Original Article

Recommendations on successful quarantine of pure exotic sheep breed at Bangladesh Livestock Research Institute in Bangladesh

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ABSTRACT

Objective: The objective of this study was to examine the newly arrived pure breed sheep considering antibody against some diseases and immunization of the animal against endemic diseases of hosting area according to species.

Materials and methods: A total of 42 pure exotic breed of sheep, originated from Australia namely Parendale (n=14), Suffolk (n=13) and Dorper (n=15), imported by the Bangladesh Livestock Research Institute (BLRI) were used in this study. Before shifting the animals to sheep farm, 1-month quarantine was done in an isolated shed by following step by step technique; first week was used for close observation, second week for immunization of animal against endemic diseases, third week for monitoring and observation against immunization, and the fourth week was used for determination of animal health status and immunity level. On day 1 and 2, the sheep remained at full rest along with supplied habituated feed, saline and drinking water. On day 3, fecal sample was collected from all animals to measure parasitic load. On day 4, blood and oculo-nasal discharge samples were collected to detect blood protozoa, peste des petits ruminants (PPR), foot and mouth disease (FMD) and other infectious zoonotic diseases like anthrax, brucellosis and tuberculosis. In the second week, immunization was done against PPR, FMD and hemorrhagic septisemia (HS) at two days interval. Farm biosecurity and biosafety for the workers and officers was maintained according to standard guideline. In the third week, the animals continued to be under close observation. On day 29, the antibody level produced due to vaccination was measured.

Results: Parasitic load was found to be very low, and blood protozoa, PPR, FMD, anthrax, brucellosis and tuberculosis were negative. The antibody titer was detected at the acceptable level in sheep on day 29. The quarantine committee decided that the sheep were ready to transfer to the farm along with some recommendations. After completion of 1-month quarantine period, all sheep could not transmit any diseases in Bangladesh.

Conclusion: Quarantine is mandatory for entering any new animal in a new area that gives the outline of different new diseases. It also acts as radar of exotic disease in a country. So, this quarantine technique serves the purpose for livestock owners effectively.

KEYWORDS

Australian sheep; Biosecurity; Pure breed; Quarantine

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INTRODUCTION

Quarantine is derived from the Italian word *Quaranta giorni*, which means forty days. This is due to 40 days isolation of people before entering the city of Dubrovnik in Croatia between 1314 and 1359. Animal quarantine is the separation and restriction of animal movement to avoid transmissible diseases (Mackowiak and Sehdev, 2002).

Each year, about 4 to 5 million cattle entered Bangladesh through border crossing, particularly before the Eid-Ul-Azha. However, buffaloes, sheep and goats are not usually imported to the country except for special purposes like high production performance and research. Bangladesh Animal and Animal Product Quarantine Act-2005 directs the control of animal diseases to improve public health and dictates what needs to be done to avoid the import of infectious disease via imported animals or animal products (<u>AAPQ, 2005</u>).

There are about 23.93 million cattle, 1.47 million buffalo, 25.93 million goat, 3.40 million sheep and 320.63 million poultry population in Bangladesh (Livestock Economy, 2017). Nill or Zero risk is the concept of quarantine. Quarantine is a separate facility of mammals, birds, reptiles, amphibians and fish to prevent disease transmission. This separation is obligatory for all animals for a specific period of time. Quarantine of animal must be done under the supervision of a veterinarian consisting of minimum 30 days (AZA, 2007).

During quarantine time, certain prophylactic measures should be taken like collection of individual or representative fecal sample for at least twice, usually two weeks apart from the examination of gastrointestinal parasites, examination of ectoparasites, and treatment should be done according to the recommedations from the attending veterinarian. Blood sera should be collected to examine blood parasite and antibody against infectious or retrospective diseases. In positive cases, treatment should be done by a veterinarian. In this period, all animals should be restrained for tagging. If the animal arrives without vaccination, immunization should be done by giving appropriate series of vaccination (AZA, 2007; http://www.maff.go.jp/aqs/english/pdf/aqs info eng.pdf).

Medical records should be maintained in all animals including environmental temperature, humidity, moisture, strict farm biosecurity, feeding, breeding and housing. If the animal dies during quarantine period, necropsy should be done under a supervision of a veterinarian and tissue sample should be collected for histopathological examination. Proper disposal of the dead animal should be done. At the end of quarantine, health certification should be done for the animals by an authorized veterinarian for further activity (<u>AZA, 2007; http://dahd.nic.in/Trade/Animal-quarantine-and-certification-services</u>).

Recently, Bangladesh imported a large number of animals to fulfill various purposes like research, high production performance by crossing with native animals as well as pure exotic breed adaptation. However, in most cases, proper scientific way of quarantine is not followed. As a result, most of the animals are being infected with various exotic diseases, which are similarly harmful for the country's domestic animals and public health. The objectives of this study was to examine the imported pure exotic breed of sheep considering antibody level against some infectous diseases and immunization of the animal against endemic diseases.

MATERIALS AND METHODS

In the quarantine period (March 2016), standard operating procedures based on risk assessment and risk management under the supervision of an authorized veterinarian are made. Risk assessment can be considered on identification and estimation of statistical probabilities and evaluation of all risks associated with imported animals and animal products. Risk management is the decision making process for identification and implementation of all measures before, during and after the animal importation to reduce the risk to an acceptable and manageable level. BLRI imported a number of 42 individuals of 100% pure exotic sheep breed from Australia through "Conservation and Improvement of Native Sheep through Community and Commercial Farming (Component-A, Research, 2nd Phase) Project"; of which 13 were Suffolk (3 Ram, 10 Ewe), 14 were Perendale (4 Ram, 10 Ewe), 15 were Dorper (5 Ram, 10 Ewe), and their age ranged from 12 to 20 months.

The sheep were health certified like vaccination and others by the supplying authority. Quarantine of the sheep at the BLRI was done by following step by step protocol as described by Animal Quarantine Guide in Japan and AQCS, India (AZA, 2007). Recommended time for the animal quarantine was 30 days. Quarantine Committee of BLRI decided the quarantine time. A newly constructed shed located 2 Km away from the main animal shed was used as the quarantine shed (**Figure 1**). The place was not previously used as pasture land, where human and animal movement was restricted. Fresh air, light and water were available at the shed. Floor disinfectant and foot bath were used to reduce infection.

The 1-month quarantine period was subdivided into 4 parts based on week. First week was considered for supervision and close monitoring. Second week was used for vaccination against endemic diseases. Third week was considered for observation against vaccination, and the fourth week was used for checking the antibody titer, hematology and finally decision making on the sheep. In During quarantine period, visitor was strictly restricted except livestock expert, quarantine team and enrolled technical workers. Strict farm biosecurity was practiced in the shed. To minimize infection or contamination, animal pasturing was also avoided.



Figure 1. Australian sheep in Quarantine shed (a, b) at the BLRI, Savar, Dhaka.

Step 1. Observation of sheep: Observation of the sheep was done through distance inspection like posture, gait, movement of animal and response to surroundings. Close observation was done through recording the temperature, respiratory rate, heart rate and presence of any clinical sign like fever, coughing and discharge. Fleece to fleece inspection was done to detect tick, mite, lice and other ectoparasites. Hooves were examined for any deformities. Register was maintained to record all activities. Environmental temperature and humidity were also recorded. The sheep were supplied habituated feed and supplementation.

Step 2. Immunization of animal against endemic diseases: Immunization of the sheep was done against endemic diseases of sheep in Bangladesh like Peste des Petits Ruminants (PPR), Foot and Mouth Disease (FMD), Hemorrhagic Septicemia (HS) and Anthrax.

Step 3. Monitoring and observation of animal: Monitoring of the animal after immunization was done by observing the temperature, oculo nasal discharge, urination and defecation.

Step 4. Determination of animal health status and immunity level: Blood sample from the sheep was collected and antibody level against the vaccine was measured. Close inspection of animal was done after the immunity level determination; the animals were certified for further study with some suggestions.

Farm biosecurity: Quarantine committee of BLRI strictly maintained farm biosafety and biosecurity to protect the exotic animals from the contamination. Necessary protective measures (apron, gumboot, face mask, gloves and disinfectant) were taken to handle the animal for the safety of both animal and personnel. Quarantine shed and farm equipments were properly cleaned with disinfectant daily. Surroundings of the shed was kept clean to protect the animal from insects. Visitors were restricted in the quarantine area and footbath with disinfectant was used. Tuberculin test was done to ensure the absence of tuberculosis in the animal as well as farm personnel.

RESULTS AND DISCUSSION

Observation and monitoring during first week (day 0 to 7): After the arrival of the animal in quarantine shed, the sheep were cared to overcome stress due to long time transportation, off-feeding and environmental variation. To reduce this stress, glucose and saline solution were supplied with drinking water. Clinical signs like coughing, slight fever, indigestion that were observed during this stae was managed by giving symptomatic treatment. On the second day, locally available oats hay, green grass (oats, maize, Australian sweet jumbo) and clean drinking water were supplied. This ration was followed for keeping similarity with their previous habituated feeding habit. On

the third day, parasitic load was examined through fecal sample examination by simple floatation technique (<u>Jatau et al., 2011</u>) and Mc master technique (<u>Sweeny et al., 2011</u>). The parasitic count was found to be very low at day 4, 5, 6 and 7. Monitoring was done to find out any clinical sign of diseases. Blood sample was collected from the jugular vein of sheep to observe the presence of blood protozoa like *Babesia spp., Anaplasma spp.* and *Theileria spp.* though Giemsa's staining method (<u>Shayan and Rahbar, 2005; WHO, 2016</u>) revealing no blood protozoa under microscope. Body temperature (morning and evening) was recorded because temperature is the mirror of disease. During this stage, the animals were fed little amount of concentrate pellet (at 100 gm/day).

Table 1. Vaccination schedule of foreign sheep breed atBLRI during quarantine

SL	Vaccine	Day of vaccine	Dose	Route
		administration	(mL)	
1	PPR vaccine	8 th day	1	s/c
2	FMD vaccine	10 th day	1	s/c
3	Anthrax vaccine	12 th day	1	s/c
4	HS vaccine	14 th day	2	s/c
-				

s/c = subcutaneous

Table 2. Ingredients and ratio of Concentrate mixture supplied to imported sheep breed

SL	Ingredients	Amount (%)
1	Maize crash	30
2	Wheat bran	30
3	Soya bean meal	20
4	Kheshari bran	16
5	Protein concentrate	1.5
6	Di Calcium Phosphate (DCP)	1
7	Salt	1
8	Vitamin mineral premix	0.5
	Total	100

Second week for immunization (day 8 to 14): Vaccination was done against most important infectious and contagious diseases of sheep prevalent in Bangladesh like PPR, FMD, Anthrax and HS. On day 8, vaccination against PPR was done using live attenuated vaccine (Livestock Research Institute; LRI, Mohakhali, Dhaka, Bangladesh) at 1 mL through subcutaneous (s/c) route. On day 10, vaccination against FMD was done using pentavalent oil adjuvant inactivated vaccine (Aftovax, MERIAL) at 1 mL, s/c. On day 12, vaccination against Anthrax was done using Anthrax spore vaccine (LRI, Mohakhali, Dhaka, Bangladesh) at 1 mL, s/c. On day 14, vaccination against HS was done using oil adjuvant inactivated vaccine (LRI, Mohakhali, Dhaka, Bangladesh) at 2 mL, s/c (**Table 1**). On day 8, blood was collected for the detection of blood protozoa using Brucella antibody test kit (Genomix Molecular Diagnostics Pvt. Ltd) as per the manufacturer's instructions, and the test result was negative. After vaccination, the sheep were kept in close observation for any reaction against vaccine.

Third week for observation and monitoring (day 15 to 21): In this week, the sheep were in close observation and monitoring to check reactions against vaccination and environmental effect as well as temperature and humidity. Sometimes they were allowed to drink molasses and table salt to minimize the environmental stress. In quarantine period, gun spray was done in morning and evening at 0.1% malathione to control tick mite flies, mosquito and other insects. Ceiling fan was used to minimize temperature and humidity. The sheep were given bath daily with clean water. Dry straw was used as bedding materials which was changed twice daily and the floor was properly washed with disinfectant.

Fourth week measurement of health status (22 to 30 day): On day 23, fecal sample was collected and examined in Parasitology Laboratory, BLRI through simple flotation method (Jatau et al., 2011) and Mc master technique (Sweenv et al., 2011) to identify parasite. The parasitic load was slightly high as compared to first week, which might be due to feeding of green grass. The parasites found were Paramphistomum spp., Strongylus spp., Monizia spp. Broad-spectrum Toxocara spp. and anthelmintics (Levamisole and Triclabendazole) were administered orally at 70 mg/Kg body weight. On day 29, 3 mL blood sample was collected from jugular vein aseptically, serum was separated for PPR (ID Screen PPR Composition competitive screening ELISA, ID.vet Innovative Diagnostics) and FMD (FMDV antibody liquid phase blocking ELISA kit, The Pirbright Institute) antibody titer in RLDL (SAARC Regional Leading Diagnostic Laboratory for PPR) and FMD Laboratory in BLRI respectively. The Optical Density (OD) value of PPRV antibody was positive (OD, 8-10%) and FMDV antibody was positive (OD value 88%) that was high antibody level to protect against PPRV and FMDV. Blood protozoa was also examined through Giemsa's Stain Method (Shayan and Rahbar, 2005; WHO, 2016) to identify the presence of Anaplasma spp., Babesia spp. and Theileria spp.; however, the result was negative. On day 30, booster dose of anthelmintics (Levamisole and Triclabendazole) was given. In 1-month quarantine, strict farm biosecurity was maintained.

Feeding: Three different sheep breeds (Perendale, Suffolk and Dorper) were grouped into six different pans

according to their breed and sex for proper management. Primarily they were fed a very little amount of compound concentrate pellet (at 200 gm/day) with ad libitum oats hay, green grass (oats, maize and Australian sweet jumbo) and clean drinking water. This ration was followed for keeping similarity with their previous feeding habit. Gradually their feeding habit was changed into hand mix concentrate feed (Table 2) and green grass from pellet and hay. Concentrate mixture was periodically increased at 50 gm/week with their requirements up to 350 gm/day. Sometimes they were allowed to drink molasses and table salt to minimize the environmental stress. Proper steps for maintaining feed biosecurity was followed for keeping them healthy. Finally, the sheep were habituated with native feeding system. Green grass (at 10 Kg/100 Kg bwt) and ad libitum drinking water were supplied daily. The green grass was washed with water and air dried in light for half an hour to minimize parasitic load.

Breeding: In early quarantine period, no animal was allowed to breed. After a certain period (last 10 days of quarantine) when brucellosis test was done and the sheep showed heat, they were bred with same breed ram. Their breeding behavior showed that they were naturally synchronized. After interpreting the post vaccination antibody titer and health status, the quarantine committee decided to shift the pure sheep in adaptation shed for their adaptation in the environment of Bangladesh along with some suggestions. After the 1-month quarantine, all imported exotic pure breed sheep did not transmit any zoonotic diseases in Bangladesh and they were suitable for farming or research study in the country. The technique of quarantine which was maintained for exotic breed at BLRI, was similar to recommended quarantine procedures (AZA, 2007).

The protocol of quarantine of BLRI also strongly supported Animal Quarantine and Certification Services, Government of India, Animal Quarantine Guide, Japan and Animal Quarantine Regulations and Quarantines (General), Part VI, OIE.

SUGGESTIONS AND RECOMMENDATIONS

a. Intense health care should be ensured at a 6 months adaptation period. Administration of FMD (A, O, Asia-1) vaccine, HS vaccine and enterotoxemia vaccine on 6 months interval, vaccination against PPR on 6 weeks after the first dose. Administration of anthelmintics thrice yearly. Ensure balance ration as per demand and supply ad libitam drinking water. Use ivermectin to minimize external parasite.

- b. Exotic breed animals should rear in a separate locations where grazing land is prepared by pasture burning and repeated ploughing.
- c. Housing should be prepared with proper ventilation and the temperature should not exceed 30-32°C and humidity should be maintained below 72%.
- d. Strict biosecurity in the shed should be maintained.

CONCLUSION

Quarantine is mandatory for introducing any new animal in a farm. It acts as radar of exotic disease in a country. As a result, farm or food producing animals can be saved from unknown diseases. Quarantine also gives the outline of zoonotic disease. Successful quarantine of 100% exotic pure sheep breed at BLRI in Bangladesh can meet the requirement of farmers, livestock entrepreneurs, researchers and also breeders to control exotic diseases, research and adaptation of exotic animals for their purpose effectively.

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CONFLICT OF INTEREST

The authors declare that there is no conflicting interest with regards to the publication of this manuscript.

AUTHORS' CONTRIBUTION

In the one month quarantine period, all Quarantine activity was leaded by MG, an Authorized veterinarian. MMR and MZH executed the health portion of exotic sheep. MY executed the nutrition and breeding portion and SA executed the nutrition portion of exotic sheep breed. ME was the Project Director also a veterinarian, and he imported the exotic sheep breed in BLRI through sheep development project.

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