CHAPTER 3

CASE STUDIES IN THE LIVESTOCK SECTOR

A Deccani flock with FecB carrier ewes being led for grazing ©NARI /Chanda Nimbkar

CHAPTER 3.1

SUSTAINABLE IMPROVEMENT IN SHEEP PRODUCTIVITY IN INDIA USING THE FECB (BOOROOLA) MUTATION

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BACKGROUND AND CONTEXT

Sheep-rearing is a traditional source of livelihood for communities in the drought-prone rural areas of India. Together, these livestock keepers tend about 70 million sheep (GOI, 2010). Most of the sheep in India are reared in the states of Andhra Pradesh, Rajasthan, Karnataka, Tamil Nadu and Maharashtra (listed in the order of sheep population size from the largest to the smallest). The major income from rearing Deccani sheep on the Deccan plateau in Maharashtra state is earned from the sale of lambs at 3 to 4 months of age. Lambs are usually sold in groups and butchers purchase them on visual inspection. As a result, a sheep owner's income depends largely on the number of saleable lambs produced per ewe per year. The reproductive rate is therefore important in such a system where lamb production is the primary product of the ewe and usually the main reason for her existence. Most of the costs of maintaining ewes have to be borne by the lamb(s) produced (Wiener, 1988). Only about 2 percent of Deccani ewes have twin lambs (Waghmode, 2007). Shepherds keep breeding rams with ewes and, with good nutrition, ewes will lamb every 9 to 10 months. The sale price of lambs has increased by 10 to 20 percent per year over the last 10 years or more, due to the increasing human population, urbanization and incomes, as well as the increasing gap between demand and supply of meat.

Mr B.V. Nimbkar, the founder of the Nimbkar Agricultural Research Institute (NARI), a non-governmental, non-profit foundation established in 1968 in Phaltan in Satara District of Maharashtra in western India, used to have his own sheep and had observed the sheep-rearing scenario around him for a long time. He was appointed in 1988 by the Government of Maharashtra as the chairman of a "Commission to study the problems of goat and sheep production in Maharashtra". He realized in the course of reading about sheep and goat production worldwide that there were prolific indigenous sheep breeds in many parts of the world and that their owners benefited from having more lambs to sell. He discovered from the renowned Australian sheep geneticist, the late Dr. Helen Newton Turner, about the prolific "Bengal" sheep that had been taken to Australia in 1792 (10 ewes and 2 rams) and in 1793 (another 100 ewes) and that were later identified as the probable source of the *FecB* (Booroola) gene, which has a powerful effect on ovulation rate, to which the fecundity of the Booroola Merino was attributed in the 1980s (Davis *et al*., 1982). Mr Nimbkar found out about the prolific Garole (Bengal) sheep in Sundarban in West Bengal state in north-eastern India in 1992, and procured 32 ewes and 12 rams in 1993 and 1994, bringing them to Phaltan over 1 500 km by train.

Breeding of Deccani ewes of the Lonand type with Garole rams (by artificial insemination because of the small size of the Garole compared with the Deccani) began at NARI in 1996 to increase the prolificacy of the Deccani. After the *FecB* mutation was identified and a DNA test for its presence developed (Wilson *et al*., 2001), it was confirmed that the prolificacy of the

Garole was indeed because of the *FecB* gene (Davis *et al*., 2002). The partially dominant mode of inheritance of *FecB* meant that after the first cross to introduce the gene, the prolificacy could be retained in the backcrosses by selecting only the carriers for breeding. The backcrossing was necessary to ensure the *FecB* carrier animals looked like the Deccani and had its larger body size, hardiness, adaptation to harsh conditions and good mothering ability.

BIOTECHNOLOGY APPLIED

The DNA test to detect the *FecB* mutation is based on the polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) procedure (Wilson *et al*., 2001). The technical capacity to perform the test in India was initially established at the National Chemical Laboratory, and from 2009 at NARI with technical guidance from Australian scientists under a project funded by the Australian Centre for International Agricultural Research (ACIAR). The PCR-RFLP assay was an essential prerequisite for the success of the breeding programme at NARI to introgress the *FecB* mutation into the local sheep breed. This is because:

1. the phenotype of *FecB* carriers (increased number of ovulations and lambs) cannot be measured in males or before the age of puberty in females;
2. without the DNA test, laparoscopic counting of ovulations (which requires skill and is tedious, labour-intensive and invasive) is the only method of assessing the *FecB* genotype of post-pubertal females;
3. the phenotype is not completely associated with genotype in females (i.e. a female that bears two lambs is more likely to carry the *FecB* mutation, but not necessarily so, and carrier ewes do not have twins at every lambing).

Now a few drops of blood of newborn lambs from NARI's or shepherds' flocks are simply collected on Whatman FTA™ classic cards and their ear tag numbers are written on the cards. DNA is isolated from these blood samples, and the PCR-RFLP *FecB* test is carried out at NARI's molecular biology laboratory established under a project funded by the Department of Biotechnology, Government of India. If the protocols are followed strictly, about 90 lambs can be genotyped in less than two days with close to 100 percent accuracy. This facilitates selection and culling decisions on lambs at a very young age.

Rams and ewes carrying the favourable *FecB* allele, but looking mostly like Deccani sheep, have been introduced into flocks of smallholders. The ewes introduced were mostly B+ (carrying one copy of the favourable B allele for prolificacy and one copy of the normal + allele) while about half the introduced rams were BB and half B+. One copy of the *FecB* gene increases the average litter size of Deccani sheep from 1.0 lamb per lambing to 1.5 lambs per lambing (i.e. 15 instead of 10 lambs per 10 ewes lambing). Better nutrition of ewes at the time of breeding can increase



from left to right
Mr Pisal in 2012 with the breeding ram carrying one copy of the FecB gene born in his flock
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Mr Kavitke (middle) with a ewe carrying two copies of the FecB gene and its 3-weeks old triplet lambs
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Mr and Mrs Pisal of Bhadali with one of their NARI Suwarna ewes in 2004
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the number of lambs born per ewe up to 1.6. Better nutrition and management of ewes and their lambs ensures the survival of most of those lambs, guaranteeing a higher income to the owner, after deducting the cost of the supplementary feed given to pregnant and lambed ewes and their lambs. The average litter size of ewes with two copies of the B allele is about 1.8 in the NARI flock, but there are very few BB ewes in smallholders' flocks so far.

IMPLEMENTATION OF THE BIOTECHNOLOGY

Rams carrying the *FecB* gene were introduced into 26 local sheep owners' flocks from 2003 onwards. Sixty *FecB* carrier (B+) ewes and 60 non-carrier ewes were introduced. The sheep owners were selected on the basis of their contact with NARI over the previous few years through NARI's field research and extension activities and their willingness to participate in the project (Prior *et al*., 2009). All animals in the participating flocks were ear tagged; all lamb births, sales and deaths and weights of all animals every two months were recorded. Another 94 *FecB* carrier ewes were purchased from NARI by 12 local smallholders in January 2010 with bank loans. *FecB*-carrier breeding rams were sent to these smallholder flocks for free by NARI. Two of these smallholders had *FecB* carrier ewes in their flocks since 2003. One of these two, Mr Dattatray

Sopan Pisal, purchased 13 more *FecB* carrier ewes from NARI again in February 2012. The current breeding ram in Mr Pisal's flock was born in the flock and genotyped at NARI to confirm its *FecB* carrier status. Mr Pisal has sold more than 50 *FecB* carrier young ewes for breeding over the last five years to other smallholders around him. NARI has now officially designated Mr Pisal's flock as a multiplier flock. At least two people have been inspired by Mr Pisal's success to take up sheep-rearing using *FecB* carrier ewes purchased from his flock (see Box for an example).

Mr Shivaji Kavitke of Kothale village in Malshiras taluka of Solapur district in Maharashtra state used to work in Mumbai, the capital of Maharashtra, as a labourer at the port trust. He retired in 2000 and returned to his village and farm. He belongs to the Dhangar (shepherd) community and decided, in 2012, to start rearing sheep again – the family's traditional occupation. He purchased 14 non-pregnant ewes carrying one copy of the *FecB* allele (B+ genotype) and 4 ewes carrying two copies (BB), from NARI in February 2012. He also brought a NARI B+ breeding ram from the flock of another shepherd participating in NARI's project and released him in his own flock. Ten months after the purchase, (we visited him on 22 December 2012), these ewes had 29 lambs - 9 lambs from the 4 BB ewes (2.25 per ewe) and 20 lambs from the 14 B+ ewes (1.43 per ewe). Twenty lambs were about 3 months of age and nine were less than one month old. There was no mortality among lambs or ewes. The 3-month weight of single born lambs was about 14.5 kg while the combined weight of twin lambs was 21 to 24 kg. Ewes that had lambed 3 or more months before, had exhibited oestrus and been mated.

Table 1: Mr Kavitke's expenditure and earned and expected income from the first lamb crop of *FecB* carrier ewes purchased in February 2012 (based on records kept by Mr Kavitke's nephew)

EXPENDITURE (US$)[a]		INCOME (US$)	
Purchase of *FecB* carrier ewes from NARI: 18 ewes at Rs.2 300 per ewe[b]	755.5	Sale of two lambs	73.0
Purchase of maize grain	73.9	Sale of sheep manure[c]	56.6
Chain link fencing	45.6	Expected sale price of 18 lambs to be sold in Jan. 2013	821.2
Tarpaulin to protect sheep from rain	13.9	Expected sale price of 9 lambs to be sold in Mar. 2013	410.6
Vet. charges and medicines	28.0		
Total expenditure	**916.9**	**Total income**	**1 361.4**

a US$1 = Rs.54.8
b The ewes sold by NARI were 'second category' and some were >4 years old. They were sold at a subsidized price.
c The remaining manure was spread on Mr Kavitke's own land.

IMPACT

Table 2 shows that *FecB* carrier ewes had 27-46 percent higher productivity in terms of 3-month old lambs produced than non-carrier ewes in smallholders' flocks.

Table 2: Live litter size of *FecB* carrier and non-carrier ewes introduced in 2003 and 2010 and born in smallholder sheep-owners' flocks within a 25-km radius of Phaltan

TRAIT	No. OF FLOCKS	No. OF EWES		YEAR	EWE'S *FecB* GENOTYPE	
		++	B+		++	B+
Live litter size at birth per ewe lambing with at least one live lamb[a]	22	959	187	2004-08	1.03 (2 406)	1.42 (325)
Live litter size at 3 months per ewe lambing with at least one live lamb[a]					0.95	1.21
Live litter size at birth per ewe lambing including stillbirths[b]	9	244	114	2010-11	1.03 (482)	1.52 (244)
Live litter size at 3 months per ewe lambing including stillbirths[b]					0.98	1.43

Figures in brackets are the number of records.

[a] Nimbkar *et al*. (2009a)

[b] Nimbkar *et al*. (2013)

During the period from 2006 to 2008, the gross margins per *FecB*-carrier ewe in Mr Pisal's flock (with 8, 41 and 50 *FecB* carrier ewes in the three years, respectively) were 37-50 percent higher than for non-carrier ++ ewes (Nimbkar *et al*., 2009a). Table 3 shows the income per ewe per year (for ewes born between 2004 and 2008) in the same flock from January 2010 to January 2012. It was Rs.1 177 per ewe for 22 non-carrier ewes and Rs.1 869 per ewe for *FecB* carrier ewes (3 BB and 31 B+ ewes).

Table 3: Income from *FecB* carrier (B+ and BB) and non-carrier (++) ewes in Mr Pisal's flock from January 2010 to January 2012 (unpublished data)

FecB GENOTYPE OF EWE	No. OF EWES	No. OF LAMBINGS (INCLUDING ABORTIONS)	LAMBS BORN ALIVE	LAMBS SOLD	WEIGHTED AVERAGE SALE PRICE PER LAMB (Rs.)	AVERAGE INCOME PER EWE PER YEAR*
++	22	33	32	19	1 850	1 177
B+ and BB	31 and 3	71	98	52	1 412	1 869

* *Average income per ewe per year* = $\frac{\text{No. of lambs alive at 3 months X weighted average price of lambs}}{\text{2 years X No. of ewes}}$

OBSTACLES / CHALLENGES ENCOUNTERED

1. Winning the confidence of deeply traditional sheep owners was a challenge. The idea of introducing twinning was novel to them and they were not so receptive to it at first; one of the reasons being they thought it involved greater risk.

2. Two-year time lag between ram introduction and twinning of ewes: Capacity-building was needed to explain to some sheep owners that it was the daughters of the *FecB*-carrier rams that would have twin lambs and not the ewes that were mated to them. Deccani ewes lamb for the first time at the age of about 18 months. If the twinning technology is introduced only through rams, there is a long time lag between its introduction in a flock and benefits in the form of twin lambs, and a further lag of three months before the lambs are sold and monetary benefits realized.

3. Carrying the FecB mutation does not guarantee twins: This biological phenomenon has impeded the acceptance of the technology. Good nutrition of the ewes at the time of breeding leads to a higher incidence of twinning among *FecB*-carrier ewes but the mechanism involved is not fully understood. Ovulation rate and litter size are probably influenced by other genes and by non-genetic factors, so these traits vary among ewes with the same genotype and among successive parturitions of the same ewe. *FecB*-carrier ewes may have singles, twins or triplets at different lambings and some ewes have twins more consistently than others. Sheep flock owners find it difficult to understand this uncertainty.

4. Sheep owners' changed preference for Madgyal rams instead of Deccani or Madgyal cross rams: Madgyal is a breed from Sangli district in Southern Maharashtra and adjoining areas of Karnataka state that is taller and larger than the Deccani. Madgyal cross lambs are therefore almost twice as large as Deccani lambs of the same age and grow much faster. From 1994, when the project began, to 2003 when the dissemination started, the Deccani sheep owners' preference for breeding rams changed markedly from using Deccani or Madgyal X Deccani rams to pure Madgyal rams, while the *FecB*-carrier rams NARI was disseminating were mostly 75 percent Deccani and 25 percent Garole. Sheep owners preferred facial features such as a narrow forehead and a Roman nose, but the *FecB*-carrier rams have the shorter stature and wide forehead of the Garole. NARI consequently started using Madgyal rams for breeding in the nucleus flock in December 2006. Madgyal rams were mated to *FecB*-carrier ewes to produce B+ 50 percent Madgyal progeny. However, this meant a reduction in the frequency of the B allele, since Madgyal rams were non-carriers of *FecB*. Inter-se mating and backcrossing were used to produce 75 percent Madgyal B+ and BB rams. NARI began to offer sheep owners B+ rams of a Madgyal-like phenotype from 2009, and BB rams similar in appearance to Madgyal from 2012. The larger size of Madgyal rams did not lead to any lambing difficulties and improved the survival of crossbred lambs. It is, however, taking time to change the earlier negative perceptions of *FecB*-carrier rams in the minds of sheep owners.

FACTORS IMPORTANT FOR SUCCESS/FAILURE

1. Pressure for early dissemination of FecB carrier rams and ewes: In an "introgression" programme such as this one, where it is desired to introduce only a single gene influencing a desired characteristic (i.e. litter size in this case) from a particular donor breed into a recipient breed, at least four generations of backcrossing with the recipient breed are recommended prior to dissemination (van der Werf, 2009). The aim of the backcrossing is to recover the genome of the recipient breed and to ensure that the introduced gene carrier animals look and perform like the recipient rather than the gene-donor breed. In this programme, there was pressure to start disseminating gene-carrier animals after only two generations of backcrossing (i.e. animals having 25 percent or more Garole genetics), in order to determine whether the encouraging results observed in the NARI flocks would also be apparent in the traditional smallholder sheep-owning environment. Because of the high Garole proportion, the animals had some undesirable attributes compared with the Deccani, such as a short stature, horns, an undesirable appearance and poor mothering ability. The sheep owners' changed preference for the Madgyal breed compounded the problem, as explained above.

2. Choosing the right sheep owners/flocks: The "twinning technology" should be introduced only to sheep owners who practice good animal husbandry and are proficient at managing the commercial aspects such as judging the right time for selling lambs and obtaining the maximum prices. We found that some sheep owners are skilled at getting ewes that had aborted or had stillborn lambs to accept other ewes' lambs. Such skill at promoting the cross-fostering of lambs is one of the keys to successfully managing an increasing proportion of twins and triplets. The technology is also more likely to succeed with farmers who can take advantage of increased lamb numbers, for example those who have irrigation to grow nutritious fodder, or enjoy access to extra labour, or those who are more settled and less nomadic (Prior *et al*., 2009). The ewes carrying twin foetuses also need to be given a small quantity of supplementary feed from about a month to 45 days before lambing until about 3 months after lambing. Maize grain is a good and reasonably priced nutritional supplement and has also become readily available in recent years. Farmers who have irrigation have started to grow it commercially because of the high yields of the new hybrids.

3. Accompanying support in key areas such as veterinary health: NARI's extension staff members have observed that sheep owners in whose flocks the twinning technology was introduced highly valued the veterinary health support they received in managing their flocks (Prior *et al*., 2009). Such support enhances the chances of success of the technology by decreasing ewe and lamb mortality. NARI has made this intervention more sustainable by training sheep owners to vaccinate their flocks and treat minor infections in their animals. Women and school or college-educated young members of the family were found to be willing and effective learners.

ISSUES TO BE CONSIDERED FOR MORE WIDESPREAD APPLICATION OF THE TECHNOLOGY

Selected competitive animals with more than 90 percent Deccani or 50 percent Madgyal genetics and carrying the desired *FecB* allele are now available from NARI. Using these, introgression into a larger number of appropriate flocks in suitable areas of the Maharashtra and Karnataka states could start straight away. It is necessary, however, to popularize the technology through publicity and by bringing sheep owners to visit NARI and smallholders that have reasonable numbers of *FecB*-carrier ewes. The owners of flocks where introgression is started should be given training, problem-solving and other kinds of support, at least for the first five years.

In other states of India where distinctly different breeds are reared, there would have to be three to four generations of backcrossing so that the *FecB*-carrier animals would have a similar phenotype as the original breed. The institution that would carry out the introgression should, therefore, have the appropriate infrastructure to maintain a large sheep flock of several hundred ewes at least, and to carry out backcrossing, data recording and genetic evaluation. The same institution, or else a separate one with a network and regular extension and training activities among sheep owners, could carry out the dissemination (Nimbkar *et al*., 2009b). Smallholder sheep owners are likely to welcome introgression if the *FecB*-carrier animals are phenotypically superior and if they find the increased lambing rate profitable.

ACKNOWLEDGEMENTS

The funding from the Australian Centre for International Agricultural Research, Canberra, Australia, from 1998-2007 under projects AS1/1994/022 and AH/2002/038 and from the Department of Biotechnology, Ministry of Science and Technology, Government of India from April 2009 to March 2012 for the *FecB* gene introgression project are gratefully acknowledged.

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A herd of Namaqua Afrikaner ewes and lambs at the Carnarvon Experimental station
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CHAPTER 3.2

SAVING THE ENDANGERED NAMAQUA AFRIKANER SHEEP BREED IN SOUTH AFRICA THROUGH CONSERVATION AND UTILIZATION

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INTRODUCTION

South Africa is home to a considerable number of indigenous livestock breeds that are well adapted to the challenging local environmental conditions. These include cattle (e.g. Nguni), sheep (e.g. Namaqua Afrikaner and Damara), and goat (e.g. Zulu) breeds that can withstand extreme temperature, low rainfall, seasonal variation in grassland quality and endo- and ecto-parasites. They fulfill an important role in subsistence farming and smallholder agriculture with regard to cultural practices, financial stability and food security (Anderson, 2003).

Indigenous breeds are a valuable genetic resource, especially in their contribution to the overall genetic diversity and stability of rural agriculture. These breeds are particularly important for their desirable traits that allow for adaptability to harsh and arid environments, which may become more widespread in future with global climate change. Unfortunately, these breeds are often disregarded because of their perceived lower production potential compared with international commercial breeds. Uncontrolled crossbreeding, changing land-use patterns and the loss of indigenous knowledge all contribute to the potential extinction of such breeds (Hanotte and Jianlin, 2005). In order to ensure the survival of indigenous breeds it is imperative to describe their observable traits (i.e. phenotypically) and production parameters. This "phenotypic characterization" is a key initial step in the conservation of indigenous breeds, based on identification and documentation of a representative sample of individual animals - see FAO (2012) for more information. A second critical aspect of breed characterization is "genetic characterization", the study of the molecular genetic diversity based on analysis of DNA samples (FAO, 2011).

The South African Namaqua Afrikaner sheep breed was on the brink of extinction and a project was therefore launched to save the breed through a combination of conservation and sustainable utilization. Characterization was a key part of this project, and provided critical information for the design of technically sound interventions.

PROBLEM STATEMENT

The Namaqua Afrikaner is one of the most robust sheep breeds in South Africa. It is well known for its longevity, and its performance under extreme conditions compares favourably with other South African breeds such as the Afrino and Dorper (Snyman *et al.*, 1993). The original Namaqua Afrikaner sheep migrated south with the Khoikhoin people and entered South Africa between 200 and 400 AD (Cloete, 1978; Ramsey *et al.*, 2000). The Nama people of the north-west Cape and southern Namibia raised these sheep as a key component of their culture and livelihoods and the Namaqua Afrikaner became well adapted to the harsh, dry climate of the area.

From left to right
Brown headed Namaqua Afrikaner ewe
©Gretha Snyman
Brown headed Namaqua Afrikaner ram
©Gretha Snyman

Despite its positive characteristics, this indigenous fat-tailed sheep is currently considered endangered according to FAO's risk classification system for livestock breeds. In 1995, there were approximately 2 000 Namaqua Afrikaner sheep left in the country and more recent surveys suggest that the population has declined in number since then (http://dad.fao.org). Moreover, poorly planned programmes aimed at improving production and uncontrolled cross-breeding have contributed to genetic dilution and the loss of genetic variation within the breed.

The main role of the Namaqua Afrikaner is to provide meat, skins, fat and hides to the rural communities in the semi-arid Northern Cape Province of South Africa. The government has recognized the breed's importance, and over the years has undertaken various support programmes. Two Namaqua Afrikaner flocks of approximately 110 ewes each are kept at two experimental stations in the Northern Cape Province. The purpose of these nucleus flocks is to provide replacement rams of good genetic quality to the keepers of Namaqua Afrikaner sheep.

During the late 1990s, the National Department of Agriculture: Directorate Grootfontein Agricultural Development Institute (GADI), tried to identify people owning Namaqua Afrikaners to establish a breeders' association. However, the majority of the Namaqua Afrikaner keepers have traditionally been smallholders, and most people only keep a few purebred animals. Only three commercially viable farmers with flocks of approximately 100 ewes each were identified. Given the importance of local breeds to the livelihoods of small-scale livestock keepers, GADI implemented a programme (GADI-Biobank) involving the conservation and improvement of South African sheep breeds. Stakeholder workshops to discuss the issue of conservation of the endangered Namaqua Afrikaner breed were held provincially and nationally in 2006 and 2007. The two departmental Namaqua Afrikaner flocks, as well as one of the privately owned flocks, were part of this initiative. These three nucleus flocks comprised most of the remaining purebred Namaqua Afrikaner sheep in the country, and formed the base population for a breed conservation and development programme.

The project was designed to address both the needs for phenotypic and genetic characterization and to ensure the utilization and conservation of this breed. First, the breed was described and its production potential was measured so as to motivate their keepers with reference to the inherent value of the animals. A genetic study based on the DNA of the base population was also performed to establish the genetic diversity of the remaining purebred animals. This information is essential to preventing inbreeding in the long term. A cryopreservation bank for the breed was also set up.

APPROACH USED

A project for the conservation and utilization of the breed was initiated in 2009-2011, during which phenotypic and genetic characterization was performed. The study was funded by GADI and the research was carried out in collaboration with the Department of Animal and Wildlife Sciences, University of Pretoria. The biotechnology used in the project consisted of DNA markers. Microsatellite markers were used due to their usefulness in providing molecular information for small stock management (Boettcher *et al*., 2010).

The genetic and phenotypic characterization was carried out using samples from animals of the two Namaqua Afrikaner flocks maintained at the Carnarvon (30°57′ S, 22°8′ E) and Karakul (28°24′ S, 21°16′ E) Experimental Stations, and of the third Namaqua Afrikaner flock kept by a commercial farmer at Welgeluk (WGK; 31°5′ S, 21°8′ E) in the Carnarvon district. These flocks are contributors to the GADI-Biobank. Blood samples were collected from 48 animals (10 rams and 38 ewes) from each flock, for a total of 144.

DNA was extracted at GADI from whole blood, and quantification was performed using a Nanodrop ND-1000 spectrophotometer at the University of Pretoria's Department of Genetics. DNA samples were amplified with 20 microsatellite markers recommended by the FAO and the International Society of Animal Genetics (FAO, 2011). Markers were selected on the basis of amplification success, the expected allelic size range and their previous inclusion in sheep characterization studies. PCR and genotyping were performed at the University of Pretoria Department of Animal and Wildlife Sciences, Animal Breeding and Genetics laboratory. Statistical programs including MS Toolkit (Park, 2001), Arlequin (Excoffier *et al.*, 2005), Genepop (Raymond and Rousset, 1995) and Structure (Pritchard *et al.*, 2000), were used to evaluate genetic diversity, population structure and inbreeding.

Standard management practices were followed for all flocks, and the production and morphometric data collected in the Carnarvon flock were analysed to describe the phenotypic characteristics. This information is vital for promoting the breed to small-scale keepers. Namaqua Afrikaner lambs tended to be light at birth (4.2 kg), but grew quickly, so that weaning weights (26.2 kg) compared favourably with other indigenous breeds, which suggests that the breed is well adapted and capable of producing lambs that are suitable for commercial production systems.

Biotechnology has also been used for the *ex situ* conservation of the breed. A cryoconservation programme for the endangered Namaqua Afrikaner sheep breed involving collection and freezing of 307 embryos was started in 2008. Cryoconservation of semen was also undertaken, but with limited success.

IMPACT OF BIOTECHNOLOGY

The molecular characterization of the various Namaqua Afrikaner flocks provided information that has been used to evaluate and maintain genetic diversity and to control inbreeding in the nucleus flocks. This has direct value for small-scale livestock keepers, ensuring that they receive good-quality sires from the nucleus flocks through the ram distribution programmes. The genetic diversity was estimated with reference to the observed heterozygosity (a measure of the total genetic variation in the population), which yielded an average value of 50 percent. In these flocks the heterozygosity was expected to be low due to the small population size and to the flocks being kept as closed populations. The genetic diversity can be increased by using new genetic material from other sources, provided that the rams used are pure Namaqua Afrikaner and unrelated to the current flocks. The population structure analyses identified the three flocks as distinct genetic groups. This implies that there is merit in keeping the three nucleus flocks separately, and that breeding stock can be exchanged among the flocks without compromising diversity.

The inbreeding (estimated using markers by computing the F_{IS} statistic) across the population was low (F_{IS} = 0.019). This result indicates that the cyclic mating system applied was successful in limiting the mating of related animals in the relatively small flocks. Current plans are to evaluate the inbreeding of these flocks every five years to determine at an early stage any unfavourable trends, so that appropriate steps can be taken to prevent further increases in inbreeding. It is also proposed that the current system of selection of replacement ewes and sires – where only animals that have physical deformities and do not conform to the general breed appearance are culled– should be continued. This will ensure that healthy breeding stock, and especially rams with high-quality genetics, can be distributed to smallholders and keepers, maintaining the levels of diversity in the national population.

As noted earlier, 307 embryos have been obtained from Namaqua Afrikaner ewes and are cryoconserved in the GADI-Biobank. These ewes were exclusively from the Carnarvon Experimental Station. Keeping in mind the genetic distance between the flocks, plans are under way to ensure that embryos from all flocks can be cryopreserved as part of future conservation activities.

PROGRESS TO DATE

Since the start of this project, another two Namaqua Afrikaner flocks have been included in the conservation programme. These flocks are also available for the cryopreservation and donation of blood for DNA storage at the GADI-Biobank. One of the flocks is kept near Calvinia in the Northern Cape Province, and the other at Barkly-East in the Eastern Cape Province. The Barkly-East flock has genetic ties with the Carnarvon flock, as the owner purchased some animals from that flock between 1994 and 1996, and again in 2010. The same set of microsatellites used in the original analysis should be used to genetically characterize animals from these two new flocks, in order to determine their genetic diversity and distance from the three flocks already characterized. If the animals in these flocks are sufficiently diverse from the flocks included in this study, the possibility of introducing rams from these flocks into the Carnarvon or Karakul flocks will also be considered in future.

The phenotypic production and reproduction performance of the Namaqua Afrikaner sheep indicated that their growth rate and reproductive performance compared well with other commercial sheep breeds. By combining the conservation effort with a commercial application, the future existence of the breed can be assured.

As an additional part of the conservation effort, a programme was implemented in 2011 where surplus young ewes and rams from the two experimental flocks are made available to farmers interested in conserving this breed, as well as to small-scale farmers in the Northern Cape Province. Sheep provided to the farmers have been selected on their production, and records are kept with regard to their original flock and distribution. Six farmers already received a total of eight rams and 50 ewes from the Carnarvon flock. Three of these are small-scale farmers, one is a commercial farmer and the other two are conservation agencies. The success of the distribution is monitored by tracking the utilization by the small breeders and obtaining feedback on the perceived value of the programme. The programme is very much at the initial stage of implementation, and there is a continuous effort to distribute more breeding stock to small keepers in the Northern Cape Province.

PROS AND CONS

Genetic biotechnology was essential in enabling the estimation of the genetic diversity of the Namaqua Afrikaner sheep. Breed and production information was collected concurrently for the accurate description and the informed and efficient conservation of the breed. Reproductive biotechnology holds a long-term benefit for the conservation of the endangered Namaqua Afrikaner breed. The GADI-Biobank has the capacity to store up to 1 000 embryos, and efforts are under way to obtain the funding necessary to support the collection of the additional material. The embryo donors will be selected based on pedigree and phenotypic data recorded within the experimental flocks.

Conserving and developing a breed such as the Namaqua Afrikaner in a country with a dual agricultural sector serving both the commercial and smallholder agriculture sectors is not an easy task. It is essential to use modern biotechnology first to gain an understanding of the breed with regard to its genetic structure, and secondly to generate commercial interest for funding. The identification of suitable farmers interested in participating in the utilization programme poses certain challenges due to different levels of education within the communities, limited infrastructure and a shortage of agricultural extension services. There is, however, a concerted public and private effort to ensure the success of this programme.

In conclusion, biotechnology has laid the foundation for developing proper strategies for the long-term genetic management of this endangered breed. The experimental populations are kept to prevent extinction and to monitor genetic variability. At the same time, the recording of phenotypic production is essential for utilization to ensure that breeding animals distributed to small farmers add to their livelihoods and food security.

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Patagonian Angora goat breeder with his flock
©Martín Abad

CHAPTER 3.3

APPLICATION OF ARTIFICIAL INSEMINATION WITH FROZEN SEMEN IN AN ANGORA GOAT BREEDING PROJECT IN NORTHERN PATAGONIA, ARGENTINA

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INTRODUCTION

This case study describes an innovative approach for improving the quality and quantity of mohair production by smallholder breeders of Angora goats in northern Patagonia (Argentina), through the provision of training, the organization of livestock keepers and the application of reproductive biotechnologies.

Northern Patagonia is the home to approximately 550 000 Angora goats. The goats are typically raised in an extensive production system with low inputs. Nearly 6 000 goat breeders base their subsistence economy on the mohair produced by these animals. Mohair production in Argentina is mainly concentrated in the Neuquén, Río Negro and Chubut Provinces. Smallholder Angora goat farming is characterized by limited resources, with restricted access to appropriate technology, credit and infrastructure. Farmers have little or no access to the benefits of formal organization through cooperatives or breeders' associations. This situation results in feeble production rates in terms of mohair quantity and quality, and a weak negotiating capacity in marketing.

The Secretaría Nacional de Agricultura, Ganadería y Alimentación created the "Mohair Programme" for the Angora goat system in 1998, the main objective of which was to increase the standard of living of the small farmers that rear Angora goats by providing technical advice and commercial and financial assistance. The following were the specific objectives:

- Improving the quality and quantity of mohair;
- Strengthening the organizations of Angora smallholders;
- Establishing a transparent and advantageous market for mohair products.

The development of a "Breeding Project for Angora Goats in northern Patagonia" as part of the Mohair Programme aimed to increase qualitative and quantitative fibre production. Although Argentina is the world's fourth largest producer of mohair, the fibre yielded per animal is low. In addition to low individual production (ca. 1 kg/animal), raw mohair in the region is discounted commercially as a result of its large medullated fibre contamination (10 percent), resulting in lower prices for farmers.

The implementation of the animal breeding project for genetic improvement required the intensive use of superior males, which were evaluated and selected with reference to key production traits. The availability of genetic material was conditioned by numerous factors such as breed, the number of available males and their level of libido and semen production

(Ritar *et al*., 1992). Additionally, the scope of the breeding project was influenced by the wide geographical dispersion of flocks and by the restricted Angora breeding season caused by the photoperiodism associated with the region's high latitude. The use of artificial insemination (AI) in the breeding project significantly reduced these drawbacks and contributed to providing a technical option for smallholders to improve their mohair production through access to genetically superior material. From another and perhaps even more important perspective, AI promoted the spread of superior genes to larger goat populations.

The logistics of the Angora breeding project were established through close collaboration and consultation with goat breeders. Meetings to promote the organization of the Angora smallholders and to develop and evaluate the project goals were conducted on a regular basis.

In addition, various aspects influencing mohair quality were addressed through training and extension activities. The activities included steps for the prevention of quality loss at shearing, the conditioning and subsequent classification of the mohair; and marketing approaches and opportunities. As farmers traditionally sold small individual lots fetching low prices, the Mohair Programme promoted the large-scale collection and storage of certified quality mohair. The marketing of larger lots and the assurance of the quality provided by the Mohair Programme boosted prices (Abad *et al*., 2002; Abad, 2007).

BREEDING PROGRAMME AND ARTIFICIAL INSEMINATION

For many years, the genetic improvement of Angora goats in Argentina was organized according to a classic genetic "pyramid" scheme, whereby most of the genetic improvement occurred within a relatively small cluster of animals at the "top" of the pyramid, and improved genetic material was then multiplied through breeding in larger, unselected populations at the "bottom". A single nucleus flock (INTA Angora goats) was the only genetic centre for the improvement of Angora breed. This nucleus herd could realistically provide no more than 30 to 40 genetically superior males for natural service per year, and even the use of AI with fresh semen could not cover high demand of smallholder goat farmers for superior genetics during the short breeding season in Patagonia (two months).

The application of two biotechnologies was therefore the best way of dealing with this challenge. First, cryopreservation of semen permitted the storage of genetic material and thus facilitated improved planning and timely insemination in outlying goat herds. Second, oestrus synchronization and laparoscopic AI (LAI) improved the efficiency and success of AI. LAI allows for the deposition of semen in the uterine horn, permitting the reduction of the sperm dose concentration relative to cervical AI (CAI) (100 vs. 200 million spermatozoa/inseminated goat) (Gibbons *et al*., 1997), while still yielding a significant increase in pregnancy rates (52 percent vs 69 percent respectively for CAI and LAI) (Gibbons *et al*., 1997). This increase was similar to that reported by other workers: a 20 percent increase in pregnancy rates when applying LAI in relation to average values of CAI (Cashmere: Ritar *et al*., 1990; dairy goats: Vallet *et al*., 1992).

The following aspects were particularly taken into consideration for the implementation of the LAI procedure with frozen semen:

1. As the combination of semen cryopreservation and LAI reduced the number of males required for reproduction, it was possible to increase the selection pressure by selecting the top 15 bucks per year, rather than the top 30 to 40. The bucks were selected each year from the INTA Angora nucleus. Firstly, genetically superior males were identified and checked clinically and serologically to ensure that they were negative for brucellosis. Then, physical evaluation for breeding soundness was performed, with special attention being given to the reproductive organs and body development. The capacity to mate and semen characteristics (quality and viability), were also considered. Finally, the capacity of bucks to be sperm-donors (willingness to be collected with the artificial vagina) and the resistance of their semen to freezing were also evaluated. Individual variation in response to freezing processes (Watson, 1995) was a major tool for the selection of males involved in the breeding project. In general, the main causes of rejection of bucks were the incapacity to ejaculate in the artificial vagina (low libido), low semen volume and sperm concentration, and low post-thaw semen quality (Gibbons, 2003).
2. The eight top goat breeders of Neuquén and Río Negro Provinces were selected, establishing the "Multipliers' group" of genetically superior material. These eight top Angora goat breeders had approximately 700 females, ensuring the production of a sufficient number of superior males to cover the required genetic demand while maintaining sufficient genetic variation to avoid inbreeding. In addition, selection criteria for females were adopted and applied. The breeding objectives included fleece weight, the rate of medullated fibre, fleece density and average fibre diameter.
3. As a consequence of LAI implementation in the multipliers' group, a more rational use of the genetic material was achieved, allowing the reduction of genetic differences between the INTA Angora nucleus and the smallholder herds.
4. Specific protocols for reproductive biotechnologies including semen cryopreservation, oestrus synchronization and AI, appropriate for the Angora breed of the northern Patagonia production system, were developed.
5. Technical staff were trained to carry out the project activities, on topics including the selection of males and females, the collection and processing of semen and the application of reproductive technologies.

As a complementary activity of the breeding project, INTA continued supplying superior males to smallholders at the bottom of the genetic pyramid. These males were used for hand-mating in pens to meet the growing demand for improved genetics.

Patagonian Angora goats in the INTA nucleus flock
©Martín Abad

The following is a summary of the reproductive methodology performed in the Angora goats breeding project carried out during three years in northern Patagonia (Gibbons and Cueto, 2011):

In the mating season (April-May), fieldwork included the animal selection, oestrus synchronization and AI of approximately 700 goats per year in the eight herds of the Angora multipliers. The oestrus synchronization procedure consisted of the insertion of intravaginal sponges with 60 mg of medroxyprogesterone acetate (MAP) for 17 days followed by the intramuscular administration of 100 IU equine chorionic gonadotropin (eCG) at the time of sponge removal. The onset of oestrus was detected with the aid of adult teaser bucks (4 males per 100 females) at 24, 36, 48 and 60 hours after sponge withdrawal.

The LAI technique was then carried out at 48 hours in goats showing oestrus at 24 and 36 hours, and at 60 hours in females that showed oestrus at 48 and 60 hours after sponge withdrawal. This synchronization and insemination scheme gave the possibility of inseminating all goats of one multiplier herd in one day only, reducing the number of workdays in the insemination schedule. Animal welfare was considered by using LAI under sedation and local anaesthesia. Ethical concerns were taken into account by adhering to local animal welfare regulations and practices.

The overall pregnancy rate in the multipliers' herds during the three-year breeding project was lower (54 percent) than that obtained in the INTA experimental flock under optimal management conditions (69 percent) (Gibbons *et al*., 1997), but the reproductive efficiency (kids born/inseminated goats) of 67 percent was deemed acceptable for the breeding project (Table 1).

Table 1. Reproductive efficiency by laparoscopic artificial insemination with frozen semen in Angora goats of northern Patagonia over three years

Goats in oestrus / Goats synchronized (%)	1 690/1 964 (86)
Pregnant goats / Inseminated goats (%)	677/1 252 (54)
Kids born / Inseminated goats (%)	842/1 252 (67)
Kids born / Pregnant goats (%)	842/677 (124)

The implementation of AI with frozen semen in the Angora breeding project in northern Patagonia allowed the intensive use of genetically superior males, producing a total of 842 offspring in three years. Female kids remained under the care of multipliers, and male kids were given to the smallholders at the bottom of the genetic pyramid.

Thus, the number of animals in natural service before the implementation of the breeding project was 503, it increased to 1 292 at the beginning of the project (hand-mated plus AI) and reached 1 930 female goats under genetic improvement in the third year of its execution (Abad, 2007).

As a consequence and with the support of the Mohair Programme, the volume of certified quality mohair increased from 4 200 kg collected from 19 smallholders in 1998, to 90 000 kg from 830 smallholders in 2006. In parallel, since 2003, a differential price for quality has been offered, promoting the growing participation of smallholders in the genetic breeding project (Abad, 2007). In 2010, a lot totalling 15 000 kg of mohair fibre of different qualities was offered in the local market and for export to South Africa. After receiving several offers to purchase the lot, the final selling price, averaged across different categories certified by fineness, length and quality, was 40 percent higher than the producers had been able to obtain by selling individual lots of unclassified mohair. The commercial gains confirm the importance of working together with organized goat breeders to obtain an improvement in the quality, quantity and price of mohair produced.

LESSONS LEARNED

The use of AI must first be evaluated with reference to its chance of implementation and its limitations in regard to the specific production system (extensive or intensive and kind of production). A good understanding of the physiology of goat reproduction is also needed to improve reproductive efficiency and establish different productive strategies. For each breed and production model, it is essential to know how changes in reproductive aspects of females (development of puberty, oestrus cyclic activity, changes in ovulation rate, etc.) and males (puberty, libido, semen quality, etc.) occur throughout the year in parallel with environmental variables.

Once the decision has been taken to address a genetic improvement programme through AI, aspects such as the nutritional and health status of females and the processes of collecting, processing, freezing and storing semen should be accounted for to avoid failures that may otherwise be wrongly attributed to animal-related factors (Corteel, 1974; Lebouef *et al*., 2000). Moreover, protocols of oestrus synchronization and AI must be adapted to different breeds and production systems to ensure that these reproductive biotechnologies become an efficient tool for each goat breeding programme.

The experience obtained during the development of this programme showed us the great importance of the participation of the goat breeders' associations in the logistics surrounding the implementation of AI as a breeding tool.

Finally, the development of the Angora goat production system has its future in the ongoing challenge to increase investment in the acquisition of valuable genetic material (bucks, semen and/or embryos), with a focus on quantitative and qualitative characteristics that can add value to its production, considering the protection of genetic biodiversity in the farming system.

ACKNOWLEDGEMENTS

The authors wish to thank the Secretaría de Ciencia y Tecnología de la Argentina, Programa de Investigación y Desarrollo and Ente Provincial de Desarrollo de la Línea Sur de la Provincia de Río Negro, for the financial support of this research. The authors also wish to thank the goat herders of Neuquén and Río Negro Provinces for their contribution to developing this project.

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Low-cost, well-ventilated housing that yields good cow comfort (Photo taken in Summer 2011 in the Chittagong district) ©CDVF /Mohammed Shamsuddin

CHAPTER 3.4

USE OF ARTIFICIAL INSEMINATION IN A COMMUNITY-BASED APPROACH TO DELIVER CATTLE PRODUCTION-RELATED VETERINARY SERVICES IN FOUR DAIRY-PRODUCING AREAS OF BANGLADESH

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BACKGROUND

Agriculture plays a significant role in the economy of Bangladesh. The contribution of livestock to the agriculture sector is 15 percent, which is 2.5 percent of the national GDP. About 15.4 percent of the total protein consumed comes from livestock products, which increased by 4.3 percent during the period between 1990 and 2008 (FAO, 2010). The country possesses 23.2 million cattle and 1.4 million buffaloes. About 25 percent of its 150 million people are directly dependent on livestock and another 50 percent receive indirect benefits from livestock for their livelihoods (Ali and Rahman, 2010).

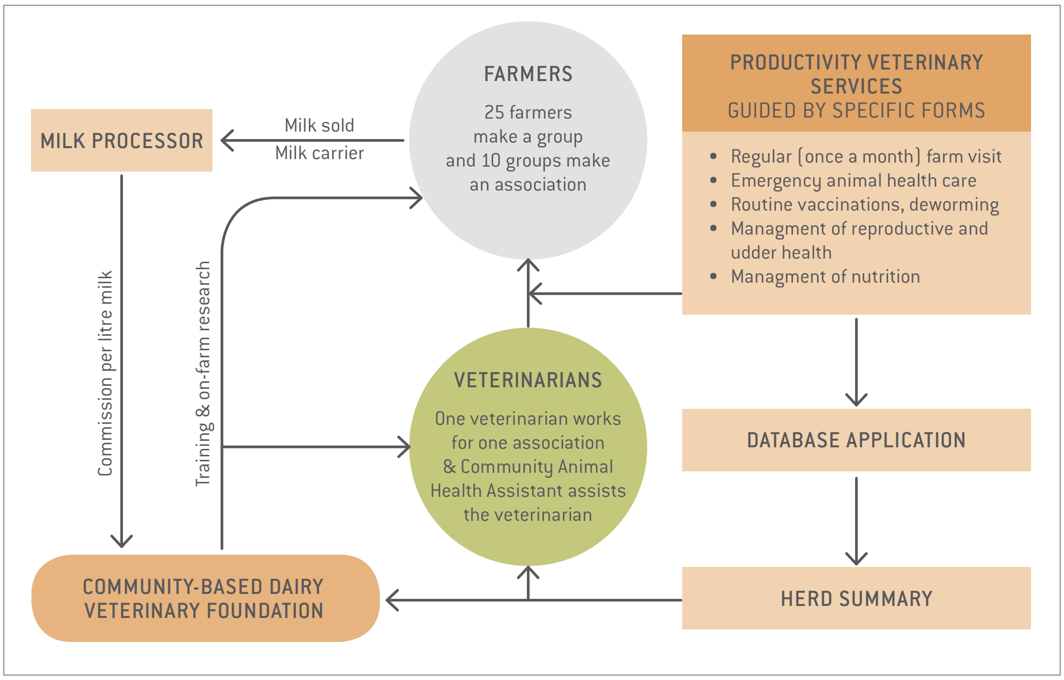
Over the last two decades, Bangladesh has experienced a major shift in the purpose of rearing cattle. Farmers now rear cattle and buffaloes primarily for milk and meat production, whereas prior to the 1990s these animals served largely as draught power for crop cultivation and rural transport. Livestock farmers in Bangladesh are overwhelmingly smallholders with one to three animals, and most of them own little or no land. The animals are mostly fed on crop residues and other by-products. The production of native zebu cattle (*Bos indicus)* of Bangladesh is low compared with crossbred (*Bos indicus* x *Bos taurus*) cattle.

Bangladesh has been practicing artificial insemination (AI) since the late 1950s.The objective was to improve the productivity of native cattle and used semen from imported zebu, *Bos taurus* and crossbred bulls. AI activities resulted in the production of about 3 million crossbred cattle in the country. However, the impact of AI on increasing milk production remained far below the expectations of stakeholders. Bangladesh has had only a modest average growth rate (2.0 percent) in milk production over the past decade, whereas a rate of at least 6.0 percent is required to achieve a goal of making 120 ml milk available daily per capita by the year 2025, considering a population growth rate of 1.6 percent (Shamsuddin and Rahman, 2009).

High-yielding cattle, especially crossbreds involving exotic breeds, suffer more from health problems and need more inputs of feed and health care than do native, locally-adapted cattle. Artificial insemination, coupled with locally based selection programmes and herd health services, has made possible manifold increases in milk production in countries with large dairy herds with stable milk markets. The owners of large herds generate enough revenue to support the purchase of supporting services. Such services have not been consistently delivered in smallholder production systems, in part because no mechanism exists for collecting revenue to bear the expenses of the services. This problem is exacerbated when no formal channels for milk marketing are available. To address this problem, starting in around 2000, scientists at the Bangladesh Agricultural University worked with local stakeholders to establish a Community-based Dairy Veterinary Foundation (CDVF) to deliver production-related veterinary services in four dairy-producing areas of Bangladesh (Satkhira, Sirajgonj, Chittagong and Mymensingh).

Figure 1 shows the model of the CDVF operation. Farmer associations deliver milk to chilling centres set up by the Bangladesh Rural Advancement Committee (BRAC) Dairy and Food Project, and revenue for the CDVF is generated through a negotiated commission per litre of milk delivered. The revenue is then used to pay dues for services to the CDVF. The CDVF delivers a package of on-farm activities, not only to prevent infectious and zoonotic diseases, but also to sustain production by improving animal nutrition, reproduction, udder health and welfare. This combination of activities is defined as a productivity veterinary service (PVS) (Shamsuddin *et al.*, 2010).

Figure 1. The model of delivering CDVF service in smallholder dairy farms



The PVS is not a substitute for traditional on-demand veterinary practices, rather it is a complementary addition. The PVS considers the entire herd and production system instead of focusing solely on clinically ill animals. The primary objective of PVS is to reduce the economic loss of farms due to diseases that result from sub-optimal management. The secondary objective of PVS is to reduce the economic loss from failure to attain optimum productivity with respect to milk, meat and calves.

The objectives of PVS are achieved by optimizing:

- the health status of a herd by improving health, reproductive efficiency and production;
- the productivity of the herd by improving management practices;
- production process in relation to animal welfare and the ecological quality of the farm environment;
- the quality and safety of dairy and meat products; and
- the profitability of the livestock enterprise, either by increasing farm income or by reducing costs, or both.

In the community-based approach to smallholder farmers, the veterinarian interacts with farmers to further increase farm profits by:

- reducing somatic cell count through mastitis prevention and control;
- increasing dry matter intake through nutrition management and improved cow comfort;
- improving reproductive efficiency;
- decreasing the age at first calving through heifer management programme; and
- advising on the effective utilization of labour and resources.

All these activities, together with guaranteed milk marketing, increase farmers' compliance with the requisites of the AI service-providers, who are now aiming to introduce genetic improvement programmes to speed up breed development.

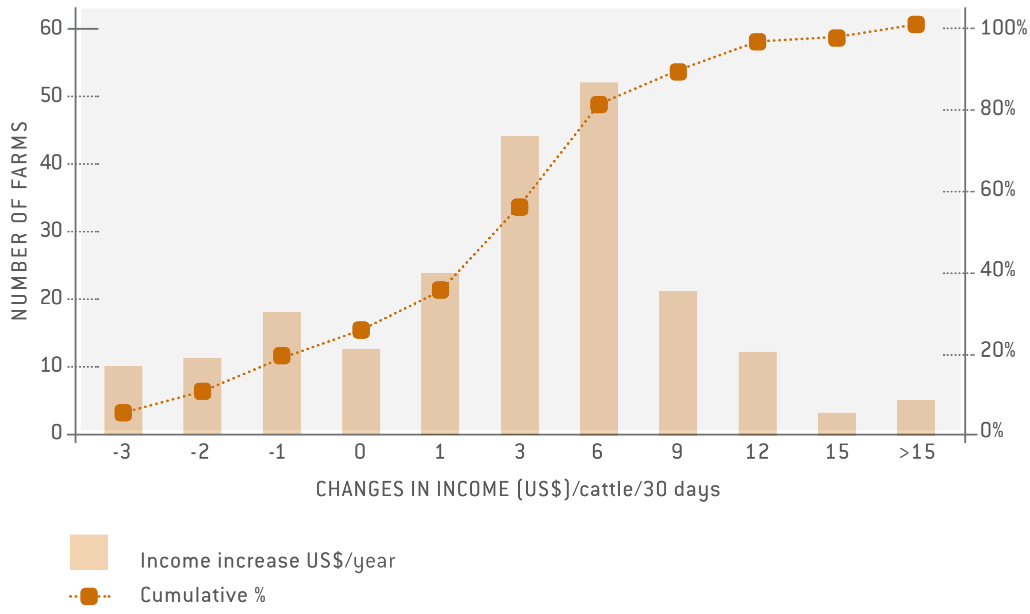
STATUS OF ARTIFICIAL INSEMINATION (AI) SERVICES IN BANGLADESH

About 4.0 million AIs were performed in cattle in 2012 in Bangladesh, of which 2.6 million were by the government through the Department of Livestock Services, 1.3 million by BRAC, an NGO, and 0.1 million by the Bangladesh Milk Producers Cooperative Union Limited, with its brand name "Milk Vita". Currently AI services can cover 40 percent of breedable cows in Bangladesh (Shamsuddin, 2011a). In a study that included major areas with dairying as an important economic activity and used the Artificial Insemination Database Application for Asia (AIDA Asia) information system to record AI data (Garcia, 2002), the conception rate in smallholder cows was 51 percent (Siddiqui *et al.*, 2013). A similar conception rate was reported earlier by Shamsuddin *et al.* (2001) who used radioimmunoassay of milk progesterone for the evaluation of the cyclicity. However, the quality of the service is not consistently high across the country. Conception rates vary widely according to the skill of the AI technician (Siddiqui *et al.*, 2013). Proper bull monitoring and culling would also improve fertility (Siddiqui *et al.*, 2008), as would improvement and standardization of semen collection, processing and storage procedures (Sugulle *et al.*, 2006).

However, there are some obstacles at the cow and herd level. The nutritional condition of cows and buffaloes significantly affects the interval from calving to first service and is often substandard (Shamsuddin *et al.*, 2001; Banu *et al.*, 2012; Siddiqui *et al.*, 2013). Poor heat detection is also an important limiting factor. In another study using progesterone radioimmunoassay, Shamsuddin *et al.* (2006) observed that oestrus was accurately detected in only 30 percent of cases. Another 30 percent of cows were detected as in oestrus when they were not (false positive) and 40 percent cows remained undetected when they were in oestrus (false negative). Poor oestrus detection has also been reported in buffaloes, where the cyclicity was evaluated by determining progesterone in milk by using enzyme-linked immunosorbent assay (ELISA) (Banu *et al.*, 2012).

A PVS can be used to propose and deliver interventions to increase the proportions of postpartum cows cycling and bred (Shamsuddin *et al*., 2010; Kamal *et al.*, 2012). From 47 to 86 percent of anoestrous heifers and cows resumed their oestrous cycles, evident by behavioural signs and per-rectal palpation of the genital tract, when treated with hormones, vitamins A, D_3 and E or nutritional supplements (Shamsuddin *et al.*, 2010). Ultrasound of genital tracts revealed that 53 percent of apparently anoestrous (silent oestrus) cows were in fact cycling (Kamal *et al.*, 2012). Farmers were subsequently able to breed 68 percent of the silent oestrous cows after treatment with hormone or nutrition supplementation or by increasing the frequency of visual heat detection and 70 percent of inseminated cows conceived (Kamal *et al.*, 2012). Thus, the PVS helped to increase the number of cows inseminated per year, which in turn led to an increased number of calves born, more cows in milk and higher income for the farmers. In a comparison to the period without the service, the PVS resulted in a per-day increase of 1.5 litres of milk per buffalo, 1.0 litres of milk per crossbred cow and 0.75 litres of milk per indigenous cow (Shamsuddin, 2011b). About 80 percent of farms that received the service in the Satkhira district saw an increase in their income, ranging from US$1 to $19.4 per cow per month (Figure 2). Moreover, with accumulated increased income, farmers were able to increase the size of their herds by purchasing new cows.

Figure 2. Effects of productivity veterinary services on farmers' income in Satkhira (n=213)



COMMUNITY-BASED APPROACH TO DELIVER PRODUCTIVITY VETERINARY SERVICES IN SMALLHOLDER DAIRY FARMS

Unfortunately, operating a PVS involves fixed costs that usually cannot be sustained by a single or small group of individual smallholders. However, although individual smallholder farmers cannot pay the cost of PVS, members of a community of 250-300 farmers that collectively produces ~2000 litres milk per day can put aside a small portion of their individual income from milk to enable them to pay together the cost of services. This concept has been proven in Bangladesh by organizing smallholder farmers and establishing the CDVF. Organization helped the farmers not only to generate a commission to support the PVS, but also to increase their bargaining power to get a better price for the milk. Furthermore, the PVS both increased smallholder farmers' income and instilled a sense of confidence in the farm community, which in turn led to a very rapid increase in the number of farmers participating and in the amount of milk produced by the community (Figures 3 and 4).

Figure 3. **Milk production in Satkhira district from April 2008 to August 2010**

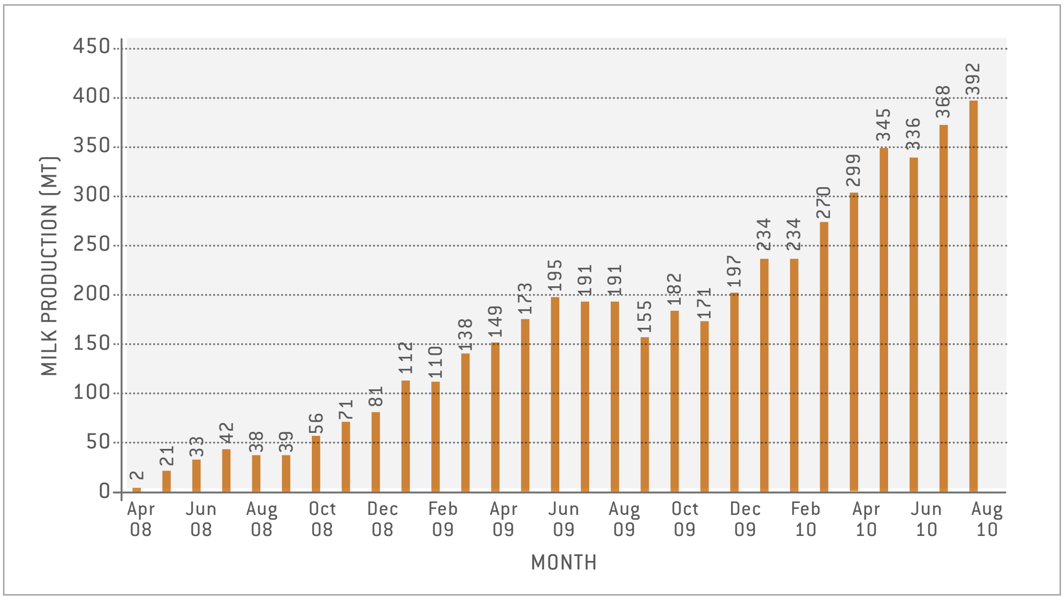
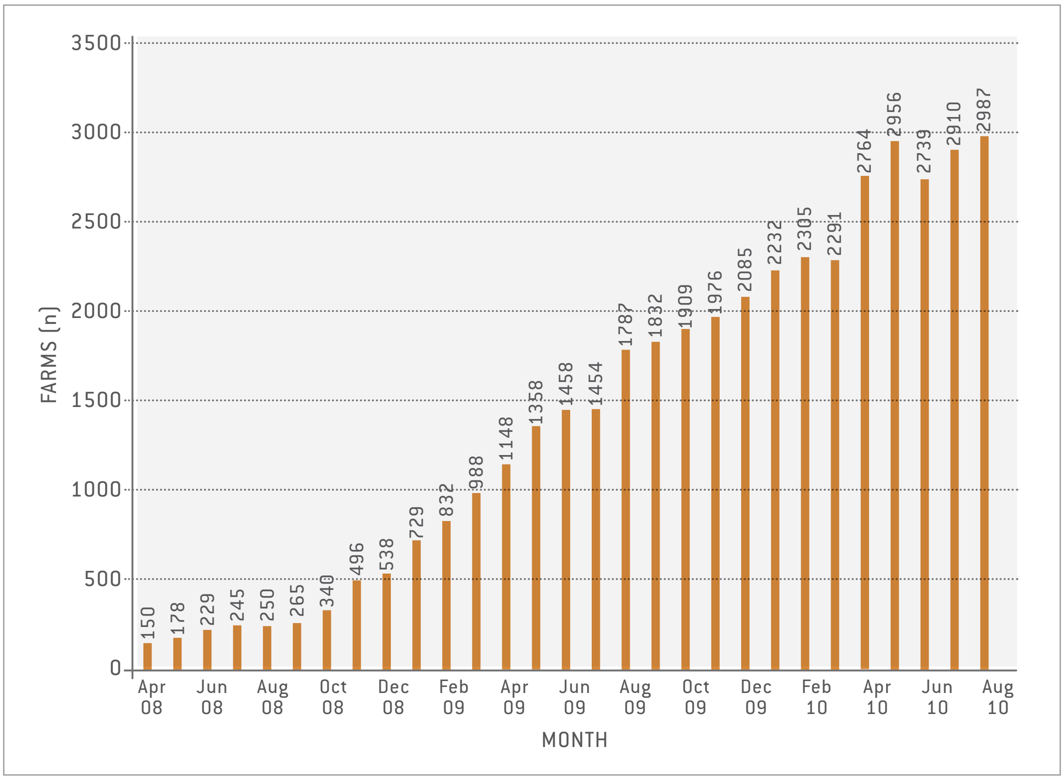


Figure 4. **Number of farmers receiving productivity veterinary services in Satkhira district from April 2008 to August 2010**



SELF-SUSTAINABILITY OF CDVF

A formal agreement between farmers' associations, veterinary service providers and milk processors is critical to the regular procurement of good quality milk, AI services and PVS delivery. Accordingly, CDVF has made an agreement with a milk processor in Bangladesh to install milk collection and chilling tanks for the CDVF areas in Satkhira and Sirajgonj. Milk collectors carry milk from the community to the chilling tanks twice daily. Each week, the milk processors pay a predetermined price for milk to the farmers on the basis of fat percentage of the quality-assured milk. In addition, the processor pays a commission per litre of milk to the CDVF for providing PVS to the community. The commission received from delivering 2 000 litres milk daily (from approximately 250 farms) to a chilling centre is enough to support the salaries of 20 milk collectors (US$62.50-75 per collector monthly), one veterinarian (US$312.50 monthly) and one field assistant (US$100 monthly), and cover the rent and maintenance of an office (US$75 monthly) in the community (unpublished data). In addition, CDVF provides vaccines and drugs for routine deworming for all farms. The CDVF activities led to increased income from dairying for farmers by increasing production and assuring a fair milk price. This agreement also benefits milk processors who are able to procure high-quality, natural milk, as they can test the fat and solids not fat (SNF) and check for adulteration at the chilling centre. The employment (milk collectors) generated by the CDVF is very important in a country like Bangladesh where rural unemployment is a big problem.

PRESENT STATUS OF THE CDVF

The success of the CDVF can be measured by its popularity among farmers. Between 2008 and 2012, the number of farms that received the PVS services in Satkhira increased from 150 to 2 935. In Sirajgonj, the number of participating farmers went from 170 in 2009 to 400 in 2012. Monthly milk production is currently 392 tons in Satkhira and 36 tons in Sirajgonj versus 2 and 12 tons respectively before the CDVF was established. In Sirajgonj, CDVF members had a greater ($P < 0.05$) economic return than non-member neighbours (Shamsuddin *et al.*, 2010). In Chittagong, the programme started in 2002 with 35 farmers and now involves 260. The monthly milk production increased from 75 tons to 360 tons in CDVF operation area in the Chittagong district.

THE WAY FORWARD

Artificial insemination will yield greater benefits to farmers if bulls are selected on the basis of the performance of their daughters in the relevant local production system. The herds in the farming communities of Bangladesh include a large number of locally-adapted crossbred cattle that have a large proportion of genetics imported from temperate developed countries. Many locally-adapted crossbred and indigenous animals show outstanding productivity and adaptability compared with the average population in the tropics. These cattle could revolutionize the dairy cattle breeding in developing countries because of their combination of adaptability and productivity. Unfortunately, because of the lack of performance recording programmes, animals such as these are not utilized in the county's breeding programme. The next step to advance the productivity and incomes of local farmers is to combine biotechnologies with telecommunication and information technologies. These latter technologies have created a nearly unlimited opportunity to not only collect and store information on the productivity and health of livestock and on the production system, but also to make it available to peers. Cell phones are now commonplace among farmers in Bangladesh. Mobile phone text messaging has been used to successfully accomplish many important surveys with national issues and could be used to collect and share production data. If customized to fit the purpose, these technologies can surely help to overcome the challenges of recording, retrieval, analysis and preparing reports on reproduction and breeding data in the smallholder dairy farms.

Other emerging technologies may also contribute to improve dairy productivity in developing countries. Genomic technologies can greatly improve the accuracy of genetic evaluations, assuming data is available for analysis. An important future research question will be to know how the genomes of these adapted crossbred animals have allowed them to maintain their adaptability to the hot and humid climate and at the same time incorporate effects of increased productivity, which had been imported from temperate climate.

Of course, the production system is an important issue, which is likely to differ between farms and between agro-ecological zones in the same country. Global positioning systems (GPS) and geographic information systems (GIS) can be utilized for the identification and niche mapping of the farms with outstanding breeding stocks to help account for precise effects of the environment. Radio frequency identification (RFID) and other electronic technology can be used to help identify animals. Robust reproductive biotechnologies such as ovulation synchronization guided by ultrasound of the genital tract and/or hormone assay and timed insemination will significantly increase the number of cows bred. Simultaneously, improved feeding will drive more cows to cycle for breeding with a higher conception rate. This holistic approach will bring a new era of implementing reproductive biotechnologies in the smallholder farms and recording quality data for selecting outstanding stocks for further developing AI services in future breeding.

Well grown heifers; the heifer on the left is two months pregnant at 15 months of age ©CDVF /Mohammed Shamsuddin

CONCLUSIONS

The coverage of AI in Bangladesh has increased over the last decade from 30 to 40 percent; however, the national average for milk production has remained far below expectations. Smallholder farmers obtain greater benefits from AI services if they are coupled with PVS and milk-marketing opportunities. Organization of smallholders into private farmers' associations has proved itself a driving force for the development of dairying in Bangladesh by increasing bargaining power and skills in the management of farm economics. In the future, a holistic approach combining AI and PVS with information technologies for performance recording of a critical number of animals as the future breeding stock, molecular technologies for genetic and genomic characterization of economically important traits, the application of reproductive biotechnologies such as ovulation synchronization guided by ultrasound, hormone assays and improvement of nutrition can provide new opportunities for the fast growing dairy industry in Bangladesh to provide milk to feed the people.

ACKNOWLEDGEMENTS

We thankfully acknowledge the USDA, Joint FAO/IAEA Division on Food and Agriculture and Government of Bangladesh for the research grants used to compile the data presented in this article.

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Harvesting of fertirrigated maize in August 2012
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CHAPTER 3.5

INTENSIVE AND INTEGRATED FARM SYSTEMS USING FERMENTATION OF SWINE EFFLUENT IN BRAZIL

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INTRODUCTION

Among the major challenges of the near-future agriculture is achieving the ability to simultaneously provide enough food and ecosystem services to urban societies, while providing "equivalent urban opportunities and benefits" to rural smallholders, who represent more than 50 percent of the global food production (Herrero *et al*., 2010; Lee *et al*., 2012). Meeting this challenge would considerably reduce rural exodus to the cities and, at the same time, achieve a more adequate point of equilibrium between food security and the provision of ecosystem services on Earth (e.g. Uriarte *et al*., 2012). Biotechnologies have a complementary role to play in achieving these goals.

Large-scale, intensive farming systems usually comprise monocultures designed to provide a limited range of product outcomes. In the case of Brazil, most monocultures are focused on the production of sugarcane for sugar and/or ethanol, eucalyptus for wood and paper industries, and soya and grains for intensive production of livestock (e.g. for milk, beef, pork and chicken) and modern industrialized human foodstuffs. Although large-scale farming has been highly profitable and has contributed greatly to the Brazilian export market, this production model has numerous drawbacks, including: the concentration of land, financial wealth and other resources; increased rural exodus; and loss of ecosystem services such as water production and purification, carbon sequestration in soils and forestry and several other biogeochemical processes involving local and regional biodiversity.

Therefore, over the last two decades, Brazil has been redesigning its rural landscape by promoting the redistribution of land to smallholders. The consolidation of democracy and wide spread of syndicalism have been the catalysts for the resettlement of many families. However, the ambiguity of the syndicalism thesis and the lack of planning in government programmes have rendered the land redistribution activities largely ineffective, primarily because:

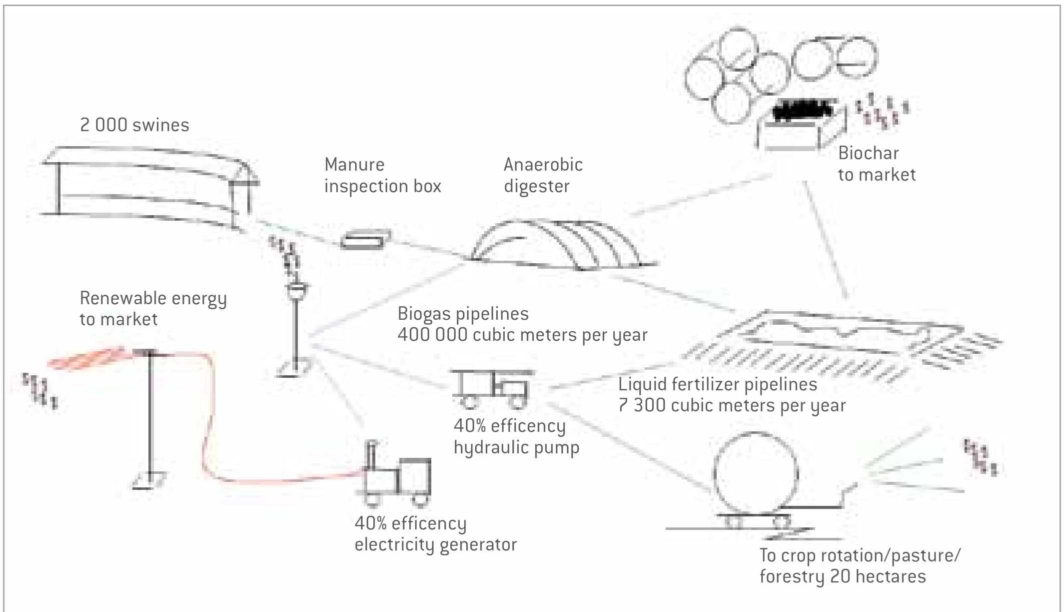
- most of the new rural settlers were already so "urbanized" that they had lost their indigenous knowledge and agricultural skills;
- the settlements were installed in areas with inappropriate or unproductive soil profiles, and, particularly;
- the services to provide technical and financial assistance to the newly settled families were grossly inadequate.

To address this problem, the Brazilian Agricultural Research Corporation (EMBRAPA) undertook a study with the Ministry of Science, Technology and Innovation (MCTI) and the Ministry of Agriculture, Livestock and Food Supply (MAPA) to identify critical factors for the successful resettlement of smallholders. The first factor noted was the need to recognize or envisage local and regional strengths and weaknesses regarding rural productivity. This can be best achieved by inspecting local and regional natural resources and noting the presence or absence of established markets and industries and assessing the available knowledge and capacity for innovation.

THE PROJECT

The project *Intensive and Integrated Farm Systems for Smallholders* in São Gabriel do Oeste, Mato Grosso do Sul, Brazil (Campanário Settlement - 19°16'46.90"S, 54°36'2.35"W), is a good example of incorporating biotechnologies into a successful programme of re-establishing families in rural settlements, while maintaining their dignity and ensuring adequate power of consumption. The Campanário Settlement comprises 142 families on 2 850 hectares (i.e. 20 hectares per family). The core scope of the project is the maintenance of smallholder families in the food production system by having access and opportunities as similar as possible to those of families living in nearby cities or metropolises.

Figure 1. Illustration of the Intensive and Integrated Farm System driven by swine effluents tackled with innovative technologies developed in São Gabriel do Oeste, Mato Grosso do Sul, Brazil



Source: illustration by Carlos Shimata, improved by Ivan Bergier

from left to right
Biodigester effluent as a source of fertilizer and biogas-driven machinery involved in fertirrigation of integrated cropfields
©EMBRAPA /Ivan Bergier
Maize and eucalyptus tree lines fertirrigated with biodigested swine manure in April 2012
©EMBRAPA /Rubia Rech

The project is a collaborative effort involving EMBRAPA, a local cooperative called the Cooperativa Agropecuária São Gabriel do Oeste Ltda (COOASGO) and a local company (Retificadora Centro Sul) and is built on the idea that it is possible to use biotechnologies to profitably transform potential swine industry pollution into profitable and useful products while simultaneously promoting favourable balances of energy, water and nutrients with social inclusion. The excess organic material and energy from swine production is converted into new products that can be commercialized by the cooperative at favourable prices and with the sharing of profits, thus substantially increasing the revenue of the settlement dwellers.

The initiative was started in 2008 by the City Hall of São Gabriel do Oeste, underlining the importance of the local government in the success of the Campanário Settlement. At that time, COOASGO, in partnership with the city government, EMBRAPA, MCTI and MAPA started a programme of social inclusion for 13 selected smallholder farmers by enabling the construction of thousand-head swine production facilities with a shared fertirrigation system (where fertilizers are applied with irrigation water). Two contiguous swine farms were chosen to implement a pilot-farm unit with the installation of a laboratory (gas chromatography, computing and water quality monitoring), in operation since May 2012, and a facility for demonstration of power and solid fertilizer production, in operation since April 2012. The selection of farms was based on the perceived capability of each family to follow the guidelines established by the cooperative to improve productivity, reduce the use of non-renewable resources and, consequently, mitigate the pollution of air, water and soil. A private company (Brascarbon) interested in certified emission reduction under the Clean Development Mechanism of the Kyoto Protocol also collaborated by constructing biodigesters and agreeing to transfer ownership of the digester to the settlers after seven years of operation. In addition, the digester effluent and a portion of the biogas generated were allowed to be used by the smallholders under the previously described *Intensive and Integrated Farm Systems for Smallholders* project, which is funded by the MCTI and the National Research Networks in Agrobiodiversity and Agricultural Sustainability programme (CNPq/REPENSA).

The controlled use of the anaerobic biodigestion or fermentation can be considered as one of the most important biotechnologies created by mankind. For example, it serves as basis for the production of numerous products for human consumption, silages and by-product feeds for livestock production and ethanol as a biofuel. In the case of effluents produced by concentrated populations of humans (cities) or animals (livestock), biodigestion has become a common technique for secure disposal and water treatment.

The commercial pig industry demands precise control of the materials and components of the swine diet. COOASGO has the advantage of producing its own feed with selected materials designed to optimize feed conversion efficiency. In the typical Brazilian production system, pigs are marketed after 120 days of confinement, meaning there are three cycles of swine production per year. The fermentation of swine effluent has two main outputs: 1) biogas for power generation and, 2) biofertilizer, which can fully replace mineral fertilizers in well-managed (i.e. kept at a high fertility level) soils of agro-ecosystems. The biogas is reasonably enriched in methane (>50 percent) that has an average heat of combustion of around 55 MJ/kg.

Considering that anaerobic biodigestion of animal waste can provide renewable energy and renewable fertilizer, its widespread integration into the swine industry and other agricultural sectors seems nearly inevitable, in particular in West Central Brazil. The anaerobic biodigestion of effluents from the swine industry is now so important that the traditional crop production areas of West Central Brazil are steadily becoming the main areas of swine production. This scenario favours the "Intensive and Integrated Farm Systems" project for the successful social inclusion of smallholder farms in Brazil. The project can be applied on both large and small farms; it is suitable for cooperatives and is extraordinarily effective at improving productivity while reducing pollution.

A local company named Retificadora Centro Sul has developed a unique engine (Rieger, 2006) to convert the biogas into either mechanical or electrical energy. The development was achieved after five years of exhaustive tests carried out on diesel engines adapted for biogas by means of special electronics and automation. The power efficiency of these engines is 40 percent, which is astonishingly superior to similar engines available worldwide. An adapted diesel power station of 100 kW can run continuously on inputs of 40 cubic metres of biogas per hour. Considering that a single adult pig has the potential to produce wastes yielding roughly 0.5 cubic metres of biogas per day (Bergier *et al*., 2012a), a population of 2 000 animals can produce 100 kW of power, which is very attractive for the alternative energy market in Brazil. However, this component of the project still requires government regulation of the market at the state level to be properly implemented.

A fraction of the power is used for dispersing the biodigester effluent onto pasture, crop, or forest land or combinations of these in different proportions. This process is called fertirrigation, where a pump draws the effluent, whose annual production from 2 000 swine is about 7 300 cubic metres (Bergier *et al*., 2012a), and moves it through stainless steel pipelines to a series of three or four manually regulated valves. At these valves, a pipeline reel has been connected that disperses the effluent over rectangular plots of three hectares each with pasture or corn, each of which are adjacent to eucalyptus groves. Retificadora Centro Sul also designed this technology, which includes a special pump driven by biogas (Rieger, 2006), so the entire processes of power generation and fertirrigation are completely driven by renewable energy. Consequently, instead of simply burning the methane to decrease the impact on climate change, the project uses it in a beneficial way, thanks to the locally-developed technology. The local knowledge in São Gabriel do Oeste was crucial not only to the sustainability of the swine production enterprise, but was also integrated into the production of other foods (such as milk, beef and grains) and material (eucalyptus wood and solid fertilizer).

THE OBSERVED AND EXPECTED OUTPUTS AND OUTCOMES

For every thousand swine, the effluent produced can safely fertirrigate an area of 10 hectares (Bergier *et al*., 2012a). The effluent has been applied to pasture, corn and soybean fields and a small eucalyptus plantation. As a result, with fertirrigation of 180 cubic metres of biofertilizer per hectare per crop cycle, the increase in yield of the pasture land has allowed the community to double its milk production, while also increasing the productivity of corn and soya. The fertirrigated eucalyptus in this study was planted in 2011/2012. With this application rate, the wood produced is expected to be available for the market in four years, substantially earlier than the standard seven years using conventional forestry technology.

The biogas and the swine effluent are also key inputs in the process of creating a novel solid fertilizer. This fertilizer is produced by pyrolysis of the nutrient-dense (especially N, P, K, Ca, Mg and other micronutrients) digested swine solids, yielding a material with biochar-like properties. The biochar is produced under special conditions that impart to it the properties of slow nutrient release and high humidity retention, allowing it to improve soil quality and carbon content, thus improving the productivity and the sustainability of the mixed livestock and cropping system. The machinery and process of production of this special biochar were developed by EMBRAPA, Retificadora Centro Sul and COOASGO and are currently undergoing the patenting process (Bergier *et al*., 2012b, 2012c). The machine for solid fertilizer production in the pilot-farm yields about 800 kilograms of biochar per month (unpublished data). Laboratory-scale biochar production from digested swine manure is discussed in Bergier *et al*. (2013).

All of these products (renewable power, fertirrigation of agro-ecosystems and biochar) are also contributing to the restoration of natural forestry for recovering the ecosystem services of a portion of the farmlands, in association with the National Institute of Colonization and Agrarian Reform (INCRA), as a strategy to help cope with the extreme weather events (droughts or floods and increased temperatures) expected under future climate change scenarios. The project has the vital collaboration of two government Ministries (MCTI, MAPA), universities (Federal University of Mato Grosso do Sul - engaged in monitoring groundwater; State University of Campinas – modelling by means of Life Cycle Analysis and Energy; and Federal University of Paraná – Principles of Integrated Farm Production: PISA), and institutes (National Institute for Space Research – greenhouse gas and atmospheric transport and Federal Institute of Mato Grosso do Sul – database development, sensor network development and electronics and automation knowledge transfer), with a grant from MCTI and CNPq/REPENSA .

The CNPq/REPENSA grant has been used for monitoring key processes (gaseous emissions, water and soil qualities) taking place in the pilot-farm. To inform the public about the project, a group of about 30 students from 9 to 14 years from the “Dorcelina Folador” state school in the settlement were familiarized with the project as part of a strategy to transfer the knowledge in open source hardware and software (Arduino), and, specifically, to promote the use of technology and innovation for achieving sustainability by the next generation of the Campanário Settlement.

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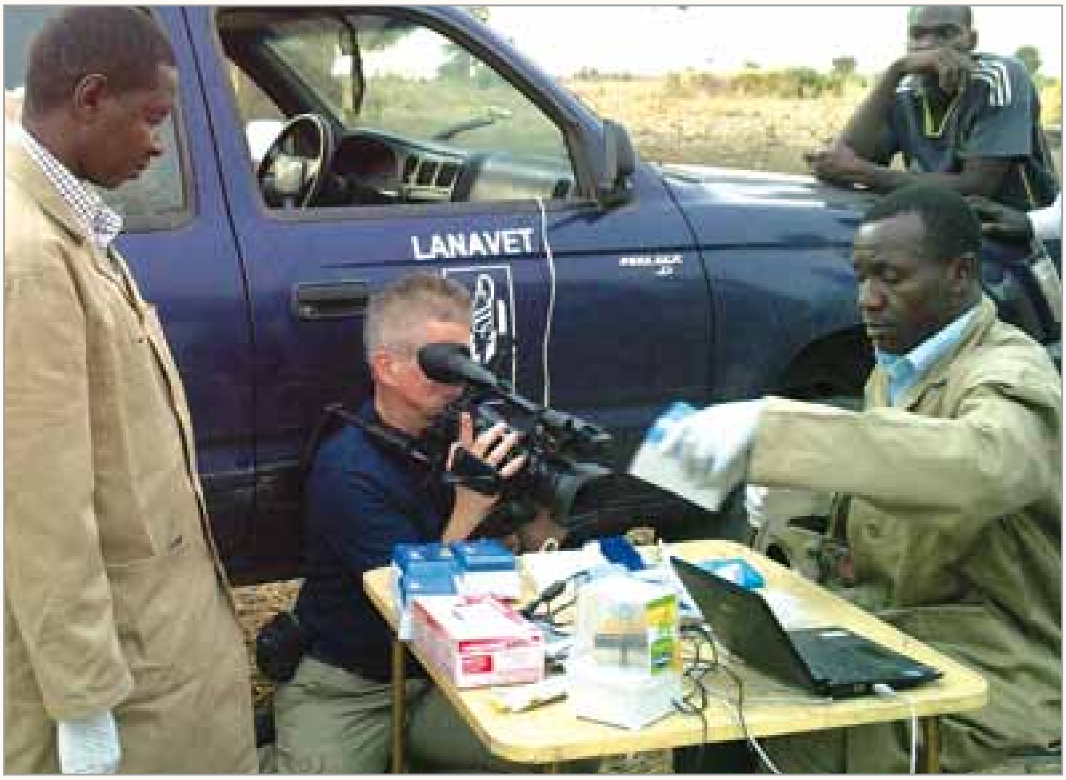

Laboratory analysis of collected samples (nasal swabs and blood) using LAMP PCR in the field. The LAMP device is connected to the cigarette lighter of the car (for energy) and to a laptop (for reading the results in real time)
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CHAPTER 3.6

TAKING THE LABORATORY TO THE FIELD: RAPID DIAGNOSIS OF PESTE DES PETITS RUMINANTS (PPR) IN CAMEROON

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INTRODUCTION

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants (especially sheep and goats). The disease is economically very significant due to its very high (close to 100 percent) morbidity and mortality rates. First identified in West Africa in the 1940s, PPR is now present throughout sub-Saharan Africa, including Cameroon, and has spread to other parts of the world as well. The wide diffusion and high virulence and contagion of PPR have led to its status as a reportable transboundary animal disease (OIE, 2012; FAO, 2012).

For years, farmers have been terrified by PPR outbreaks in sub-Saharan Africa and one factor limiting its control is the lack of an early detection system complementary to the sophisticated laboratory-based diagnostic procedures. Such a tool could help stop the spread of the disease at an earlier stage, limiting its impact.

The PPR virus is an RNA virus and thus has to be reverse-transcribed before the nucleic acid can be amplified using the polymerase chain reaction (PCR). Although scientists have developed several methods of diagnosing this disease, they were all found to be technically challenging, not sufficiently robust, time-consuming, expensive and not well adapted to African reality in the field. The rapid diagnosis of a pathogen during an outbreak investigation is not only essential to ascertain the cause but, more importantly, allows fast decision-taking for the application of preventive and control measures to stop its rapid spread.

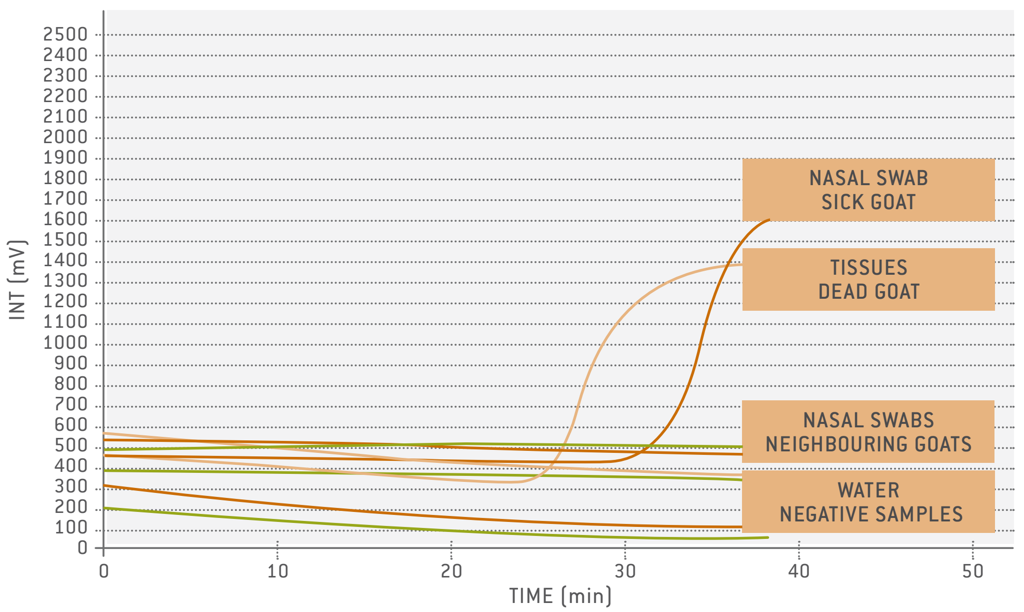
Loop-mediated isothermal amplification (LAMP) is a novel method for nucleic acid amplification in which the target nucleic acid is continuously amplified by a single enzyme at a constant temperature. In contrast, standard PCR requires a thermal cycler, a specialized machine that alternates between low and high temperatures to allow repeated turns of DNA synthesis and denaturation. Because LAMP PCR is undertaken without the need of thermal-cycling equipment, it has attracted quite some scientific interest as a practical diagnostic tool in the field, especially in developing countries (Fu *et al*., 2011). The technique uses four primers that recognize six regions on the target nucleic acid, so that the specificity is extremely high. The LAMP PCR method is also highly efficient and enables the synthesis of large amounts of DNA in a short time (less than an hour including sample preparation). The accumulation of amplified target DNA results in increased turbidity of the reaction solution, or changes in fluorescence if specialized marker dyes are incorporated. These changes can be detected with a photometer and even measured in real time to allow for a quantitative analysis. As the readings have quantitative values, they can be sent via mobile phone to a reference laboratory for confirmation and further study. Protocols have been developed for detection of PPR virus via LAMP PCR (Li *et al.*, 2009; Li *et al.*, 2010).

In an attempt to use this technology to assist countries under the umbrella of the International Atomic Energy Agency (IAEA) "Food for the Future" initiative (IAEA, 2012), a diagnostic procedure based on LAMP PCR was developed through a coordinated research project operated by the joint FAO/IAEA Programme for Nuclear Techniques in Food and Agriculture (AGE) in Vienna. Scientists at AGE developed a specific "Master mix" of reagents that can be transported at room temperature and used to apply LAMP PCR directly to biological samples without the need for DNA or RNA extraction. Through this project, the LAMP PCR technique was transferred to the Laboratoire National Veterinaire (LANAVET) and several other laboratories to evaluate its fitness for purpose and suitability as a field diagnostic device. In addition to these reagents, the project also distributed to participants an adapted "tube scanner" photometer, a fluorescence measurement system based on next-generation technology, for detection of the pathogen and quantification of the amplified DNA. The tube scanner used in the project is manufactured by the ESE/Qiagen company (Düsseldorf, Germany), and distributed free of charge to the countries participating in the project. These two "ingredients" provided the foundation for building simple "mobile laboratories" consisting of the tube scanner and a laptop computer for visualization of the results – both connected to a 12-volt car battery and vials of reagents and two pipettes allowing for a very rapid real-time test (results obtained within 40 minutes) with only three pipetting steps. In addition, this real-time method allows the amplification of RNA in a single closed tube. The assay system is cost-effective: the tube scanner costs about US$6 000 and reagents about US$2 per sample. This report presents the success story of using the mobile laboratory concept in the field to investigate two outbreaks of PPR in Cameroon.

DESCRIPTION OF THE CASES

The first case occurred in January 2012 after the death of one goat and the observation of clinical signs in several other members of a herd of sheep and goats in Vélé centre, Vélé sub-division, Mayo-Danai division in the Far North region of Cameroon. The local delegate of the Ministry of Livestock, Fisheries and Animal Industries (MINEPIA) informed the Director General of LANAVET via telephone a few minutes after the death of the first goat in the afternoon of 29 January 2012. The mobile team arrived the following morning (11.00 hours) from the LANAVET offices in Garoua, about 325 km away. After clinical examination of the herd by the LANAVET team, PPR was proposed as the disease responsible. Samples were immediately collected from sick goats and the carcass was examined to confirm the clinical diagnosis. Samples were subjected to LAMP PCR carried out in a tube scanner (LAMP device). Positive results confirming the PPR diagnosis were obtained within 30 minutes (Figure 1). The local veterinary officer of MINEPIA was immediately alerted and rapid control measures were taken. A ring vaccination programme against PPR was organized immediately the next day for all susceptible animals in the entire village, including the neighbouring countryside. Although the diagnosis was unable to save the animals in the flock where the outbreak occurred, the quick action limited the outbreak to the original farm.

Figure 1. PPR LAMP PCR results for outbreak in Vélé: the two upward curves are for positive samples. Negative samples are linear (water negative control and swabs from goats of neighbouring farms)



The second outbreak was in Gabarey Waka (about 25 km from the first site) in the same division, along the Logon River less than two kilometres from Koumi village in Chad. It was reported to the Director General of LANAVET in the same manner as the previous one on 26 March 2012, and was also diagnosed using LAMP PCR. The subsequent implementation of a vaccination programme again contained the outbreak to the original flock and mortality in that flock was limited to 30 percent.

In addition to its simplicity, requiring neither thermal-cycling equipment nor DNA/RNA extraction, LAMP PCR is also flexible in terms of the source of biological samples. In the first outbreak, nasal swabs from a sick goat and tissues (lung and intestines) from the dead goat were used for the analysis. In the second outbreak, nasal swabs and whole blood samples were collected from four clinically sick goats and analysed using LAMP PCR. Samples of blood for sera were also collected for further analysis.

The real-time graphics for the outbreak in Vélé is presented in Figure 1. A clear real-time curve of the amplification plots of the two positive samples was observed already after 25 minutes in Figure 1, indicating that a specific reaction for the PPR genome had happened.

DIFFERENCE MADE

The impact of the field diagnosis was very positive. It was successful not only in identifying the pathogen at the earliest stage possible, confirming the concerns of the farmer, but also and more importantly, in triggering rapid control measures (ring vaccination in communities around the infected farm, the gathering and sensitization of farmers of the risk and of the need to respect the "no-in, no-out" principle to avoid disease spread), which halted the spread of PPR at the farm level. As noted previously, all goats on the first farm contracted PPR and died, while 30 percent mortality was observed in the second flock within two weeks of the initial diagnosis. These results demonstrate the very destructive effect of that PPR viral strain. Nevertheless, with the benefit of the rapid diagnosis, damage was limited to these flocks only. Without this rapid response, thousands of sheep and goats would likely have succumbed to the disease during these outbreaks, leading to millions of CFA francs in losses. Such a fast reaction would not have been possible if the conventional procedure had been followed of taking samples to the laboratory for analysis and then waiting for results before implementing control measures.

For example, just a few years earlier, PPR destroyed almost all the goats in the nearby village of Sinasi (a locality in the Mayo-Rey division), before control measures could be implemented. Similarly, another outbreak was recorded in Guider (North region) where most of the community's goats died from infection by the PPR virus before control action could be taken. In addition, an acute contagious bovine pleuro-pneumonia outbreak in Douang Gouvra (Mayo-Danai division) caused the death of 12 cattle (40 percent) within one week in five small household herds, with most of the rest infected by the time that control measures were finally implemented. Although one cannot be certain, it is likely that application of the LAMP PCR diagnosis procedure could have limited the damage in these cases.

Arrival of the LANAVET mobile laboratory team at the outbreak site, installation of working materials while farmers bring sick sheep and goats for sampling in order to get the diagnosis *in situ*. The senior veterinarian (Abel Wade) calls the Director General of LANAVET after clinical examination of a few animals to say that PPR is highly suspected and urgent laboratory analysis is needed
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As is nearly always the case with application of biotechnology, the success achieved was not entirely the result of the LAMP PCR technology, but depended also upon appropriate training by AGE of LANAVET staff and continual interaction among farmers, veterinarians and staff of LANAVET and MINEPIA. When farmers see how the veterinarian is conducting sample analysis in their farm, they are motivated to collaborate in control measures. As a result of this communication, the AGE was able to develop the technology to meet exactly the expectations of veterinarians doing fieldwork and performing their tasks far away from laboratory infrastructure. In terms of lessons learned, the principle of taking the laboratory to the field is an attractive option for the surveying and control of transboundary animal diseases, especially in developing countries.

CONCLUSIONS

LAMP PCR has the potential to catalyse a revolution in the diagnostic methods used in disease surveillance and investigation in developing countries. This was the first known report of an in-the-field molecular diagnosis of a transboundary animal disease outbreak, and the response time was cut to within a few hours from the initial disease reporting. This technique should be further exploited, as its implementation will improve disease reporting and allow the rapid application of control measures if coupled with vaccination and targeted culling. Livestock farming is the main economic activity for the majority of poor households, so early disease diagnosis (taking the laboratory to the field) and the rapid control of outbreaks, as in this case study, have the potential to support poverty alleviation. Faster access to veterinary services, containment of outbreaks at the early stage and limitation of mortality rates can improve the economic situation of small livestock holders.

ACKNOWLEDGMENTS

The authors are grateful to all the scientists who contributed to development and implementation of the LAMP technique. Special thanks go to AGE for knowledge transfer, supply of LAMP reagents and financial aid for field visits to LANAVET and to ESE/QUIAGEN for providing the tube scanner.

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The eradication of the tsetse fly Glossina austeni and the disease trypanosomosis from Unguja Island allowed the maintenance of upgraded cattle breeds that produced significantly more milk
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CHAPTER 3.7

APPLICATION OF THE STERILE INSECT TECHNIQUE IN ZANZIBAR TO ERADICATE TSETSE FLIES, THE VECTORS OF TRYPANOSOMOSIS

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BACKGROUND

In 36 countries in South Saharan Africa, tsetse flies (*Glossina* spp.) transmit blood parasites of the genus *Trypanosoma,* causing a deadly disease called trypanosomosis, also known as "sleeping sickness" in humans and "nagana" in livestock. An area of 8.7 million km² is infested by at least one of the more than 30 tsetse fly species and subspecies, exposing approximately 50 million African cattle to the bite of tsetse flies.

The disease is a major bottleneck to agriculture and rural development, as productive livestock needed for animal traction to plough the land and for transporting commodities to markets, as well as for the production of milk, meat and fertilizers, often succumb to the disease. Found only in Africa, tsetse flies and the disease they transmit are responsible for over US$1 billion in direct losses to livestock every year, while the lost potential from not-realized production in agriculture and livestock systems is estimated at US$4-5 billion per year. This is because the nagana infection – even when not fatal – weakens animals to the point that they are no longer able to produce or provide draught power for agriculture and other purposes.

The continuous treatment of livestock with trypanocides and insecticides is not sustainable in view of the high costs and increasing resistance to these drugs. Vector eradication campaigns using traps, spraying and covering livestock with "pour-on" insecticide have been effective in suppressing tsetse populations, but in areas of denser vegetation, where insecticide penetration and attraction to the traps are limited, eradication has not been achieved. Under such situations, the sterile insect technique (SIT) can be an important final component of area-wide integrated pest management (AW-IPM) campaigns, with sterile males locating and mating with the remnant wild virgin females to render areas under agricultural development completely free from the tsetse and trypanosomosis disease.

ZANZIBAR

One example of successful application of the SIT for control of tsetse and trypanosomosis is the Unguja Island of Zanzibar in the 1990s. Prior to this programme, trypanosomosis prevalence among the mostly indigenous cattle averaged around 19 percent. The rural farming communities were unable to maintain livestock as a basis for productive mixed farming, for providing sufficient and good-quality food crops and vegetables, milk and meat to their families, or for income-generation through marketing agriculture and livestock products.

During several years of preparatory activities, a monitoring network was established and the fly population was suppressed using insecticide-based control tactics such as pour-on formulations on livestock and stationary targets that attract and kill flies. This period was followed by a four-year operational AW-IPM campaign involving the systematic monitoring and weekly aerial release of sterile male flies. After the last wild female fly was trapped in September 1996, weekly sterile male releases and monitoring were continued respectively through 1997 and 1999 to ensure that the tsetse was indeed eradicated and disease transmission had stopped.

The SIT is a type of birth control for insects. It is a biotechnological tactic that integrates biological and engineering techniques to produce on an industrial scale and then release, usually by air, reproductively sterilized (usually via irradiation) insects of the target pest. Virgin female individuals of the pest population that are mated and inseminated by released sterile male insects do not produce any progeny. If the repeated inundative releases of sterile males allow them to out-compete wild males for mating, the wild population declines. The SIT acts in an inverse density dependent way. Sterile males become increasingly effective with the declining pest population in finding and mating with the remaining wild females. In situations where populations are isolated and systematic releases over the whole target pest population are sustained long enough, the population can eventually disappear. Other modern biotechnological tools such as molecular population genetics are used to study the degree of gene flow between various pest insect populations, which provides indicators regarding their relationship and potential isolation. This information on particular pest populations leads to better feasibility assessment and planning of AW-IPM campaigns with an SIT component.

To meet the demand for sterile insects, a mass-rearing facility was established at the Tsetse and Trypanosomiasis Research Institute in Tanga, Tanzania, and a colony of more than one million tsetse females was maintained during the operational phase. Because tsetse flies feed only on blood, a system to collect blood from slaughtered animals was established, and a process to decontaminate the blood from micro-organisms developed. The decontamination of blood was achieved through irradiation, using the same gamma chamber used to sterilize the males, but at a different dosage. Starting in 1994, weekly aerial release of sterile males took place over Unguja Island, following established flight lines. At the peak of the eradication campaign, 110 000 sterile males were released each week over the entire island, which drove the population to extinction. An intensive monitoring network confirmed that the wild tsetse fly population had declined and had been finally eliminated, and that transmission of trypanosomosis to livestock had disappeared (Vreysen *et al*., 2000).

The project was financed by the Government of the United Republic of Tanzania, IAEA, FAO, and various donors including Belgium, Canada, China, Sweden, the United Kingdom, the United States of America, the International Fund for Agricultural Development [loan to Zanzibar] and the OPEC Fund for International Development.

For decades, the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, with its Agriculture and Biotechnology Laboratories in Seibersdorf, Austria, has been developing, refining and promoting this environmentally friendly technique for controlling populations of major insect pests. Since 1970, at the request of African countries suffering from this deadly disease of humans and warm-blooded animals, the feasibility of using the SIT for tsetse species was assessed. Mass-rearing, sterilization, transporting and release procedures were developed for seven tsetse-fly species of major economic importance, including an *in vitro* artificial membrane blood-feeding system, thus avoiding the large-scale use of live animals for tsetse feeding. The Tsetse and Trypanosomiasis Research Institute in Tanga further refined these techniques for the target tsetse-fly species in Zanzibar.

Over the years, the Government of Tanzania, along with external partners, had made several attempts to eradicate populations of different tsetse-fly species in the country using traps, ground spraying and “pour-on” insecticide formulations, but the suppressed fly population always rebounded. It was only after the SIT was added to the traditional methods used in Zanzibar as part of an AW-IPM campaign, that the *Glossina austeni* population was finally eradicated from the island. A centrally organized full-time management team and structure under the Ministry of Livestock and Fisheries Development of the Federal Republic of Tanzania and the Ministry of Agriculture of Zanzibar, was established to manage the fly production in Tanga and the area-wide field activities on Unguja Island and to coordinate the implementation of the campaign with livestock owners and other stakeholders.

CHALLENGES

One of the main challenges in area-wide programmes is organizing farmers into associations and getting the commitment and support for the eradication campaign from government authorities, donors and other stakeholders. Another challenge is the mass-rearing of insects, which is a seven-days-a-week/365-days-a-year effort, requiring good technical, engineering and managerial support. One of the challenges for the Zanzibar campaign was an army ant attack one night on the tsetse colony, which delayed colony build-up and, consequently, the campaign [tsetse flies have a slow rate of reproduction].

After the removal of the tsetse fly *Glossina austeni* and the disease trypanosomosis from Unguja Island, agricultural productivity increased significantly mainly due to the availability of draught oxen
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The operational budget of the AW-IPM project with the SIT component in Zanzibar was approximately US$3.5 million spread over four years, which included insecticide-based tsetse suppression, veterinary and entomological monitoring, sterile male production and weekly aerial release. An additional US$2 million included substantial insectary refurbishment, operational research, and many other activities that have also benefited other projects. An earlier major eradication attempt by other institutions, which spent over US$3 million on only applying insecticide-treated targets and pour-ons on livestock, contributed to suppressing the tsetse population on Unguja, but was unsuccessful in achieving eradication. The successful eradication therefore clearly involved a substantial investment, but costs for the entire AW-IPM campaign must be weighed against the many benefits obtained so far and expected in the future, and against the cost of permanently living with the problem and investing in continuous suppression based on insecticides.

IMPACTS

With the complete disappearance of trypanosomosis, farmers on Unguja were able to integrate livestock with cropping activities in areas where this had been impossible before. Overall, the quality of people's lives increased substantially thanks to increased livestock and crop productivity, animal use for transport and traction, etc. As summarized by Feldmann *et al*. (2005), two economic surveys conducted two and five years after the completion of tsetse eradication operations (Tambi, Maina and Mdoe, 1999; Mdoe, 2003) confirmed the following.

- The number of small farmers holding indigenous cattle increased from 31 percent in 1985 to 94 percent in 2002.
- There was high demand for improved livestock breeds (mostly crossbred), and the number of farmers holding improved cattle breeds increased from 2 percent in 1985 to 24 percent in 2002.
- From 1985 to 1999, milk production nearly tripled and the proportion of farmers selling milk from indigenous cattle increased from 11 percent in 1985 to 62 percent in 1999.
- The portion of small farmers using oxen for ploughing increased to 5 percent in 2002 but was expected to increase thereafter, significantly increasing the crop productivity of their farms.
- From 1999 to 2002, the average monthly income of farming households increased by 30 percent. The proportion of households with a monthly income of over US$25 increased from 69 to 86 percent; and the proportion with a monthly income of over US$50 increased from 22 to 36 percent. This can be associated with tsetse and trypanosomosis eradication since a strong correlation was observed between household income and milk yields, milk sales, and the use of manure and animal power for cultivation and transport.

In addition, the removal of the tsetse population from the Jozani forest reserve, where tsetse flies represented a major threat to adjacent livestock and agricultural systems, facilitated preserving this endangered habitat. Efficient wildlife management practices implemented after tsetse eradication even resulted in an increase in the numbers of some rare and protected wildlife species, such as the Zanzibar red colobus monkey, *Procolobus kirkii*.

For a long time, the tsetse and trypanosomosis problem did not get the attention it deserved, because it affects only rural Africa. The Zanzibar success raised the hope of African governments and stimulated similar campaigns on mainland Africa. At the African Summit of 2000, the Pan-African Tsetse and Trypanosomiasis Eradication Campaign (PATTEC) was established under the African Union Commission, calling for eradication of the tsetse from Africa over the next decades (Feldmann, 2004). In conjunction with technical support provided by the Joint FAO/IAEA Division, PATTEC has been supporting follow-up programmes that aim at integrating the

SIT for creating trypanosomosis- and tsetse-free zones in selected areas. Among the most advanced programmes are ongoing tsetse eradication campaigns in the Southern Rift Valley in Ethiopia and the Niayes region of Senegal.

CONCLUDING REMARKS

Success was the result of effective technology transfer and dedicated technical support by the FAO/IAEA, local and expatriate staff dedicated full-time to the campaign, training and commitment by the government and several donors. Other essential components of these area-wide eradication campaigns were: adequate public awareness and education; flexible management of the complex logistics; adequate baseline data collection; good sterile fly quality and back-ups for the fly colony; available spare parts for key equipment, and adequate infrastructure for the mass-rearing facility, including a gamma source and reliable access to electricity, water and other supplies. The isolated nature of Unguja Island and its distance of 35 km from the Tanzania mainland made it favourable to sustaining the status of eradication, as reinvasion was not a concern.

The eradication of tsetse from Unguja Island is not the only case of a successful AW-IPM programme with an SIT component. The New World screwworm fly, *Cochliomyia hominivorax*, which causes myiasis in warm-blooded vertebrates, including humans, livestock and wildlife, has also been successfully eradicated from all of North and Central America, as well as from an outbreak in Libya (Vargas-Terán, Hofmann and Tweddle, 2005). This pest is currently being contained with sterile males along the Panama-Colombia border. The SIT has been also very effective in suppression or eradication programmes against a number of major crop insect pests such as fruit flies and moths (Dyck, Hendrichs and Robinson, 2005).

To be able to move more decisively towards freeing larger areas in sub-Saharan Africa from tsetse, more capacity-building in all activities related to the area-wide implementation of the SIT will be required, as well as the establishment of a number of large tsetse fly mass-rearing facilities that should preferably be managed by the private sector.

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