cost of shipment would depend on the quantity of product and the area of Nepal that has imported the product. The Duty on the samples are the Canadian dollar value of the shipment x 4% then that value plus the Canadian dollar amount x 5% those two values are the duty on the samples (Canadian Border Agency, 2015). In 2011 agricultural exports made up 3 percent of Canada's GDP (Global Affairs Canada, 2013).

Import

The product after having been imported would be received in China. From this location, the product would travel to its final destination in rural Nepal. The Nepalese collect a customs duty as it contributes to the total tax revenue (Government of Nepal, 2016). The importers are required to be registered with Health Canada (Health Canada, 2010). The purchasers of the blood test in Nepal nay come together and make bulk orders so to lower the cost.

Documentation

Upon importation, the product is required to meet the nations guidelines, imports to China must present the following documentation, (CIE Sourcing Service)

- Customs Declaration Form
- Inspection Certificate
- Veterinary (Health) Certificate
- Health Certificate
- Sanitary Certificate
- Inspection Certificate of Quantity and Weight
- Quality Certificate
- Certificate of Origin

The product must also include the "Export Permit, Quote or License" for any item to be accepting into China (CIE Sourcing Service)

Trade Barriers

Canada and China have a bilateral trade relationship (Global Affairs Canada, 2013), trade with Nepal is also bilateral. Two-way trade with Nepal varies between thirteen million and 23 million dollars annually (Government of Canada, 2013). By implementing a Canadian product in the Nepalese agricultural industry, the import export trade would be further strengthened. Nepal is a landlocked country and this makes it difficult for Canada to participate in direct trade with Nepal. Both major airlines and boats must land in either Inia or China before the product reaches the destination country.

Benefits for Exporting Nation: Canada

In 1952 Canada indirectly pledged its assistance to development in Nepal (Government of Canada, 2013), by providing the country with agricultural technology Canada is helping to further Nepal's development. Scrapie detection would improve agriculture and in turn contribute to the economy and the progression of the country.

Company

Expansion of Amorfix's, technology internationally would change the companies dynamic. Each country in itself has a wealth of natural resources, when nations participate in bilateral trade each country offers the other something that plentiful be it wealth or tangible products (Phillips, Isaac, 2001). This a mutually beneficial market for both the company as it gains in research and revenue as well as the country as the research Amorfix was performing was intended to benefit the general public.

Education/Progression/Research

Expansion of the Canadian company to international borders would grow the pool of data and may lead to a break through involving a cure for scrapie that has not as of yet been discovered (CFIA, 2014). This will allow the company to grow in research and profitability. If there is success in expanding the field Canadian farmers will be able to prevent and cure the outbreak of scrapie in their livestock as well.

The Nepalese would have to create a registration that could function alongside the Canadian system as and livestock that exhibit symptoms of scrapie must be entered in the data base. Similar to the United States Department of Agriculture Scrapie Flock Certification Program (USDA SFCP) (CFIA, 2016). This would aid in keeping organised the data that is collected from samples.

Government

The trade the Canadian product rapeseed otherwise known as canola aided in the evolution of international trading rules (Phillips, Isaac, 2001). As this technology, would appear as the method of scrapie detection this product use internationally could potentially revolutionise the standards for veterinary blood testing kits and the small ruminant industry through policies and international agreements. In the case of canola, the World Trade Organisation became involved and lowered the tariffs of developing nations by a total of 36 percent (Phillips, Isaac, 2001). This could also be the case with scrapie blood tests and the veterinary pharmaceutical industry.

Benefits to the Importing Nation: Nepal

Economy

The importation of a Canadian is beneficial to the Nepalese economy in serval fashions, the first being that of increased GDP. With globalization, the GPD of developing countries specifically Asian countries rises by an average of 6.1 percent annually (Nayyar, 2015), this was found in a study conducted over more than twenty-five years. An increase in employment in underdeveloped countries is also witnessed as a trend in succession of globalization (Nayyar, 2015).

Tourism

Scrapie is a disease that can be contracted by humans through contact of bodily fluids or consumption of animal by products, scrapie is a potential risk to ecotourism in Nepal. If Children come in contact with human contractible pathogens they are highly susceptible to transmission (Muehlenbein, M., 2016). Scrapie detection would prevent tourists from coming in contact with infected animals so as the disease is to spread. Visiting tropical agricultural institutions already presents challenges at customs detection would validate the presence of lack there of the disease

to make the actual entrance and exit of the country much simpler. The elimination of disease ridden livestock also creates a much more desirable travel destination.

Agriculture

The use of scrapie detection methods would improve the entire small ruminant agricultural sector in Nepal. The removal of scrapie and creation of a genetically pure and resistant flock of sheep or herd of goats could eventually be achieved, although the test does not ensure complete freedom of infection (New Scientist, 2005). Having a nearly disease free herd will create less loss in production and therefor greater profit margins.

Expansion

As China expands in population the amount of available arable land has been significantly reduced. Between the year 1990 and 2000 the urban development in the Beijing, Tianjin and Hebei region of China expanded by at least 71% (Tan, Li, Xie, Lu, 2004). In order for this expansion to occur agricultural land had to be consumed by urban sprawl. Of the urban development that took place in China 74% of the land expanded onto was previously arable (Tan, Li, Xie, Lu, 2004). With the exponentially growing population there is an immediate demand for resources. Nepal would have the opportunity to present itself as a local resource for small ruminant by products. This opportunity would allow small farms within Nepal to grow to meet China's demands.

Feasibility

Cost Analysis

The exchange rate of the Canadian dollar to U.S. dollars is 0.7443 as of November, 29th 2016 (Bank of Canada, 2016). The unit conversion of 1.00 US dollar to Nepalese Rupees is 1.096 as of November, 29th 2016 (Nepal Rasta Bank, 2016). The average income per person in Nepal varies through the topographical regions in rural Nepal average income per capita is 7 305 rupees (Paul, Thapa, Prennushi, 2012).

Table 1- A rough estimate of the projected cost per animal to test for the presence of scrapie. Not included is the cost of development or trucking costs as the price is dependent on cost and quantity of the item(s).

	Cost per Unit USD ($)	**Cost per Unit Cad dollars ($)**	**Cost per unit NR**
Test kit	495.00	665.03	542.52
Needle	8.00-70.00	10.75 - 94.04	8.77 – 76.72
Syringe	0.25	0.34	0.27
Shipping	81.88 – 534.43	110.00 or 718.00	89.74 – 585.74
Taxes and Tariffs	27.18 -Value of shipment x 4% -New value plus value of shipment -This value x 5% is the Canadian Duty (calculations done in Canadian dollars)	665.03+10.75+0.34 =676.12 x 0.04 =27.04+676.12 =730.02x0.05 =36.51	29.79
Estimated Minimum Total	530.43	712.63	581.35

As the average income, per capita is 7 305 rupees (Paul, Thapa, Prennushi, 2012), in Nepal to test the presence of scrapie in only one animal it would cost a over a twelfth of the farmer's average income. This product is to expensive to appease the needs of local farmers in rural Nepal. As this product is purely conceptual the numbers may not have complete accuracy however even with a large margin of error the product is not one of potential in Nepal. This product unique in the field of this disease and as such it has no current competition.

Canadian Loans and Grants

The government of Canada offers loans to Canadian citizens to aid in the area of agriculture these are available only nationally and can not exceed 350 000 dollars (Agriculture and Agri-

Food Canada, 2016). This grant program if extended to Nepal may aid in the start up of this program, however if a loan is granted it must be paid back and as such the product must be able to sustain and profit for itself.

References

Adams, Dr. George. CNW (2016) *Amorfix detects scrapie in prions in blood from asymptomatic sheep.* Retrieved from Online Journal

Agriculture and Agri-Food Canada. (8 April 2016). *Canadian Agricultural Loans Act program*. Retrieved From: http://www.agr.gc.ca/eng/?id=1288035482429

Air Canada (November 2016). *House Air Waybill Fees.* Retrieved From: Air Canada Cargo

Ayres, C. (29 November 2016). *Custom Peptide, Protein, Antibody, ELISA Provider*. Retrieved From: Personal Communications.

Bain, R., Joshi, B. and Gauchan, D. (2000). Nepal assessment report — development and testing of an integrated approach to the control of gastro-intestinal parasites of small ruminants in South and Southeast Asia (TAG 433). Report and findings of a consultancy to examine the importance of helminth infections as a constraint to the sustainable livelihoods of small ruminant producers in Nepal, International Livestock Research Institute, April.

Bank of Canada. (29 November 2016). *Daily Currency Converter.* Retrieved From: http://www.bankofcanada.ca/rates/exchange/daily-converter/

BioChemed Services. (2016). *Animal Blood Testing.* Retrieved from Company Website: http://www.biochemed.com/animal-products/animal-whole-blood/sheep-whole-blood/

Canada Border Services Agency. (2015, November 10). *Step-by-Step Guide to Importing Commercial Goods into Canada.* Retrieved from Government Website: http://www.cbsa asfc.gc.ca/import/guide-eng.html

Canadian Food Inspection Agency. (2014, November 9). *Requirements for Small Ruminant Imported from the United States for Breeding, Domestic, or Captive Purposes*. Retrieved from Canadian Food Inspection Agency Legislation Online pdf: http://www.scrapiecanada.ca

Canadian Food Inspection Agency. (2016, November 9). *Requirements for Small Ruminant Imported from the United States for Breeding, Domestic, or Captive Purposes*. Retrieved

from Canadian Food Inspection Agency Legislation Online pdf: http://www.scrapiecanada.ca

Canadian Sheep Federation, Canadian Sheep Breeders' Association, Canadian National Goat Federation, Agriculture and Agri-Food Canada, Canadian Food Inspection Agency. (2016, February 1) *Scrapie Canada*. Retrieved from: http://www.scrapiecanada.ca

Chapagain, T. (2016). *Agriculture and Agri-Food Systems in Nepal*. Retrieved From: Power Point, Personal Communications

CIE Sourcing Service. (2016). *China Import and Export Documents Forms and Samples Complete List*. Retrieved From: http://www.chinaimportexport.org/china-import-and export-documents-forms-and-samples-complete-list/

CNW. (30 June 2015). *Amorfix Life Sciences, Ltd. Announces Results of Annual and Special Meeting of Shareholders with Overwhelming Support for all Resolutions, Including Change of Name to ProMIS Neurosciences, Inc.* Retrieved From: CNW Group

FAO (Food and Agriculture Organization of the United Nations) (2000). *Livestock statistics*. Retrieved From: Rome, FAO. http://apps.fao.org/cgi-bin/nph-db

Global Affairs Canada. (5 September 2013). *Canada-China Economic Complementarities Study*. Retrieved From: http://www.international.gc.ca/trade-agreements-accords commerciaux/agr-acc/china-chine/study-comp-etude.aspx?lang=eng

Government of Canada. (August 2013). Canada-Nepal Relations. Retrieved From: http://www.canadainternational.gc.ca/india-inde/bilateral_relations_bilaterales/ canada_nepal.aspx?lang=eng

Guomin, S. (December 2010). Immunoassays in Agricultural Biotechnology. Retrieved From: http://site.ebrary.com.subzero.lib.uoguelph.ca/lib/oculguelph/detail.action?docID=1043 400

Health Canada. (5 April 2016). Drugs and Health Products: Export & Import. Retrieved From: www.hc-sc.gc.ca

Hoag, D.L., Thilmany, D.D., Koontz, S.R. (2006). *Economics of Livestock Disease Insurance Principles, Issues and Worldwide Cases.* Retrieved From: http://www.cabi.org/cabebooks/FullTextPDF/2008/20083078030.pdf

Jackman, R., Everest, D.J.1., Schmerr, M.J., Khawaja, M., Keep, P., Docherty, J., (May 2006). *Evaluation of a Preclinical Blood Test for Scrapie in Sheep Using Immunocapillary. Electrophoresis.* (Vol. 89, pp. 720-727) Retrieved From: Journal of AOAC International

Kiania, A., Alstrup, L., Nielsenb, M.O. (October 2015). *Differential metabolic and endocrine adaptations in llamas, sheep, and goats fed high- and low-protein grass-based diets.* (Vol. 53, pp. 9-16). Retrieved From: Domestic Animal Endocrinology

Lamichhane, D.K., Shrestha, S. (August 2012). *Determinants of farmers' choice for veterinary service providers in Nepal Mountains*. (Vol. 44 (44) pp. 1163-1168). Retrieved From: Tropical Animal Health and Production

Maltsoglou, I., Taniguchi, K. (2004). *Poverty, Livestock and Household Typologies in Nepal.* Retrieved From: Pro-Poor Livestock Policy Initiative

McLeod, R. (May 2001). *Control of Footrot in Small Ruminants in Nepal.* Retrieved From http://ageconsearch.umn.edu/bitstream/47694/2/IAS16.pdf

Mena Report. (18 March 2016). *Rate contract for purchase hcv elisa test , hiv elisa kit, glass slide blood transfusion deptt*. Retrieved From: Academic OneFile

Muehlenbein, M. (October 2016). *Disease and Human/Animal Interactions*. (Vol. 45 pp. 395 416). Retrieved From: Annual Review of Anthropology

Nayyar, D. (March 2015). *Globalization and Employment*. (Vol. 58 (1) pp. 87-97). Retrieved From: Indian Journal of Labour Economics

Nepal, Ageing, ARDS Nepal, NASCIF. (2013, September). *Nepal Report on World Alzheimer's Month.* Retrieved from: http://ageingnepal.org/

Nepal Rasta Bank. (29 November 2016). *Foreign Exchange Rates.* Retrieved From: https://nrb.org.np/fxmexchangerate.php

New Scientist. (2005). Scrapie Mark II. Retrieved Fromhttp://search.proquest.com.subzero.lib.uoguelph.ca/docview/200385204/abstract/B4807674976F4535PQ/1?accountid=11233

Ortiz-Pelaez, A., Kelly, L., Adkin, A. (1 December 2012). *The Risk of Introducing Scrapie From Restocking Goats in Great Britain*. (Vol. 107 (3-4) pp. 222-230). Retrieved From: Preventive Veterinary Medecine

Paul, S., Thapa, P., Prennushi, G. (2012). *Spatial Dimensions of Income Inequality in Nepal*. (Vol. 46). Retrieved From: Journal of Developing Areas

Phillips, P.W.B., Isaac, G.E. (2001). *Regulating international trade in knowledge-based products.* (Chpt. 13 pp. 243-269). Retrieved From: The biotechnology revolution in global agriculture: innovation, invention and investment in the canola industry.

ProMIS Neurosciences (2016). Company and Mission. Retrieved From: http://promisneurosciences.com/

Properzi, F., Pocchiari, M. (2013). *Identification of misfolded proteins in body fluids for the diagnosis of prion diseases.* (pp. 480). Retrieved From: International Journal of Cell Biology

Sherman, D.M. (2011). The spread of pathogens through trade in small ruminants and their products. Retrieved From: Revue Scientifique et Technique-OIE

Shipley, L. *Grazers and Browsers: How Digestive Morphology Affects Diet Selection.* Retrieved From: http://nature.berkeley.edu/classes/espm186/Unit_II_(cont)_files/grazer%20v.%20rowser.pdf

Shrestha, R., Wegge, P. (17 July 2008). *Wild Sheep and Livestock in Nepal Trans-Himalaya: Coexistence or Competition.* (Vol. 35 (2) pp. 125-136). Retrieved From: Foundation for Environmental Conservation

Shrestha, S., Pandey V.P., Chanamai, C., Ghosh D. K. (December 2013). *Green, Blue and Grey Water Footprints of Primary Crops Production in Nepal.* (Vol. 27 (15) pp. 5223-5243). Retrieved From: Water Resources Management

Son, Y.S. (November 1999). *Production Uses of Korean Native Black Goat.* (Vol. 34 (3) pp. 303-308) Retrieved From: Small Ruminant Research http://www.sciencedirect.com/science/article/pii/S0921448899000814

Tan, M., Li, X., Xie, H., Lu, C. (July 2005). *Urban land expansion and arable land loss in China—a case study of Beijing–Tianjin–Hebei region.* (Vol. 22 (3) pp. 187-196). Retrieved From: Land Use Policy

Tsunoda, K., Namikawa, T., Sato, K., et al. (2010). *Prion Protein Polymorphisms and Estimation of Risk of Scrapie in East Asian Sheep.* (Vol. 48 (1) pp. 13-25). Retrieved From: Biochemical Genetics

Valley Vet Supply. (2016). Syringes and Needles. Retrieved From: https://www.valleyvet.com /c/livestock-supplies/goat-sheep/health-care/syringes-needles.html

Veterinary Record (9 May 2015). Veterinary Relief Efforts Begin After Nepal Earthquake. Retrieved From: http://veterinaryrecord.bmj.com.subzero.lib.uoguelph.ca/content /176/19/479.full.pdf+html

World Bank (2016). *Nepal*. Retrieved From: http://data.worldbank.org/country/nepal?view=chart